UNIVERSITE D'AUVERGNE **UNIVERSITE BLAISE PASCAL**

N° D.U. 2214 Année 2012

ECOLE DOCTORALE

DES SCIENCES DE LA VIE,

SANTE, AGRONOMIE, ENVIRONNEMENT

N° : 566

Thèse

Présentée à l'Université Blaise Pascal

Pour l'obtention du grade de

DOCTEUR D'UNIVERSITE

Spécialité : SCIENCES DES ALIMENTS

Khaled ABBAS

Effet de traitements thermiques sur les propriétés fonctionnelles de fromages traditionnels : le cas des pâtes persillées

Soutenue le 13 Janvier 2012 devant le jury :

Pr. MICHAUD Ph.	Polytech'Clermont-Ferrand, Univ. B. Pascal	Président
Pr. DUFOUR E.	DGER, Ministère de l'Agriculture et de la Pêche	Rapporteur
Pr DHULSTER P.	IUT A, Université Lille 1	Rapporteur
Dr. ALLAIS I.	AgroSup, Dijon	Rapporteur
Pr. DEGHIDI M.	Université de Fayoum, Egypte	Examinateur
DR. BERDAGUE J-L.	INRA Centre de Clermont-Ferrand-Theix	Examinateur
Pr. LEBECQUE A.	VetAgro Sup Campus agronomique de Clermont	Directrice de thèse
Dr. AÏT KADDOUR A.	VetAgro Sup Campus agronomique de Clermont	Co-encadrant de thèse

Unité de Recherche CALITYSS

VetAgro Sup Campus Agronomique de Clermont

89 Avenue de l'Europe – F-63370 Lempdes

REMERCIEMENTS

Cette thèse a été financée par une bourse de l'enseignement supérieur attribuée par l'Ambassade d'Egypte et l'université de FAYOUM ; elle a été réalisée au sein de l'Unité de Recherche « **C**onsommateur-**Ali**ment **Ty**pique-**S**anté-**S**écurité » de VetAgro Sup. Je souhaite remercier Monsieur le Ministre de l'enseignement supérieur égyptien ainsi que Monsieur le Président de l'université de Fayoum (Egypte) de m'avoir accordé cette bourse et m'avoir permis de me former à la recherche.

Je souhaite également remercier Monsieur Stéphane MARTINOT, Directeur de VetAgro Sup, Messieurs Georges GOSSET et Etienne JOSIEN, Directeurs généraux adjoints de VetAgro Sup Campus agronomique de Clermont, ainsi que Madame Isabelle CHEVALLIER, responsable de l'UR CALITYSS, de m'avoir accepté au sein de leur laboratoire.

Je tiens à exprimer mon entière reconnaissance au Professeur Annick LEBECQUE ma directeur de thèse ainsi qu'au Dr Abderrahmane AÏT KADDOUR (co-encadrant de thèse). Je les remercie vivement pour leur grande disponibilité et leurs nombreux conseils tout au long de ce travail et pour m'avoir offert par leurs compétences scientifiques et pédagogiques les moyens de mener à bien ce projet. Je remercie également le Professeur Eric DUFOUR, pour m'avoir accepté dans son laboratoire la première année de thèse.

Je suis très sensible à l'honneur que me font Mme Irène ALLAIS de AgroSup Dijon, M. Pascal DHULSTER de l'université de Lille et Monsieur Eric DUFOUR de la DGER pour avoir accepté d'être rapporteurs de mon travail. Je remercie Monsieur Philippe MICHAUD de Polytech Clermont-Ferrand pour avoir accepté d'être président de mon jury et Messieurs Jean-Louis BERDAGUE de l'INRA de Theix et Mahmoud DEGHIDI de l'université de Fayoum pour leur qualité d'examinateurs dans ce même jury.

Je remercie Dr Tahar BOUBELLOUTA pour ses collaborations internes, ses conseils scientifiques surtout dans le domaine d'analyse des données et également pour les agréables moments que nous avons passé ensemble durant les années de cette thèse.

Mes sincères remerciements vont également à Cécile BORD pour son aide et ses connaissances en analyses sensorielles sans oublier Delphine, Shaïmaa et Catherine qui ont participé à la réalisation d'une partie des expériences de cette thèse.

Je remercie également les personnes des laboratoires Arlette, Marcelle, Sylvie et merci aussi au personnel du secrétariat, aux ingénieurs d'étude, ainsi qu'aux enseignants-chercheurs du département Qualité et Economie Alimentaires. Merci à Georges HULLO, François RAPHANEL et Béatrice DAVI pour leurs aides en Word et à Endnote.

Je remercie également les doctorants de l'unité de recherche CALITYSS avec qui j'ai partagé le bureau.

Mes remerciements vont à ma famille et tout particulièrement à mes parents et à mes amis qui m'ont soutenu tout au long de mon parcours.

Je garde mes remerciements amoureux pour ma femme *Shaïmaa* qui m'a soutenu au quotidien durant toutes ces années, sans elle je ne serais jamais arrivé à ce stade. Mon amour va également à mes enfants Mohamed, Ahmed, Ziad et Saif qui ont changé ma vie.

VALORISATIONS DES TRAVAUX

ARTICLES REDIGES :

K. ABBAS, E. DUFOUR, A LEBECQUE (2008-2009). Current methods for assessment of melted cheese functionalities and their correlations with sensory attributes. A Review.

K. ABBAS, S. OTHMAN, C. BORD, A. AIT KADDOUR, A. LEBECQUE (2010-2011). Characterization of physiochemical, textural, and sensory characteristics of different French Blue-veined cheeses.

K. ABBAS, R. Karoui, A. AIT KADDOUR (2011). Application of synchronous fluorescence spectroscopy for the determination of some chemical parameters in PDO French blue cheeses. (Accepted: European Food Research and Technology)

K. ABBAS, S. OTHMAN, A. AIT KADDOUR, A. LEBECQUE (2010-2011). Potential of synchronous fluorescence spectroscopy to determine the physicochemical changes of four French blue cheeses during heating and cooling: correlation with rheological characteristics.

K. ABBAS, C. BORD, S. OTHMAN, A. AIT KADDOUR, A. LEBECQUE (2010-2011). Heat-induced textural characteristics of Blue cheeses: instrumental and sensory methods.

COMMUNICATIONS A DES CONGRES (POSTERS) :

K. ABBAS, A. LEBECQUE, A. AIT KADDOUR. Synchronous fluorescence Spectroscopy for monitoring physicochemical changes during heat-treatment of blue cheeses. JED Université de Plaise Pascal, le 6-7 mai 2010, Clermont Ferrand, France. Communication écrite.

K. ABBAS, A. AIT KADDOUR, S. OTHMAN, A. LEBECQUE, Synchronous fluorescence spectroscopy for monitoring physico-chemical changes during heat-treatment of Blue cheeses. Food Innovation Congrès, octobre 25-29, Valencia, Espagne, 2010. Communication écrite.

RESUME

Les ventes des fromages AOP subissent un affaiblissement. L'utilisation de ces fromages sous forme d'ingrédient représente une alternative intéressante à la dégustation et permettrait de maintenir une activité durable aux producteurs de ces fromages.

L'objectif de ce travail est d'explorer les potentialités de 4 catégories de fromages à pâte persillée AOP du Massif-Central à être utilisés comme ingrédient à chaud pour répondre à des propriétés telles que « fondant, nappant, filant, gratinant». Les fonctionnalités recherchées pour ce type d'usage ont été prospectées à l'aide de méthodes physico-chimiques et instrumentales classiques mais également à l'aide de méthodes spectrales et sensorielles afin d'enrichir les observations de la macro et microstructure fromagère et de la perception du consommateur.

Dans un premier temps, les caractéristiques physicochimiques, rhéologiques et sensorielles à froid et certaines propriétés à chaud ont été décrites pour les 4 catégories de fromages. Ces premiers résultats ont permis de cataloguer les fromages selon des caractéristiques essentiellement liées à l'organisation matricielle des composants tels que les protéines, les matières grasses, les minéraux. L'analyse sensorielle a permis de décrire précisément certaines propriétés à froid qui ne sont pas mises en évidence par d'autres analyses.

Les pâtes persillées ont une grande hétérogénéité de pâte due à la présence de « veines » de moisissures. La seconde partie est une étude sur la capacité de la SFS en tant que méthode d'étude de la micro structure, à prédire la diversité de composition de ces fromages. Malgré la forte hétérogénéité des fromages, la SFS permet une « empreinte » identifiable des fromages et prédit certains mais pas la totalité des paramètres physicochimiques.

L'évolution de la macro et microstructure de ces fromages au cours du chauffage et du refroidissement a ensuite été étudiée par SFS et par test de compression dynamique. Ces méthodes décrivent bien la fonte de la matière grasse et de la matrice fromagère. Elles sont corrélées entre elles et démontrent une relation entre la structure moléculaire et les propriétés rhéologiques de ces fromages.

Une étude comparative des propriétés sensorielles des fromages a été menée à froid et après chauffage. Les attributs décrivent des caractéristiques de texture différentes entre les 2 conditions. Cette démarche a permis de bien différencier les 4 catégories de fromages et de mettre en évidence certains attributs de qualité ou de défaut pour une utilisation à chaud.

En conclusion, ces 4 catégories de fromages à pâte persillée ont des potentialités à être utilisés comme ingrédient pour usage à chaud. L'analyse sensorielle a caractérisé les perceptions des consommateurs vis à vis de ces fromages chauffés et ainsi aider à préciser leurs fonctionnalités. Les analyses rhéologiques et spectrales ont apportés des explications liées à ce traitement au niveau de la macro et microstructure de la matrice fromagère.

Mots clés : fromage ingrédient, pâte persillée, traitement thermique, propriété fonctionnelle, test de compression dynamique, spectroscopie de fluorescence synchrone, analyse sensorielle, chimiométrie.

ABSTRACT

Sales of PDO cheeses undergo a decline. The use of these cheeses in the form of ingredient represents an interesting alternative to the tasting and would allow to maintain a sustainable business activity to the producers of these cheeses.

The objective of this work is to investigate the potentialities of four categories of Blue cheese PDO of the Massif Central as an ingredient to meet their properties during heating such as "melting, stretchability, browning". The functional properties desired for this type of cheese have been investigated by physico-chemical methods and instrumental classics and also by spectral method and sensory evaluations to enrich the observations of the macro and microstructure of the cheese and the consumer perception.

First, the physicochemical, rheological, sensory characteristics and certain properties during heating have been reported for four categories of Blue cheese. These initial results allowed us to characterize the cheese studied according to their characteristics essentially which were related to the organization matrix constitutes such as proteins, fats, minerals. The sensory analysis allowed to describe exactly certain properties which are not revealed by the other analyses.

Blue-veined cheeses have a great heterogeneity due to the presence of "veins" of mold. The second part is a study on the ability of the SFS method to study the micro structure and to predict the diversity of composition of these cheeses. Despite the high heterogeneity of cheeses, SFS allows an identifiable 'fingerprint' of cheese and predicts some but not the totality of the physicochemical parameters.

The evolution of the macro-and microstructure of these cheeses during heating and cooling was then studied by SFS and dynamic shear test. These methods describe well the melting temperature of the fat and the cheese matrix. They are correlated between them and demonstrate a relationship between the molecular structure and the rheological properties of these cheeses.

A comparative study of the sensory textural properties of cheeses was carried out in the non- and heated forms. The attributes describe different characteristics of texture between the two conditions. This approach allowed to differentiate well the four categories of cheeses and to highlight on certain attributes of quality or defects to be used in cooking application.

In conclusion, these four categories of Blue-veined cheeses have the potential to be used as an ingredient in cooking application. The sensory analysis characterized the perceptions of the consumers in both non- and heated forms and thus helps to specify their features. The rheological and spectral analyses have provided explanations related to this treatment at the level of the macro and microstructure of the cheese matrix

Keywords: Cheese ingredient, Blue-veined cheese, heat treatment, functional properties, dynamic shear test, synchronous fluorescence spectroscopy, sensory analysis, chemometrics.

LISTE DES ILLUSTRATIONS

LISTE DES TABLEAUX

LISTE DES FIGURES

LISTE DES ABREVIATIONS

AOP: Appellation d'Origine Protégée

AOC : Appellation d'Origine Contrôlée

PP : Pâte Persillée

ANOVA : Analyse de Variance

AFNOR : Association Française de Normalisation

TPA : Profil d'Analyse de la Texture

UV: Ultra-violet

QDA: Quantitative Descriptive Analysis

ACP: Analyse en Composantes Principales

ACC: Analyse Canonique des Corrélations

CHA: Classification Hiérarchique Ascendante

PLS: Régression par les moindres carrées partiels (Partial Least Square)

PLDA: Analyse discriminante par les moindres carrés partiels (Partial Least Square Discriminante Analysis)

G': module élastique

G'': module visqueux

Tan δ: Viscoélasticité

η *: Viscosité complexe

T_{dp} : Température de point de goutte

T_{sp} : Température de point de ramollissement

SFS : Spectroscopie de Fluorescence Synchrone

u.a : unité arbitraire

μm : micrométre

mg : milligramme

LISTE DES ANNEXES

TABLE DES MATIERES

INTRODUCTION

INTRODUCTION

Le fromage est l'un des plus anciens aliments manufacturés. Plus d'un millier de variétés de fromages sont répertoriées dans le monde. Cette étonnante diversité est la conséquence de multiples facteurs, notamment le type de lait utilisé (de vache, de buffle, de chèvre, ou mouton), le procédé de fabrication et les pratiques et les préférences locales. Le fromage est un produit fermenté obtenu par coagulation sous l'effet d'une acidification à caractère lactique et/ou par apport d'enzymes coagulantes. Le gel obtenu est ensuite égoutté puis éventuellement affiné après salage. La France est l'un des seuls pays à avoir une aussi grande variété de fromages, aujourd'hui plus de 400 sortes sont dénombrées, dont 46 bénéficiant d'une Appellation d'Origine Protégée (INAO, 2001). La France est réputée comme étant le "pays du fromage", tant au niveau de la production (1,82 millions de tonnes- INRA 2007- tableau n°1) que de la consommation. Les français sont les premiers amateurs de fromages, avec une consommation annuelle moyenne de 24,5 kg/habitant en 2008 (C.N.I.E.L, 2009). Parmi les fromages au lait de vache, la préférence des français va, en premier, aux fromages frais, puis, par ordre décroissant, aux fromages à pâte molle, aux fromages à pâte pressée cuite, aux fromages à pâte pressée non cuite et aux fromages à pâte persillée. La production française de fromages AOP représente environ 16% des fabrications de fromages affinés (SSP, 2008) et 83% des fabrications de fromages au lait cru. 60% des volumes sont couverts par la production de 5 catégories de fromages : le Comté, le Roquefort, le Reblochon, le Cantal et le Saint-Nectaire. En 2008, le tonnage des fromages AOC a atteint 1492 795 tonnes avec une augmentation de 66% par rapport à 1998. Toutefois on observe une stagnation sur les dernières années : 0% entre 2007 et 2008 (INAO/CNAOL). Les professionnels inquiets de voir les ventes s'affaiblir cherchent de nouveaux débouchés en innovant pour valoriser leurs fromages AOP. Les fromages à Pâte Persillée AOP sont au nombre de 7 dont 5 AOP dans le Massif-Central représentant 98% de la production nationale. La production nationale est de 34 109 tonnes dont 15 974 au lait de vache (47%). Leurs ventes subissent le même ralentissement malgré des campagnes de communication incisives (2010/2009 : -9,8% pour le bleu d'Auvergne, -4,8% pour la Fourme d'Ambert) (ODG/INAO/CNAOL).

Au cours des dernières décennies, le fromage a pris une importance commerciale croissante dans l'industrie alimentaire du fait de son utilisation comme ingrédient dans les plats cuisinés notamment en garniture sur des pizzas, en tranches dans les sandwichs ou incorporés dans des sauces froides ou chaudes. Les fromages fondus sont les plus utilisés dans des applications en tant qu'ingrédients mais une proportion croissante d'autres types de fromages prennent la même voie de diversification valorisante. Bien que de nombreuses variétés traditionnelles possèdent des propriétés sensorielles et technologiques qui se prêtent bien à leur utilisation directe comme ingrédient (l'emmental, le comté), des fabrications spécifiques à cet usage ont vu le jour. Il est probable que d'autres types de fabrications fromagères vont évoluer à l'avenir pour répondre aux exigences relatives à leur usage en tant qu'ingrédient. Il est donc intéressant actuellement d'étudier les potentialités de certains fromages traditionnels aux débouchés incertains à être valorisé partiellement sous une autre forme que la dégustation.

Les industriels utilisent des fromages ingrédients capables de répondre à plusieurs utilisations (froid, chaud, coupé, râpé….). Ils recherchent des fonctionnalités technologiques liées à leurs traitements industriels mais également aux attentes des consommateurs. Ces caractéristiques fonctionnelles doivent de plus être constantes dans le temps et répondre à des objectifs de performance très spécifiques. Dans la plupart de ces applications, les caractéristiques physiques et rhéologiques du fromage sont souvent plus importantes que les attributs de saveur ou d'aspect.

Le développement de fromages avec des propriétés fonctionnelles à chaud bien identifiées représente l'une des clés de l'utilisation de ces produits comme ingrédient dans les plats cuisinés. Pour contrôler les propriétés fonctionnelles de ces produits ou les prévoir, nous avons besoin de méthodes de caractérisation rapides et fiables. Actuellement, ces propriétés sont le plus souvent évaluées à l'aide des tests empiriques imitant certaines techniques d'appréciations sensorielles fournissant une évaluation globale de ces propriétés.

Dernièrement, des techniques instrumentales telles que la spectroscopie de fluorescence et le test de compression dynamique ont montré un potentiel intéressant pour la caractérisation respectivement de la structure moléculaire et de la structure macroscopique des fromages. Ces différentes méthodes ont permis de montrer que les propriétés de texture des fromages sont déterminées à la fois par les caractéristiques physico-chimiques des fromages mis en œuvre et par les paramètres technologiques de fabrication. En effet, des corrélations assez étroites ont été mises en évidence entre l'évolution de certains paramètres physico-chimiques et les modifications des propriétés texturales.

Dans cette thèse, nous avons choisi de travailler sur les potentialités des fromages à pâte persillée traditionnels à être utilisé comme ingrédient à chaud. Ces fromages sont déjà utilisés en tant qu'ingrédient par les industriels sous une forme adaptée de fabrication.

Cependant aucune étude n'a été publiée à ce jour sur ce domaine à notre connaissance. Nous avons choisi d'étudier des fromages de fabrication traditionnelle au lait de vache, issus du Massif Central et sous signe de qualité AOP dans l'optique de rechercher une valorisation alternative à la dégustation.

La démarche vise à étudier les phénomènes produits par la chaleur sur les fromages tant au niveau de la structure qu'au niveau organoleptique par des méthodes croisées d'études classiques (physicochimique, rhéologique) ou le développement d'approches innovantes (Fluorescence frontale, développement des descripteurs sensoriels). La nouveauté et l'originalité portent également sur l'étendue de l'étude en suivant la dynamique du produit de froid à chaud jusqu'au refroidissement.

L'objectif de cette thèse est :

(i) d'identifier les indicateurs qualité (sensoriels, instrumentaux, physicochimiques) représentatifs des qualités attendues de fromages à pâte persillée

(ii) d'étudier dans un premier temps l'intérêt de la spectroscopie de fluorescence synchrone pour suivre l'effet de la chaleur sur les matrices fromagères

(iii) d'évaluer la potentialité de la spectroscopie de fluorescence synchrone pour déterminer les changements physicochimiques et les corrélations avec les caractéristiques rhéologiques des fromages au cours du chauffage et du refroidissement

(iv) d'étudier l'évolution des caractéristiques sensorielles texturales suite au traitement thermique

(v) de mettre en relation l'analyse sensorielle en parallèle avec les méthodes instrumentales (spectroscopie et rhéologie) et physico-chimiques afin d'expliquer au mieux les phénomènes observés.

Les enjeux de ce travail pour l'industrie laitière sont les suivant :

1. Décrire et caractériser les qualités technologiques et sensorielles de 4 types de fromages à pâte persillée AOP du Massif-Central par des méthodes sensorielle, rhéologique, spectroscopique et chimique.

2. Améliorer les connaissances sur les changements structurels de matrices fromagères avant et après chauffage au cours de son utilisation comme ingrédient dans les applications alimentaires.

3. Développer des méthodes sensorielles pour distinguer et caractériser les propriétés fonctionnelles des divers fromages à pâte persillée ayant subis un traitement thermique et étudier les relations entre les données des différentes techniques.
4. Essayer d'apporter quelques réponses tant aux producteurs de pâte persillée sous AOP qu'aux attentes des industriels ou des consommateurs en terme de qualité et de potentialité d'utilisation sous forme d'ingrédient pour des utilisations à chaud.

Ce travail a été réalisé au sein de l'équipe COQUA de l'Unité de recherche CALITYSS et s'inscrit dans le programme conventionné de développement et de transfert de méthodes instrumentales appliquées aux produits laitiers vers l'Université de Fayoum en Egypte.

Ce manuscrit est organisé en deux parties principales. La première partie est une revue bibliographique faisant le point sur les caractéristiques des fromages et des pâtes persillées en particulier, sur les fonctionnalités des fromages ingrédients ainsi que sur les méthodes disponibles pour étudier ces propriétés. La deuxième partie présente sous forme de publications scientifiques, la démarche, la méthodologie, les résultats et les conclusions et perspectives de ce travail.

REVUE BIBLIOGRAPHIQUE

I. Définition, structure, caractéristiques physico-chimiques et sensorielles des fromages

I.1. Définition des fromages

I.1.1. Généralités

Parmi les 400 variétés de fromages obtenus à partir de lait de vache, de chèvre, de brebis et de bufflonne, dix grandes familles de fromages peuvent être distinguées : les pâtes fraîches, les pâtes molles à croûte fleurie, les pâtes molles à croûte lavée, les pâtes persillées, les pâtes pressées non cuites, les pâtes pressées cuites, les fromages fondus, les pâtes filées, les fromages de brebis et les fromages de chèvre. Les quantités de fromages produites varient beaucoup d'un type de fromage à l'autre. Cependant, trois grandes classes de même grandeur se partagent la production : 34% de la production totale de fromage pour les fromages frais, 30 % pour les pâtes molles et 32 % pour l'ensemble des pâtes pressées et pressées cuites.

Quarante six fabrications fromagères françaises bénéficient actuellement d'une Appellation d'Origine Protégée ou d'une AOC, 29 au lait de vache, 14 au lait de chèvre, et 3 au lait de brebis (INAO, 2011). Contrairement aux marques commerciales, la dénomination AOP reflète un patrimoine collectif et peut être utilisée par tous les producteurs d'une zone géographique définie par un arrêté. Les fromages traditionnels AOP, dont les caractéristiques sont liées à une zone géographique définie et à des savoir-faire locaux d'élevage, de transformation et d'affinage représentent dans la plupart des pays des volumes significatifs par rapport à l'ensemble des productions totales de fromages. Les fromages AOP du Massif-Central sont au nombre de 8 pour les fabrications au lait de vache et représente 34% de la production française des AOP. La recherche a démontré que de nombreux facteurs contribuent à une qualité spécifique des fromages AOP : le pâturage, la race, le lait cru, les procédés de transformation, l'affinage en caves naturelles ou non (Coulon et al, 2004).

Cette grande diversité de fromages est obtenue grâce aux variations de certains paramètres de fabrication (Figure I).

I.1.2. Principales étapes de la fabrication fromagère

La dénomination « fromage » est réservée au produit fermenté ou non, affiné ou non, obtenu à partir de matières d'origine exclusivement laitière, utilisées seules ou en mélange, et coagulées en totalité ou en partie avant égouttage ou élimination partielle de l'eau (extrait du décret n°88-1206 du 30/12/1988). Les principes de base de la fabrication des fromages sont les mêmes pour presque toutes les catégories de fromages. La fabrication consiste à enlever l'eau du lait avec pour conséquence la concentration de six à dix fois d'une partie des protéines, lipides, minéraux et vitamines avec l'expulsion de lactosérum. Les processus impliqués sont : la préparation du lait, la coagulation, l'égouttage, le salage, l'affinage.

- **Préparation du lait**

Le lait est un système complexe légèrement acide (pH 6,7) constitué d'une phase aqueuse avec des molécules solubles (lactose, protéines sériques, sels minéraux, vitamines), une émulsion de globules gras (matière grasse complexe, figure n°2) et une suspension colloïdale de protéines complexes sous forme de micelles (caséine et sels minéraux, figure n°3 et 4) (Walstra et al 2005).

Le lait a une composition qui varie en fonction de nombreux facteurs zoologiques et d'élevage (Grappin et 2006). L'équilibre de ce milieu complexe est fragile, il varie également en fonction des techniques de conservation (tel que la réfrigération) et du développement de la flore primaire qui l'habite. De ce fait, le lait doit subir ou non une préparation avant sa conversion en fromage. Cette étape peut concerner :

- l'homogénéisation des globules gras : elle permet une meilleure répartition de la matière grasse au sein du caillé et favoriser une couleur plus blanche du caillé. Elle peut toutefois accélérer les phénomènes de lipolyse, d'acidification et d'oxydation
- la standardisation de la composition chimique du lait en matières grasses (retrait ou ajout de MG) et en matières protéiques (caséinates de Na, Ca, protéines de sérum…)
- la correction technologique de l'état de minéralisation final du caillé : le refroidissement précoce du lait engendre des déplacements de l'équilibre du Calcium lié aux caséines vers le Calcium libre. Une correction de la teneur et de l'équilibre en Ca lie/libre est établi généralement par un apport en chlorure de calcium
- les traitements thermiques : ils visent à détruire la majorité de la flore banale et pathogène tout en conservant au mieux les qualités sensorielles du lait et en créant des conditions favorables aux étapes technologiques suivantes de fabrication fromagère.

La thermisation est un chauffage qui entraine une réduction globale de Log3 ou 4 la flore du lait. Ce traitement laisse subsister de la phosphatase, au contraire de la pasteurisation, qui élimine quant à elle toute la phosphatase alcaline (Codex alimentarius).

- **Coagulation**

Les protéines du lait peuvent être dénaturées par coagulation acide à l'aide de microorganismes acidifiants ou par coagulation enzymatique par ajout d'enzymes protéolytiques (présure). Ces 2 méthodes sont souvent complémentaires dans des proportions variables qui permettent de différencier les caillés lactiques des caillés type présure donnant des gels à caractère très différent.

La coagulation acide par les bactéries lactiques est progressive et permet d'obtenir un gel lisse et homogène. Au fur et à mesure de l'abaissement lent du pH, les résidus acides libres fixent des protons et de ce fait augmentent la solubilité du phosphate micellaire du calcium (Le Graet et Brulé, 1993). La neutralisation des charges négatives en surface des micelles de caséines entraine une agrégation des micelles entre elles, ce qui produit une augmentation du diamètre moyen des micelles par chainage. Lorsque le point isoélectrique des caséines est atteint (pH 4,65), la totalité du phosphate de calcium est dissout et les micelles sont complètement déstructurées. La charge nette des micelles est pratiquement nulle et les répulsions électrostatiques sont inexistantes. Les protéines déminéralisées sont totalement dénaturées. Le gel de type acide est formé par des liaisons hydrophobes, hydrogènes et électrostatiques. Il est composé de micelles en réseau peu répulsives les unes par rapport aux autres. C'est un gel friable, ferme, cassant (figure n°5). Ce gel n'a pas le pouvoir de se contracter (synérèse) et présentera un égouttage très limité (Le Graet et Brulé, 1993).

La coagulation par voie enzymatique (présure = 80% chymosine, 20% pepsine) se fait par hydrolyse de la caséine kappa située en périphérie de la micelle. L'attaque enzymatique se fait sur la liaison peptidique 105 (phénylalanine) -106 (méthionine) qui libère une partie hydrophile de la caséine kappa et une partie restante hydrophobe la paracaséine kappa rattachée à la micelle. Cette fraction hydrophobe forme un coagulum de micelles sous forme de gel de paracaséine par floculation et agrégation (Amiot et al 2002). L'hydratation diminue, des liaisons hydrophobes et électrostatiques s'établissent entre les micelles modifiées. Enfin les micelles agrégées se réorganisent avec l'apparition de liaisons phosphocalciques et des ponts disulfures entre les paracaséines. Ce gel est structuré, souple, élastique, imperméable, peu friable avec un fort pouvoir de rétention d'eau permettant un relargage de sa fraction aqueuse lors de l'égouttage par synérèse. Les facteurs influençant la coagulation sont nombreux : la composition du lait, la concentration en enzymes et la température d'emprésurage, les traitements technologiques… (Vétier et al, 2000).

- **Egouttage**

L'égouttage est une étape de concentration de certains constituants du gel par un phénomène physique de rétraction (synérèse) et l'évacuation passive du lactosérum liée à la porosité et la perméabilité du gel (Walstra et 1985). Sous l'effet conjugué de la présure, de l'acidité et de la température, les liaisons moléculaires qui se créent entre les caséines et les minéraux provoquent une contraction du réseau qui expulse l'eau et les solutés (protéines sériques, minéraux solubles, lactose, composés azotés non protéiques). Le caillé peut subir différentes actions mécaniques qui accélèrent le phénomène (brassage, tranchage, broyage, pressage) et donnent un grain et une composition chimique de caillé final variables selon le type de procédé.

- **Salage**

Le salage a un rôle sensoriel en donnant une saveur marquée au produit et un rôle technologique en complétant l'égouttage et en limitant l'acidification et la déminéralisation. L'ajout de sel permet également la sélection de la flore d'affinage (Hardy, 1997). Le salage se fait à l'aide de sel fin ou de gros sel par saupoudrage, immersion en saumure ou par salage direct du caillé.

- **Affinage**

L'affinage est une étape clé pour le développement des qualités spécifiques de chaque fromage. Sous l'action d'enzymes de diverses origines, le caillé est fermenté, hydrolysé, transformé en une pâte d'aspect, de texture, de saveur et d'arôme complètement modifiés. Cette étape dépend de la composition et de la structure du caillé, de la durée d'affinage, de la composition de la flore interne et de surface ainsi que du contexte environnementale de la cave : aération, humidité, température, microbisme de la cave, cave contrôlée ou naturelle…) (Herbert, 1999). L'affinage est complexe et difficile à contrôler.

I.2. Cas des pâtes persillées

I.2.1. Les différentes catégories de pâtes persillées

Les pâtes persillées AOP au lait de vache sont le Bleu d'Auvergne, le Bleu des Causses, les Fourmes d'Ambert et de Montbrison pour le Massif-Central ainsi que le Bleu de Gex-Haut-Jura, le Bleu du Vercors-Sassenage. Le Roquefort est le seul fromage persillé AOP au lait de brebis. Par ailleurs, il existe des fromages persillés sans appellation AOP tel que le Bleu de Laqueuille, le Bleu de Thiézac, le Bleu de Termignon, le Bleu de Bresse, le Saingorlon. Certains fromages persillés ont été crées par des industriels sous différentes marques (Saint-Agur…).

L'étude présentée porte uniquement sur les ***4 fromages à pâtes persillées AOP du Massif-Central.***

I.2.2. Procédés de fabrication des fromages persillés

Les fromages à pâte persillée sont au lait de vache emprésuré, à pâte non pressée, non cuite, fermentée, salée.

Le lait cru ou thermisé ou pasteurisé, est emprésuré, une préparation de souches de *Penicillium roqueforti* spécifiques à chaque fabrication est ajoutée. Le caillé est brassé, égoutté, dans certains cas émietté-salé puis découpé en cubes de grosseur variable et placé dans des moules. Il n'est ni pressé, ni chauffé. Après démoulage, les fromages sont salés à l'extérieur par frottage ou saupoudrage. Ils sont ensuite placés dans des hâloirs, un local ventilé dont le degré d'hygrométrie et la température sont contrôlés. Le piquage des caillés par des aiguilles de dimensions variables permet l'oxygénation des moisissures internes. Cette étape favorise le développement des moisissures et permet de répartir harmonieusement les marbrures bleues ou vertes dans la pâte. L'affinage s'effectue dans des caves froides (6 à 12°C) et très humides (90 à 98% d'humidité relative). L'affinage des bleus des Causses se déroule en partie en caves à ventilation naturelle. Les fromages persillés sont ensuite conservés entre 0 et 6°C jusqu'à la fin de l'affinage. Celui-ci dure au minimum de 1 à 2,5 mois selon les formes et les formats. (tableau n°2).

Rôle du Penicillium roqueforti : les caractéristiques organoleptiques des fromages à pâte persillée sont dues principalement à l'activité métabolique de *P. roqueforti*. Ces microorganismes se développent suite à l'apport d'oxygène par piquage du caillé drainé mais

non compact, en début d'affinage. De nombreux auteurs ont montré que les différentes souches présentaient des activités métaboliques différentes en ce qui concerne la dégradation des protéines et les matières grasses du lait. *P. roqueforti* possède des lipases intra et extra cellulaires qui libèrent des acides gras transformés en méthyl-cétones, alcools secondaires et esters responsables de la flaveur de ces fromages. Les différentes souches de *P. roqueforti* sont également protéolytiques et participent à l'évolution de la texture au cours de l'affinage. En fin d'affinage, les teneurs en azote soluble à pH 4,6 et en azote non protéique sont respectivement voisines de 50 et 30% de l'azote total (Desmazaud *et al.*, 1976).
Une importante production de peptides de haut et bas poids moléculaires ainsi que d'acides aminés libres a été identifiée. Cette libération de composés azotés marque bien le rôle majeur des *Penicillium* dans l'évolution de la matrice au cours de l'affinage des fromages concernés. Les acides aminés libres résultent de l'action des exopeptidases, la large spécificité des carboxypeptidases à sérine devant leur permettre de libérer à la fois des acides aminés apolaires, acides ou basiques (Cerning J. et al, 1987).

I.2.3. Caractéristiques sensorielles et composition des pâtes persillées

D'après les cahiers de charges de ces 4 catégories de fromages persillés, le groupe des fourmes se différencie des bleus par :
- leur forme de cylindre haut comparé au cylindre plat,
- une croûte grise pour les 2 catégories exceptée l'orangé pour la fourme de Montbrison
- une texture souple, légèrement ferme comparée à une texture fondante, onctueuse, fine pour les bleus
- une saveur de laitage fruitée et une flaveur de *Pénicillium* moins marquée comparée à celle des bleus plus intense, plus typée.

Les tableaux n°2 et 3 résument les caractéristiques de ces 4 types de fromages. Cependant chaque catégorie de fromages persillés AOP étudié se différencie des autres, ceci étant dû à la fois aux choix technologiques et aux souches de *Pénicillium roqueforti* utilisées.
La technologie de fabrication de la Fourme de Montbrison se différencie des autres fabrications par l'absence d'homogénéisation du lait, un grain de caillé petit (grain de blé), un léger pressage, un émiettage avec un salage dans la masse, un repos sur chéneaux de résineux, un piquage tardif. Ces éléments différenciateurs donnent un produit spécifique avec une croûte typique orangée due aux pigments des résineux, une texture compacte, homogène sans

ouverture, légèrement ferme et un marbré plus qu'un persillé de la pâte dans des tons bleu clair au lieu de bleu verdâtre des autres catégories. La flaveur « bleu » est peu marquée, elle est à tendance « saveur laitage fruité »

Le bleu d'Auvergne est le fromage persillé le plus typé tant pour sa texture de pâte fondante et onctueuse que pour sa flaveur intense et typée de sous-bois et champignon avec une amertume légère. La technologie du bleu des Causses est très spécifique de cette fabrication avec un affinage long de 10 semaines en partie dans des caves naturelles, en partie au froid et en anaérobiose. Le temps d'affinage est un facteur primordial pour le développement des activités enzymatiques et la répercussion sur les qualités organoleptiques des fromages : texture fondante, faible amertume…Ces caractéristiques donnent un fromage à persillage régulier, une texture onctueuse et une saveur équilibrée sans excès de perception salée ou d'amertume.

Les articles scientifiques décrivant les caractéristiques sensorielles de fromages persillés sont très peu nombreux (Lawlor J.Ben et al, 2003). Nous n'avons trouvé aucune information sur les fromages étudiés.

Les ***compositions*** des fromages en matière sèche oscillent entre 50 et 53% du produit frais et celles en matière grasse entre 50 et 52% de la matière sèche. Ces compositions sont très proches, la fourme de Montbrison étant légèrement plus grasse que les autres et le bleu des causses légèrement plus sec que les autres (tableau n°4).

II. Fonctionnalités des fromages

II.1. Définition des fonctionnalités d'un aliment

Apparu pour la première fois au Japon au milieu des années quatre-vingt, le terme « functional food » désignait alors des denrées dans lesquelles l'ajout d'un ingrédient lui conférait une dimension supplémentaire : influencer de manière favorable une fonction de l'organisme. Aujourd'hui, il n'y a toujours pas de définition légale de ce terme qui est pourtant très utilisé. Un aliment fonctionnel contient une multitude de composants classifiés ou non comme nutriments qui peuvent remplir différentes fonctions, c'est pour cela qu'il n'existe pas de définition simple et universelle de l'aliment fonctionnel. D'après Diplock AT et al (1999), *l'aliment fonctionnel* est avant tout un *concept* centré d'avantage sur les *fonctions à moduler* que sur les produits à développer. C'est avant tout un aliment qui est destiné à une utilisation particulière, il ressemble à un aliment traditionnel consommé dans un cadre de nourriture habituelle.

Pour avoir une idée *des fonctionnalités d'un produit alimentaire,* il faut envisager ses fonctions d'usage, c'est-à-dire la fonction attendue pour répondre au besoin d'utilisateurs donnés. Depuis les années quatre-vingt dix, de nombreux travaux ont été menés sur l'intérêt et la définition de l'aliment fonctionnel « qui peut fournir un bénéfice pour la santé au-delà de ceux dépendant des nutriments traditionnels qu'il contient » (Thomas PR & Earl Reds, 1994) (Méjean et al., 2006, Hyardin et al., 2009, Antoine, 2009). La fonctionnalité d'un aliment apparaît donc comme sa capacité à intervenir sur les fonctions de l'organisme, pour en moduler l'activité. Depuis, ce lien à la santé et au bien-être de l'homme a évolué vers une utilisation plus large du terme « fonctionnalité » en l'appliquant aux fonctions sensorielles (fonctions gustatives, d'appétence…), nutritionnelles mais aussi technologiques (thermo-fonctionnalité, aptitude culinaire…), cette dernière étant très souvent liées aux fonctions sensorielles perçues par le consommateur.

II.2. Fonctionnalités des fromages ingrédients

II.2.1. Principe et contexte d'usage

Les fromages ont la capacité de satisfaire une large diversité de demandes nutritionnelles et sensorielles et d'usages divers de la part des industriels ou/et des consommateurs. Les fromages sont délivrés aux consommateurs à travers 3 circuits différents : par l'achat direct, au travers de la restauration hors foyer ou par le secteur industriel. Le secteur fromager se développe actuellement dans l'industrie alimentaire par l'utilisation des fromages sous forme d'ingrédient dans des préparations culinaires chaudes ou froides. De ce fait, certaines propriétés fonctionnelles des fromages sont particulièrement recherchées pour répondre aux contraintes industrielles et pour satisfaire le consommateur. Des modulations de formulation par ajout d'ingrédients, d'additif ou d'auxiliaires jusqu'à des variations de procédés, de nombreuses techniques sont apparues pour répondre à ces besoins ainsi qu'aux contraintes économiques. Certains industriels ont fait le choix de rechercher des produits nobles tels que les fromages AOP pour se différencier et apporter une valeur ajoutée. Toutefois, les cahiers des charges des AOP induisent des contraintes fortes de fabrication et il est nécessaire de connaitre les aptitudes de ces fromages à ce type d'utilisation.

Guinée T.P (2002) considère que les propriétés fonctionnelles des fromages peuvent être regroupées selon 3 catégories : 1- les propriétés rhéologiques des fromages à froid (caractéristique de rupture), 2- les propriétés culinaires (fluidité), 3- les propriétés liées au pouvoir aromatisant.

Les propriétés rhéologiques des fromages à froid permettent les actions suivantes : découper, trancher, râper, étaler, effriter pour des usages tels que le portionnage, les sauces froides, la sandwicherie, les salades traiteur. Ce sont des aptitudes qui sont liées aux propriétés d'élasticité, de fermeté, d'adhésivité et de friabilité des fromages (tableaux 5 et 6).

Les propriétés culinaires correspondent à des thermo-fonctionnalités recherchées dans le cas d'utilisation des fromages à chaud. Ces aptitudes sont liées aux propriétés entre autre de coulant, fondant, nappant, filant, gratinant recherchées pour des usages tels que les sauces chaudes, les pizzas (les Français sont les deuxièmes plus gros mangeurs au monde avec 8 kg/an/habitant après les Américains - (Agra media, 10/08/2011).

Ces deux types de propriétés sont liés aux propriétés texturales des fromages d'ordre technologique et sensoriel. Nous n'aborderons pas les propriétés aromatisantes.

II.2.2. Fonctionnalités des fromages à chaud

Les fonctionnalités des fromages à chaud ont été abondamment étudiées depuis les années quatre-vingt dix essentiellement en utilisant la Mozzarella puis le Cheddar comme modèles d'étude (McMahon et al, 1993a ; Kindstedt 1995 ; Rowney et al, 1999, Guinée 2002) (tableaux n° 7 et 8) puis plus récemment en étudiant le Comté et la Raclette française. (Boubellouta et Dufour 2010) Les caractéristiques fonctionnelles des fromages recherchées par les industriels et appréciées par les consommateurs sont donc : le coulant, le fondant, le nappant, le filant, le gratinant.

De nombreux facteurs affectent les fonctionnalités des fromages à chaud :

- Le prétraitement du lait : pasteurisation, homogénéisation,
- Le procédé : variation de pH, température de chauffage, pH à l'égouttage,
- La composition : taux de matière grasse, de protéine, de matière sèche, calcium libre/lié ;
- L'affinage : protéolyse, lipolyse…

La plupart de ces facteurs exerce un effet sur l'organisation de la microstructure de la matrice et sur la physico-chimie des protéines (degré de liaison avec le calcium, degré d'agrégation ou d'hydratation, degré d'hydrolyse de la caséine), celle de la phase de la matière grasse (émulsification, lipolyse…). La matière grasse se liquéfie autour de 30°C et la matrice fromagère fond autour de 60°C (Famelard et al, 2002).

Etalement (couvrant, nappant, fondant) : les trois termes couvrant, nappant et fondant désignent un même phénomène : l'aptitude du fromage à fondre sous l'action de la chaleur de façon uniforme et homogène et à recouvrir le support (aliment, assiette) (J.M. Reparet, 2000). De même, un fromage ayant un faible taux de matières grasses tendra à être plus dur et moins fondant qu'un autre plus gras (Fife R.L et al, 1996). Les fromages ayant un haut taux d'humidité sont plus nappants, plus coulants que les autres. Le rapport calcium libre/lié aux protéines et donc la proportion de calcium insoluble associé aux caséines interfère également sur la qualité du fondant et la capacité d'étalement des fromages à chaud (Lucey J.A. 2008). La facilité d'écoulement des fromages augmente avec le temps d'affinage du à la protéolyse et la perte de calcium insoluble. La fonte proprement dite des fromages est due à une réduction de la force d'interactions des caséines avec l'augmentation de la température et la contraction du réseau (Guinée T.P et al, 1999).

Exsudation de matière grasse : ce terme représente la matière grasse qui exsude du fromage lorsqu'il est chauffé. Cette matière grasse exsudée joue un rôle lubrifiant pour le fromage à chaud et facilite l'étalement. Elle joue également un rôle protecteur lors de la cuisson en limitant notamment le gratinage. Cette matière grasse (libre ou issue des petits globules gras) est libérée au cours de l'affinage. Les traitements mécaniques et thermiques du lait également peuvent ou non générer des MG libres. Les fromages issus de laits riches en acides gras insaturés exsudent à des températures plus basses que les autres. Les acides gras insaturés ont un point de fusion plus bas que les saturés, en conséquence ces matières grasses présentent une mobilité plus importante et assouplissent plus rapidement les pâtes. Cette particularité apparaît avec la saisonnalité des fabrications d'hiver et d'été liée au mode d'alimentation des animaux.

Filant : ce terme représente l'aptitude du fromage à former des fils sous l'action d'une force et de la chaleur (Kindstedt P.S., 1993). La qualité du filant est importante puisque la plupart des consommateurs ne souhaite pas des fils trop longs. La longueur, la tension et le type de fil correspondent à des qualités attribuées au fromage qui sont détériorées avec le vieillissement de celui-ci. Le filant est une caractéristique de la Mozzarella.

Gratinant : durant le traitement thermique, la couleur de certains fromages augmente. Cette coloration est due aux réactions de Maillard entre les sucres résiduels (ex lactose) et certains acides aminés (fonction NH_2 libre). La coloration peut aller du jaune brun au noir selon la sévérité du traitement thermique, de la concentration en sucre disponible et du type de four utilisé.

III. Méthodes d'étude des fonctionnalités des fromages à chaud

Ce paragraphe est présenté sous forme d'un article : « Current methods for assessment of melted cheese functionalities and their correlations with sensory attributes. A Review » K. ABBAS, E. DUFOUR, A LEBECQUE (2008-2009) (Article n°1 non publié)

III.1. Méthodes empiriques

III.1.1. Test de Schreiber : étalement et exsudation des matières grasses

Le test de Schreiber (P.S.Kindstedt, 1990, J.M.Reparet, 2000) a pour principe d'évaluer, à l'aide de la mesure du diamètre ou de la surface, l'étalement du fromage et l'exsudation de matière grasse d'un échantillon circulaire de fromage posé sur un support et chauffé. (figure n°6). La bibliographie (Gunasekaran., 1999, Reparet, 2000) présente des températures allant de 60°C à 232°C, reparties essentiellement en dessous de 100°C et au dessus de 200°C, avec des temps variant de 30 secondes à 15 minutes.

Le fromage est généralement moulu. La façon d'obtenir le fromage moulu et la régularité de la température dans le four sont les principaux facteurs impactant sur la reproductibilité de cette méthode. La répétabilité du test est généralement faible (Reparet, 2000).

III.1.2. Test de Arnott : *fondant*

Le test d'Arnott (Arnott et al 1957) consiste à comparer la hauteur d'un cylindre de fromage avant et après traitement thermique à 100°C pendant 15 minutes (figure n°7). Cette méthode très empirique et aux résultats aléatoires est une des premières méthodes utilisées pour évaluer le fondant des fromages en simulant le comportement du fromage en situation culinaire.

III.1.3. Test à la fourchette : *filant*

Le filant est empiriquement apprécié par un test à la fourchette (McMahon et al , 1996). Le fromage chauffé est étiré avec une fourchette jusqu'à la rupture des fils, la longueur mesurée du fil à la rupture constitue la valeur de la propriété « filant ».

La bibliographie (P. S. Kindtedt et al., 1989 et 1991 ; R. L. Fife et al. 1994, Reparet, 2003) fait état de la mesure du filant pour la Mozzarella, en utilisant un viscosimètre hélicoïdale et une barre mobile.

III.2. Méthodes analytiques et instrumentales

III.2.1. Points de ramollissement et de goutte : *fusion de la matrice fromagère*

Le point de ramollissement correspond à la température à partir de laquelle la première goutte d'un échantillon de fromage commence à se former, lorsque le fromage est chauffé. Le point de goutte correspond à la température à laquelle la goutte se détache lorsqu'un échantillon de fromage est chauffé. (Blumenthal A. et al 1976, Reparet J.M. et al 2000). Ces 2 points permettent d'identifier les températures de fusion et d'évolution de la matrice fromagère susceptible d'être perçue par le consommateur. Ces 2 techniques sont automatisées; généralement le fromage est chauffé lentement de 30 à 140°C avec une vitesse de 2°C par minute (Eberhard et al., 1986).

III.2.2. Méthode butyrométrique : *relargage de la matière grasse*

La méthode butyromètrique permet de mesurer la matière grasse relarguée à chaud par un échantillon de fromage (Kindstedt P.S. et al 1991, Richoux R. et al., 2001). Cette méthode consiste à extraire la phase huileuse par chauffage (57,5°C pendant quelques minutes) et centrifugation dans un mélange eau/méthanol. Ce paramètre peut servir de méthode d'estimation d'exsudation de gras lors de la fonte de l'échantillon de fromage.

III.3. Méthodes rhéologiques : *propriétés mécaniques et structure des matrices fromagères*

La rhéologie, science de la déformation de la matière, est l'étude des relations entre une contrainte exercée sur un échantillon et la déformation résultante sur une période de temps donnée. Les relations contrainte/déformation sont décrites en utilisant différents termes rhéologiques : l'élasticité, la viscosité, la viscoélasticité, la contrainte ou la déformation à la rupture... Ces termes fournissent des indices sur les propriétés rhéologiques liées à la texture du fromage et notamment lors d'opérations de réduction de taille (fermeté, friabilité…lors du portionnage), de préparation culinaire (viscosité liée à la liquéfaction de la graisse, l'agrégation des protéines…) ou de consommation (fermeté, friabilité, déformation…liés à la mastication).

Ces propriétés rhéologiques des fromages sont fonction de :

- La composition (les niveaux d'humidité, de matières grasses et de protéines)
- La microstructure, représentant la distribution spatiale (intra-et intermoléculaires) de ses composantes
- La macrostructure, représentant la disposition des macro-composants (particules de lait caillé, poches de gaz, veines…) et déterminant la présence d'hétérogénéité
- L'état physico-chimique de ses composants (degrés d'agrégation, d'hydratation ou de dégradation des protéines…).

III.3.1. Test de pénétration

Le test de pénétration consiste à enfoncer une aiguille ou une tige dans l'échantillon à une vitesse constante (Vassal et al, 1987). La force nécessaire à la pénétration est enregistrée. La fermeté est estimée à partir du travail effectué pendant l'enfoncement de l'aiguille jusqu'à une profondeur de pénétration fixée.

Les pénétromètres ont été utilisés entre autre pour déterminer la fermeté des fromages à pâte molle fraîche et pour montrer l'influence des différentes étapes de traitement, tels que l'homogénéisation, le refroidissement du lait sur les propriétés de texture de ces fromages (Sanchez et al, 1996).

III.3.2. Test de compression uniaxiale : *élasticité, contrainte, déformation*

Le test de compression uniaxiale a été utilisé dans de nombreuses études réalisées sur des fromages à pâte pressée. (Creamer et Olson 1982, Ak et Gunasekaran, 1992). L'échantillon doit être de forme cylindrique ou cubique avec des surfaces planes. Il est comprimé uniaxialement (selon un axe principal) entre 2 plateaux parallèles dont l'un avance vers l'autre à une vitesse constante prédéterminée (Van Vliet, 1991), un capteur enregistre la force déployée par l'échantillon durant la déformation jusqu'à la fracture. Les paramètres calculés sont le module d'élasticité, la contrainte (σ_F) et la déformation (ε_F) au point de fracture et l'énergie de la fracture (W_F) (Fénelon et Guinée, 2000) (Figure n°8).

III.3.3. Profil d'Analyse de la Texture (TPA) : dureté, élasticité, cohésion, adhérence, simulation de la mastication

Le « Texture Profil Analysis » est un test visant à reproduire globalement mais partiellement le phénomène de mastication (Hardy et Scher, 1997). Un cylindre d'échantillon est comprimé entre deux plaques d'acier deux fois de suite pour simuler deux morsures (Figure 9). A partir de la courbe obtenue représentant la force en fonction du temps, quatre paramètres de texture peuvent en être déduit : la dureté TPA, l'élasticité TPA, l'intensité de la cohésion TPA, l'intensité de l'adhérence TPA (Figure 9). Cependant, l'utilisation de ce test est limitée car la majorité des paramètres se calculent une fois que la fracture a eu lieu. (Van Vliet, 1991).

III.3.4. Test de compression dynamique : *viscosité, élasticité, viscoélasticité*

Le test de compression dynamique, basé sur l'application d'une contrainte de façon sinusoïdale, permet de décrire et d'expliquer les modifications structurales susceptibles d'intervenir dans un échantillon de fromage entre autre lors de l'application d'un traitement thermique. (Reparet, 2000, Richoux et al 2001, Famelard et al 2002). La technique d'oscillation est non destructive et elle permet de mesurer simultanément les comportements visqueux et élastique de l'échantillon. Cette méthode doit être mise en application dans la région viscoélastique linéaire. Les paramètres rhéologiques (G', G" et tan δ) sont calculés. G' est le module élastique : il sera important pour un échantillon de prédominance élastique ou fortement structuré. G" est le module visqueux, il est important pour un échantillon de prédominance visqueuse. Le rapport G''/G' correspond à Tan δ, celui-ci représente l'angle de déphasage de perte. Ce rapport est un indice du caractère viscoélastique de l'échantillon à une température T et une fréquence ω données : Tan δ augmentera avec la viscosité et diminuera lorsque l'élasticité augmentera. (figure n°10).

Le test de compression dynamique présente l'avantage d'être rapide et de permettre plusieurs types de balayage (température, temps…). Le balayage en température peut varier de -10 à 100°C pour les appareils équipés d'un système à effet Peltier.

III.4. Méthodes spectroscopiques : *structure de la matrice fromagère*

III.4.1. Généralités sur la spectroscopie

Les techniques de spectroscopie sont rapides, d'un coût de fonctionnement faible et peuvent apporter de nombreuses informations. (Birlouez-Aragon et al. 2002; Karoui et al. 2005). Ces techniques sont considérées comme sensibles, non destructives, rapides, non invasives. Ces méthodes nécessitent une préparation simple des échantillons et elles peuvent être prédictives. (Karoui and Dufour 2006; Karoui et al. 2008; Woodcock et al. 2008). Ces dernières années, des méthodes spectroscopiques ont été développées pour contrôler la qualité des productions en incluant différentes mesures couvrant une large partie du spectre électromagnétique : absorption dans le visible et l'ultra-violet, émission de fluorescence, absorption dans le moyen et proche infrarouge, résonnance magnétique nucléaire, absorption par microonde…Ces différentes méthodes ont permis de suivre simultanément plusieurs paramètres de qualité (Belton 2000, Kulmyrzaev and Dufour 2008).

Le moyen-infrarouge a été utilisé pour étudier les produits laitiers pour par exemple suivre l'évolution des interactions protéiques au cours de l'affinage de fromage (Mazerolles et al. 2001).

La spectroscopie de fluorescence a permis de suivre la qualité des laits au cours de différents traitements : l'homogénéisation, le chauffage ou l'acidification des laits, l'affinage des fromages, le chauffage de fromages à pâte pressée, l'origine géographique des fromages mais également la qualité de nombreux autres produits issus de différents secteurs de production (Karoui et Blécker, 2010).

Du point de vue technique, la spectroscopie photonique repose sur l'étude de l'interaction d'une onde électromagnétique avec la lumière. Le spectre électromagnétique est divisé en diverses régions en fonction de la longueur d'onde des radiations (figure n°11). Chaque région est associée à un type de transition atomique ou moléculaire différentes mettant en jeu des énergies différentes. Les photons de la lumière transportent de l'énergie liée à la fréquence de radiation. Lorsque la lumière traverse une matière non transparente, elle est absorbée partiellement. Dans le domaine de l'UV ou du visible, l'énergie lumineuse absorbée par la matière fait passer les électrons d'une molécule d'un état fondamental stable à un état de plus haute énergie instable. Lorsque la molécule retourne à son état fondamental, elle cède de l'énergie au milieu soit sous forme de lumière (luminescence), soit sous forme de chaleur ou soit sous forme de fluorescence.

III.4.2. Spectroscopie de fluorescence : *structure moléculaire de la matrice fromagère*

La fluorescence correspond à l'émission de photons par certaines molécules excitées à l'aide d'une radiation lumineuse dans l'ultraviolet et le visible (Lakowicz, 1983). Le temps (10^{-9}à 10^{-7}secondes) durant lequel la molécule reste excitée, est caractéristique de celles-ci. Cette émission de photons est appelée fluorescence, le signal enregistré à différentes longueurs d'onde constitue le spectre d'émission de fluorescence. Le photon émis a moins d'énergie que celui d'excitation. Donc pour une molécule donnée, les longueurs d'onde d'émission seront supérieures à celles d'excitation et l'énergie des photons de fluorescence sera plus faible que celle des photons d'excitation.

La spectroscopie de fluorescence donne des informations sur la présence de molécules fluorescentes et sur leur environnement. Elle permet d'observer l'évolution de la conformation d'un constituant contenant ces molécules et de ses interactions (protéine, lipides, eau…) en fonction du traitement qu'il subit dans son environnement : hydratation, coagulation, protéolyse et lipolyse de l'affinage, fonte ou cristallisation de triglycéride…Les acides aminés aromatiques (Dufour et al 1994), les produits d'oxydation des lipides (Gatellier et al 2007) ont des propriétés de fluorescence. Ils ont été utilisés pour étudier la structure des protéines et des lipides et leurs diverses interactions. Ceux sont des fluorophores intrinsèques aux produits qui sont utilisés comme marqueurs spécifiques d'un constituant tels que les résidus tryptophane de la caséine (Fox, 1989), les vitamines comme la vitamine A des membranes des globules gras (Dufour et al 2001), les cofacteurs enzymatiques comme le NADH et la riboflavine révélatrice de l'état d'oxydation des produits car très sensible à la lumière et à la présence d'oxygène (Kristensen et al 2000). L'ensemble de ces sondes intrinsèques se retrouve dans les fromages, mais il existe de nombreuses autres possibilités de composés ayant des propriétés de fluorescence : certains terpènes issus de la flore botanique, les acides gras insaturés conjugués, les composés phénolique si le produit est fumé… Il est possible également d'utiliser des sondes extrinsèques spécifiques de certains constituants (Herbert et al, 1999).

La ***fluorescence à angle droit*** a été utilisée pour étudier des solutions diluées. Les produits agroalimentaires solides ou viscoélastiques ne peuvent pas être étudiés par cette méthode.

La ***spectroscopie de fluorescence frontale*** permet de contourner ce problème en étudiant uniquement la surface de l'échantillon (Genot et al 1992b) (figure n°12). Les photons émis sont collectés sous un angle de 54° par rapport à l'échantillon pour minimiser la collection de photons réfléchis.

La ***spectroscopie de fluorescence synchrone*** présente l'avantage de pouvoir caractériser plusieurs fluorophores à partir d'un seul spectre. Cette technique très répandue dans le domaine de la pétrochimie n'a été que récemment exploitée en agroalimentaire : pour discriminer des huiles issues de différents espèces variétales (Poulli, 2007), pour étudier les changements structurales des laits au cours du chauffage ou de l'acidification et des fromages à pâte pressé au cours de la fonte (Boubellouta, 2008). Pour cette méthode, la longueur d'onde d'excitation et celle d'émission varient simultanément tout en gardant entre elles un décalage constant $\Delta\lambda$. Pratiquement, on choisit une longueur d'onde d'émission de départ supérieure de quelques nanomètres à la longueur d'onde d'excitation, puis les monochromateurs défilent à la même vitesse (Patra et Mishra, 2002) (figure n°13, 14).

III.5. Méthodes sensorielles : *caractéristiques sensorielles*

Il existe plusieurs méthodes de description sensorielle dont l'objectif est de déterminer le degré et la nature des différences entre des produits alimentaires afin de fournir une carte d'identité sensorielle de ces produits. Ces méthodes utilisent généralement un groupe de sujets sélectionnés et entraînés à quantifier leurs perceptions sensorielles. Les premières méthodes telles que le profil de flaveur ou le profil de texture ont été développées dans les années 50 et 60. Ces méthodes permettaient de décrire et quantifier une unique dimension sensorielle. Dans les années 70, la méthode QDA (Quantitative Descriptive Analysis) a été développée pour permettre l'établissement de cartes d'identités sensorielles sur lesquelles pouvaient être représentées simultanément plusieurs dimensions sensorielles. Cependant ces méthodes sont lourdes à mettre en place, notamment en raison du temps nécessaire à l'entraînement du groupe de sujets. De ce fait, des méthodes plus rapides ont été développées dans les années 80 avec le profil libre choix et dans les années 2000 avec le profil flash. Pour ces méthodes, la phase d'entraînement est réduite au minimum dans la mesure où les sujets utilisent leur propre vocabulaire.

III.5.1. Analyse descriptive quantitative

La méthode d'analyse descriptive quantitative ou méthode QDA a été développée par Stone et *al.* (1974) et améliorée en 1980 par ces mêmes auteurs. Elle consiste à établir un profil sensoriel des produits étudiés à la suite de la quantification individuelle de la sensation perçue pour chacun des descripteurs par des juges entraînés. La démarche est la suivante :

- le panel est entraîné pour une seule gamme de produit,
- toutes les caractéristiques sensorielles du produit sont évaluées (aspect, texture, odeur et flaveur),
- la mise en place de la liste de descripteurs est réalisée par consensus du panel avec l'aide des produits généralement de la gamme (références internes),
- le panel est entraîné à quantifier la sensation perçue pour chaque descripteur à partir de la gamme de produit à évaluer,
- l'évaluation de chaque descripteur est recueillie sur une échelle d'intensité,
- trois à six répétitions sont réalisées,

- les performances des sujets et les résultats finaux sont obtenus à la suite du traitement statistique des données par l'analyse de variance et par des techniques d'analyses multidimensionnelles comme l'analyse en composante principale (ACP).

La méthode recommandée par la norme AFNOR (AFNOR, ISO 13 299, 2003) est issue de la méthode QDA. Mais elle diffère sur la mise en place de la liste des descripteurs (procédure faisant intervenir des analyses statistiques) et sur les échelles d'intensité à utiliser.
L'avantage de cette méthode est que disposant d'un panel entraîné sur une liste de descripteurs normalisés pour une gamme de produit, les résultats de différents profils réalisés à différentes périodes sur cette même gamme de produit peuvent être comparés. Cette méthode est très utile pour le suivi de la qualité organoleptique des produits. Son inconvénient majeur réside dans le temps important dédié pour l'entraînement du panel et dans le coût important que cela représente.

III.5.2. Profil de texture

La méthode du profil de texture a été développée par la société General Food Corporation (Brandt et *al.*, 1963 ; Szczesniak, 1963). Cette méthode est issue de la méthode du profil de flaveur et vise comme son nom l'indique à définir et quantifier les sensations perçues relatives à la texture des aliments. A ses débuts, cette technique examinait les caractéristiques mécaniques, géométriques et d'autres caractéristiques comme l'humidité ou le caractère gras des aliments. Puis elle a été étendue à l'étude des caractéristiques des produits semi-solides (Civille et Szczesniak, 1973 ; Civille et Liska, 1975) ou pour l'étude des sensations en surface des produits (Nogueira-Terrones, 2000). De nos jours, la norme AFNOR (AFNOR, ISO 11 036, 1995) fournit des descripteurs précisément décrits en tenant compte de la propriété structurale correspondante. Cependant la limite de cette méthode est la grande diversité de termes utilisés pour des concepts voisins et la diversité de définitions associées à un même terme (Giboreau, 2001).

III.5.3. Spectrum

La méthode Spectrum a été développée par Civille et *al*. (1999). Elle a pour objectif de fournir un outil descriptif universel basé sur l'utilisation de références absolues valables pour tout type de produits (Montet, 2001). Le choix de la méthodologie de profil n'est pas imposé, par contre une banque de données regroupant des références absolues associées à des descripteurs d'aspect, d'odeur, de texture et de flaveur doit être utilisée. Ainsi, les échelles utilisées associées aux références absolues sont censées estimer des intensités absolues pour un descripteur.

Du fait des références absolues, la méthode permet de comparer les intensités correspondantes à des descripteurs différents. L'inconvénient est le temps nécessaire (plusieurs semaines à plusieurs mois) aux sujets pour mémoriser le nombre important de références.

IV. Analyses des données - Chimiométrie

La chimiométrie peut être définie comme l'application des outils mathématiques, en particulier statistiques, pour optimiser les procédures d'obtention et de traitement de données de la chimie analytique, afin d'en extraire le maximum d'informations pertinentes. Cette science fait l'objet d'ouvrages généraux (Sharaf et al 1986 ; Martens et Naes 1989). Les méthodes chimiométriques peuvent avoir trois objectifs principaux : la description des données sous une forme synthétique (méthodes descriptives), la prédiction (méthodes prédictives) ou la planification expérimentale.

IV.1. Pré traitements des données spectrales

Les spectres enregistrés peuvent être entachés de défauts que l'on peut tenter de réduire *a priori*. Les défauts les plus courants sont la présence de bruit aléatoire, la variation de la ligne de base, la redondance de l'information. Ainsi les données spectrales brutes, telles qu'elles sont acquises par un spectromètre, ne revêtent pas obligatoirement la forme la plus adaptée aux traitements chimiométriques ultérieurs. Différents prétraitements peuvent être appliqués aux spectres afin de réduire ces défauts. Parmi les prétraitements les plus utilisés, on trouve la déviation normale standardisée (SNV) (Barnes et al., 1989; Gendrin et al., 2007), la Multiplicative Scatter Correction (MSC), la Correction du Signal Orthogonal OSC (Niazi & Goodarzi, 2008), les lissages et les dérivées (Bertrand & Vigneau, 2006).

IV.2. Méthodes descriptives ou exploratoires

Les méthodes exploratoires ont comme objectif de décrire les données, sans introduire d'éléments de connaissances *a priori.* Les méthodes exploratoires comprennent à la fois des techniques élémentaires qui ne sont pas à négliger (représentations graphiques, indices de localisation, indices de dispersion…) mais également des approches plus sophistiquées, telles que l'analyse en composantes principales et la classification (Bertrand and Dufour, 2006).

IV.2.1. Analyses en composantes principales

L'Analyse en Composantes Principales est une méthode statistique de projection de données multidimensionnelles, utilisée pour la réduction de la dimension de données. Cette méthode mise en place par Hotelling (Hotelling, 1933) est basée sur une technique décrite par Pearson (Pearson, 1901). La méthode s'appuie sur l'hypothèse selon laquelle il existe de fortes corrélations entre les données étudiées. Le but de l'Analyse en Composantes Principales

(ACP) est de condenser les données originelles en de nouveaux groupements, appelés nouvelles composantes ou composantes principales ; et orthogonales entre elles, de façon à ce qu'elles ne présentent plus de corrélation entre elles et soient ordonnées en terme de pourcentage de variance expliquée par chaque composante. Ainsi, la première composante contient les informations relatives au pourcentage maximal de variance, la deuxième contient les informations relatives au pourcentage de variance suivant. Le processus peut être répété jusqu'a l'obtention de la dernière composante. A l'aide de l'ACP, on peut souvent condenser la collection spectrale dans des proportions très importantes : dix à vingt composantes sont en général largement suffisantes pour résumer l'information utile, et la taille de la matrice des données peut être réduite par un facteur compris entre 10 et 100. L'analyse en composantes principales (ACP) est particulièrement adaptée à l'étude exploratoire des données spectrales. Elle permet une représentation synthétique et visuelle en cartes dites cartes factorielles ou cartes de ressemblance dans lesquelles chaque spectre est représenté par un point sur un graphique. Deux spectres présentant une similitude se placent à des positions voisines sur ces cartes. Les données condensées par l'ACP peuvent servir de variables de base à d'autres traitements statistiques tels que la régression ou l'analyse factorielle discriminante. Pour de nombreuses méthodes supervisées, l'orthogonalité des variables rend les calculs numériques très simples et plus fiables (Bertrand et al., 2006).

IV.2.2. Analyse Canonique des Corrélations (ACC)

Le but de l'ACC est d'étudier les relations linéaires entre deux groupes de variables quantitatives observées sur un même ensemble d'individus. De façon précise, on cherche une combinaison linéaire des variables du premier ensemble et une combinaison linéaire des variables du deuxième qui soient les plus corrélées possibles (Bouroche et Saporta, 1998). Dans le cas où ce premier couple n'est pas suffisant pour résumer les relations entre les deux ensembles de variables, nous cherchons, selon le même principe, un deuxième (puis un troisième, etc.) couple de variables canoniques qui complète l'information donnée par le premier couple (Vigneau et *al*., 2000). Ainsi le but de l'ACC est de déterminer des combinaisons linéaires des variables pour chacun des groupes, appelées variables canoniques, de façon à ce que les variables canoniques du premier groupe soient les plus corrélées possible aux variables canoniques du second groupe. Les coefficients de corrélations calculés entre les différentes variables canoniques sont appelés "coefficients de corrélation canonique". Comme pour l'ACP, les variables canoniques créées pour chaque tableau doivent être orthogonales entre elles. Des cartes de ressemblance des individus peuvent être également tracées pour les ensembles de données. Ces cartes sont sensiblement identiques lorsque les coefficients de corrélation canonique sont élevés.

En pratique l'application directe de l'analyse canonique à des spectres infrarouge et/ou de fluorescence engendre des artefacts du fait des redondances importantes existant entre les variables spectrales. Dans ce cas, une solution consiste à appliquer l'ACP à chaque tableau pour enlever, d'une part, toutes corrélations internes et s'assurer que les corrélations observées soient uniquement des corrélations entre les données obtenues par les différentes techniques, et, d'autre part, de faciliter l'interprétation des résultats de l'analyse canonique (Muller, 1982). Cette méthode a été appliquée par Devaux et *al*. (1993) pour le traitement des spectres infrarouge.

IV.2.3. La Classification Hiérarchique Ascendante (CHA)

La classification hiérarchique ascendante fait partie des méthodes exploratoires de type classification. Ce sont des méthodes d'apprentissage non supervisées ayant pour but de segmenter la population étudiée en groupes, sans connaissance à priori d'une structure. La classification hiérarchique ascendante (CHA) consiste à procéder de manière séquentielle à des regroupements successifs des individus étudiés en classes emboîtées (Bertrand et al., 2006). Cette méthode produit une séquence complète de partition. Au sommet, on retrouve un grand nombre de classes et, à la base, l'individu étudié est représenté dans une classe unique. Les résultats peuvent alors être mis sous la forme de graphes, appelés dendrogrammes. Les individus sont donc regroupés selon leurs similitudes ou leurs dissimilitudes. Il existe différents types de calcul de distance (distance euclidienne, distance de Minkowski, coefficient de Pearson, etc…), et différentes méthodes d'agrégation (méthode de ward, distances moyennes entre groupes, etc …).

IV.3. Méthodes prédictives

Les méthodes prédictives ont comme objectif de prédire (ou estimer) au mieux à partir des données, les valeurs d'une ou plusieurs variables pour de nouveaux échantillons. Dans les applications analytiques, le cas le plus fréquemment rencontré correspond à la prédiction d’une variable quantitative, telle que la concentration d’un composant présent dans le produit étudié. La méthode très connue appelée Partial least square (PLS) est bien adaptée aux données spectrales. On peut chercher à prédire une variable qualitative qui traduit l’appartenance de l’échantillon à un groupe qualitatif donné. Par exemple, la spectroscopie peut être utilisée pour vérifier la conformité d’un produit et garantir qu’il est bien d’une nature ou d’une qualité attendue. C’est le cas lorsqu’on cherche à authentifier les produits étudiés. Il est très souvent utile de connaître la nature spectrale de l’information qui est exploitée par un modèle prédictif. La plupart des méthodes sont à l’origine de modèles spectraux qui sont des outils d’interprétation pour le spectroscopiste (Bertrand and Dufour 2006).

IV.3.1. La régression par les moindres carrées partiels – (Partial least Square- PLS)

La régression PLS est actuellement la méthode la plus connue et la plus utilisée dans de nombreux domaines (Vigneau et al., 2006). Elle a été introduite par (Wold, 1966) et a fait l’objet de nombreuses adaptations et développement.

Comme pour l’ACP, la PLS est basée sur la construction de facteurs à partir des données spectrales initiales. Le but de cette méthode est de réduire la quantité de données et d’éviter ainsi les problèmes de surentraînement sans éliminer les informations utiles. Les composantes ou variables de la nouvelle base vectorielle, dites variables latentes, sont des combinaisons linéaires des variables initiales. Mais la différence avec l’ACP est que la PLS construit ses facteurs en tenant compte de la corrélation entre les variables prédictives X et les variables prédites Y (Agnar, 1988; Wold et al., 2001). La condensation des données se fait donc suivant les directions les plus pertinentes en terme de prédiction des variables Y (Geladi & Kowalski, 1986). La régression sur la matrice des variables latentes est utilisée pour construire l’équation de prédiction.

IV.3.2. Analyse discriminante par les moindres carrés partiels (Partial Least Square Discriminante Analysis - PLSDA)

L'analyse discriminante PLS (PLSDA) est une méthode de classification supervisée. Elle est maintenant très couramment utilisée dans le domaine de l'agroalimentaire (Downey, 2006).

L'objectif de la méthode est, comme pour l'analyse factorielle discriminante (AFD), de séparer le mieux des groupes d'individus. Son principe fondamental repose sur la création de nouvelles variables Y, de dimension n x k, formée par les indicateurs des groupes où n et k représentent le nombre d'individus et de groupes, respectivement, et d'appliquer la régression PLS2 sur ces nouvelles variables. Considérons, l'individu i appartenant au groupe k. La ligne i de Y est un vecteur dont tous les éléments valent 0, à l'exception de l'élément en position k, qui prend la valeur 1. Comme dans le cas de l'ACP et la PLS, les composantes discriminantes de la PLSDA peuvent être représentées sous forme de cartes factorielles. Le nombre de variables latentes choisies pour construire le modèle influencera directement celui-ci. Ce choix du nombre de variables latentes résulte d'un compromis entre stabilité et précision, entre complexité du modèle et les problèmes de sur-interprétation des données. Un modèle établi avec un faible nombre de dimensions risque de donner une erreur résiduelle assez élevée, mais le modèle sera fiable. A l'opposé, avec un trop grand nombre de dimensions, le modèle sera plus précis mais reposera sur des informations associées à des phénomènes de faible intensité. Un pourcentage de bonne classification peut être calculé, permettant ainsi d'évaluer la bonne prédiction de l'appartenance d'un individu à un groupe qualitatif.

ARTCICLE 1: CURRENT METHODS FOR ASSESSMENT CHEESE FUNCTIONALITY AND ITS CORRELATION WITH SENSORY ATTRIBUTES (A REVIEW)

Khaled ABBAS, Éric DUFOUR, Annick LEBECQUE*.

U.R. CALLITYSS, VetAgro Sup de Clermont,

89 av. De l'europe-BP-35-63370 Lempdes, France.

*Corresponding author: lebecque@enitac.fr - Tel: 33.(0)4.73.98.13.41

1. INTRODUCTION

The popularity of cheese in the diet, either as a food or ingredient, is continuing to grow on a worldwide basis as a result of changing lifestyles and the discovery of new applications for cheese as an ingredient such as, appetizers, souap, sauces, casseroles, crackers, fillings in pastry and pie, pizza, cheese slice on hamburgers and cold sandwiches. There have been several reviews on the functionality required of cheese when used as a food ingredient [38,75]. On cooking applications, cheese may be required to melt, flow, stretch and oil-off to varying degrees depending on the application because of its flavour and heat induced functionality. The functional attributes of cooked cheese generally have a major impact on the quality of foods in which cheese is included as an ingredient, e.g., pizza pie. Table 1 summarized major functional attributes of heated cheese used as an ingredient.

Cheese is a viscoelastic material. The viscoelastic properties of foods can be determined by applying small stress or strain at levels do not cause significant irreversible changes in the cheese [77]. The physicochemical properties of cheese have always been an important component in assessing cheese quality and value. The assessment was usually done by sensory evaluation which was quite adequate because cheese was generally consumed in its “original” state. Development of heat-processed cheese products prompted some research on studying the physical properties of cheese and the need for adequate methods to measure the stretchability, melt, flow, and consistency characteristics of cooked cheese [49,73,85,89,94,97].

Sensory analysis is widely used by researchers and professionals in order to assess food quality and to predict consumer acceptance. Sensory evaluations require a large panel of trained assessors, are time consuming, and so limited to a restricted number of samples. Instrumental methods may be used to characterise the texture of cheeses. For example, rheological measurements allow access to mechanical attributes of texture. Several studies dealing with the relationships between rheology and sensory properties have been published [45,75,84].

The aim of this review is to describe the common methods which utilised in assessments functional properties of heated cheese and its relationships with sensory attributes.

2. MEASURING CHEESE MELTABILITY

Melt is the ability of the cheese to flow and spread as well as the loss of (visual) integrity of the individual cheese shreds upon heating [42]. The flow of cheese upon heating can be determined by their rheology for small strain analysis (G', G"). Characteristics of meltability are: (1) the cheese should melt in a certain temperature range, (2) it should remain homogeneous both in appearance (no fat separation) and mouthfeel (no granular texture), and (3) the apparent viscosity should be low enough for material to flow clearly and high enough not flowing too fast; hence there is an optimum viscosity [101].

Melting behaviour (ease to melt and extent of flow) is dependent on both heat transfer and the thermal phase change characteristics (ease to melt) of the solid cheese and rheological flow properties of the melt (extent of flow), which are highly interdependent [33,92]. The melting property of cheese is important not only for texture, but also for the mixing property of cheese in food preparation. It is affected by change in viscosity and the nature of protein-protein and protein-lipid interactions at elevated temperature. Many attempts have been made to measure and control cheese meltability. Meltability tests can be divided into empirical and objective instrumental methods. The empirical methods depended on measuring dimensional changes in the cheese samples upon melting such as diameter, height,…etc [10,67]; the objective tests rely on determining intrinsic rheological properties of the cheese such as viscosity, viscoelasticity,..etc [69,88,111].

2.1. EMPIRICAL METHODS

Empirical tests are used to make qualitative measurements and are dependent upon the measuring conditions and apparatus**.** Empirical techniques are generally employed in industry for determining cheese meltability due to their simplicity and speed.

2.1.1. Oven method

Arnott test [10,73] in which meltability was determined by "exposing a standard cylinder of cheese to 100°C for 15min. Measurements of cylinder height before and after treatment were used as a basis of comparison". The height of the centre of the cylinder was measured. The meltability was a relative measurement expressed in terms of percent decrease in cylinder height after the heat treatment.

2.1.2. Tube test

In the following year, Olson and Price [89] reported two potential problems in applying Arnott and several other similar methods then in use: (a) film formation on the surface due to exposure to air during heating, and (b) uneven flowing of melted cheese. They proposed what is new known as the "tube method". A glass tube holds the sample during the test. One end of the tube is closed with a rubber stopper but is vented by a 1-mm-diameter glass tube. A reference line is etched on the glass 27.5mm from the opposite end of the melting tube. A measured quantity (15g) of the sample is placed at this end and closed with a rubber stopper. The tube with the sample is held horizontally in a rack and heated in an oven at 110°C for 6 min. Finally, the rack is tilled to stop further flow of the sample. The distance of flow from the reference line is quickly measured. The tube is heated for an additional 2 min in the horizontal position, and the distance of flow is measured again. The total distance (in mm) covered by the sample in 6+2 min of heating is called "cheese flow".

Subsequently, several reports have appeared introducing modifications of the above test either in terms of sample size or heating conditions. However, none of these methods gained wide acceptance. Kosikowski [67] reported a method known as the Schreiber test, in which the circumference of a melted cheese disc is taken as an index of meltability and has become the most popular test in the industry for evaluating cheese meltability for the following reasons: (a) simplicity both in terms of sample preparation and skills needed by operators; (b) ability to tests multiple sample simultaneously; and (c) reasonable correlation with perceived melt quality of several cheeses [42].

According to the original Schreiber test, a cylindrical cheese specimen (41mm in diameter, 4.8mm in height) put onto the center of a clean glass Petri dish and centered on a concentrically numbered target-type graph is heated in an oven set at 232°C for 5 min; record numerically the outer edge of the flow line, the longest flow line from the center to the edge of the melt is used as the cheese meltability. As the cheese melts uniformly and easily, its diameter and flow line number increase. Cheeses attaining a value of 4 or higher are acceptable. Cheeses with values below 4 are rejected and corrective action is immediately instituted. Major drawbacks of this procedure are excessive and uncontrolled heating, drying, crust formation, and browning, and inaccurate flow line measurement in case of noncircular melting [42,80]. However, this method suffers from three shortcomings: (a) excessive heat treatment; (b) uncontrolled heating; (c) measurement of flow line. Muthukmarappan et al. [82] proposed some modifications to the Schreiber test to evaluate the meltability of Mozzarella. They recommended heating the cheese plug in an oven set at 90°C for 5 min on an aluminium plate and measuring the area of spread of melted cheese as an indicator of cheese meltability. Note that the modified Schreiber tests conditions proposed by Muthukumarappan were empirically determined and should be considered suitable only for testing regular-fat Mozzarella cheeses [42].

2.2. OBJECTIVE METHODS

Many efforts have been made to measure meltability objectively by several researchers. These efforts range from semi empirical methods to fundamental rheological measurement using instruments such as dynamic rheometers, viscometers, and compression analysers (such as a Universal Testing Machine).

Rheological properties can be determined by applying small stresses (force per unit area) or strains (deformation per unit length) at levels that do not cause significant irreversible changes in the cheese. Objectives rheology methods have usually been applied without considering their relationship with sensory attributes.

Flow behavior of materials is characterized by their viscosities, and accordingly, measurements of cheese viscosity was a major focus in an effort to objectively quantify meltability (ease to flow and extend of flow). Ease of flow can be determined from the stress causing flow, and extend of flow can be related to strain rate. Viscosity, the ratio of stress to strain rate, can help estimate the combined behavior, the meltability [42].

2.2.1. Cheese Melt Profile

During heating cheese undergoes a phase from solid to liquid. This transition plotted as “cheese flow vs. heating time,” is known as the cheese melt profile, the transition of cheese flow from the “flow initiation zone” to “flow termination zone” via “rapid flow zone”, where most of the flow occurs. The cheese temperature vs. heating time may also be plotted along with the melt profile to assist in graphical determination of cheese melt/flow parameters.

The major advantage of the cheese melt profile measurement is that it facilitates determining several parameters, in addition to cheese meltability, that are relevant to end-use application of cheese and knowing the mass and thermal properties of cheese, one can calculate thermal energies involved in different flow ranges [42].

When cheese is first put into an oven, the cheese temperature increases, but the shape does not change. Once the cheese reaches a critical temperature called the softening point, it begins to flow and change shape. The cheese matrix collapses and becomes one semisolid mass. The last critical point the cheese reaches in its melt profile is the complete melt point. After this point, height changes are minimal and cheese temperatures eventually reach the oven temperature. Softening can be related to the loss of elasticity, which occurs when all cheeses are heated. Flow behaviour may occur when the viscous modulus become greater than the elastic modulus.

Blumenthal et al. [14] probably were first to use the standard dropping point method to characterize the melting behavior of Raclette cheese. They used the same equipment and technique as described in various official standards (ASTM D 3461-76) for fat characterization. The dropping point corresponds to the temperature at which the first drop of melted sample falls off a nipple with an orifice diameter of 2.8 mm. In addition to the dropping point, a softening point (Ts) was defined and determined for the same samples. The Ts was temperature at which the first drop started to flow out of a nipple with an orifice diameter of 6.4 mm. It was found that the dropping point better discriminates between cheeses of different melting quality than the softening point.

The method was successfully applied to Raclette cheese and allowed characterization of the melting quality [32,33]. However, the dropping point of only a few cheese varieties can be measured without major problems [98]. For many cheese varieties, oiling-off occurs before the temperature of the dropping point is reached or the drop does not fall off at all, probably because of high viscosity. With exception of very hard cheeses, including Parmesan and Sbrinz, the softening point can be measured without difficulties. It was found that the dropping point better discriminates between cheeses of different melting quality than the softening point. Difficulties are encountered when testing cheeses such as Mozzarella, which contain droplets of residual whey [101].

Softening point [83] of cheese increased with reduction in fat content of cheese and increased oven temperature. It appeared that if the cheese was heated quickly a higher temperature could be reached before it softened. In general, the softening point of cheese decreases with age of cheese.

2.2.2. Dynamic shear rheometry

The dynamic shear rheometry (DSR) and the small amplitude osciallatory (SAOS) tests are used to determine the elastic and viscous components of viscoelastic materials. The amplitude of strain is usually kept small to stay within a linear viscoelastic region [35]. In this analysis, the elastic modulus (G′), viscous modulus (G″), and tan δ (G″/G′) =1 are determined and used to quantify cheese melt and flow properties [104,115]. Additionally, dynamic methods allow for an understanding of short-range interactions such as conformation and structure of the casein particles. Such tests vary either the stress or strain harmonically with time in an oscillatory type movement.

Nolan et al**.** [86] used dynamic, small strain rheological methods to differentiate natural and imitation Mozzarella cheese. They found that measurements of the viscous and elastic components of the cheeses were very sensitive to calcium citrate concentration. Additionally, this group observed that specimen slippage in the instrument was occurring possibly due to the migration of milk fat onto the surface of the cheese at room temperature. They concluded that the use of cryanoacrylate glue to bond the cheese samples to the rheometer plate surfaces was most appropriate. Even cheeses that are difficult to differentiate using other analytical techniques can be easily classified using small strain methods. By measuring the viscoelastic properties of Mozzarella and Cheshire cheeses, small strain methods are more appropriate to distinguish these cheeses than methods that show differences in protein content, specific heat values, and microstructural properties [108].

Horne et al. [50] use a dynamic shear test with temperature range from 20 to 80°C to quantify the viscoelastic properties of a control and Mozzarella cheese containing denatured whey protein. They observed significant differences in the values of shear moduli, reflecting the harder, more brittle and crumbly nature of the cheese.

Ustunol et al. [110] have used a dynamic rheological (G′,G" and G*) test with temperature-sweep from 25 to 90°C to evaluated meltability of Mozzarella cheese with varying fat content (0-34.3%) and in comparison with Arnott method. They have found a correlation between the complex modulus and meltability indices.

Ma et al**.** [75] compared viscoelastic properties of reduced-fat and full-fat Mozzarella cheese, specifically observing how lecithin incorporation affected viscoelasticity on reduced-fat cheese. They observed that the full-fat cheese had a greater elastic (G′) and loss (G″) modulus over the same stress region as the reduced fat samples. Lecithin addition increased the elastic modulus of the reduced fat samples, but did not change the loss modulus. They speculated that the addition of lecithin to the reduced-fat cheese changed the three dimensional structure of the cheese since the elastic modulus increased with the lecithin addition, but the loss modulus did not change.

Recently, Mounsey and O'Riordan [81] have found a correlation between the maximum tan δ values and the meltability indices of imitation cheese. Dynamic rheology may be a useful fundamental method to assess the meltability of cheese products.

Guinee et al. [40] wanted to understand the mechanism of the melting process in order to be able to develop cheeses with predictable melting characteristics. They observed the microstructure of three different commercial cheeses using confocal scanning laser microscopy and then looked at how the viscoelasticity of these same cheeses changed as the cheeses were heated. As expected, the phase angle (δ) increased as the cheeses were heated indicating that the cheeses were becoming more viscous and less elastic.

2.2.3. Squeezing flow rheometry (compression tests)

The squeezing flow technique has been widely used to study cheese meltability [3,21,22]. The sample and test geometry of the squeeze flow method also make it suitable for performing creep and stress relaxation tests. With this method a biaxial elongational viscosity can be determined. It can be measured by compressing a cheese sample between lubricated plates in a uniaxial instrument such as Universal Testing Machine. Under this configuration, assuming perfect slip, shear stress at sample-platen interfaces is zero [111].

Olson and Nelson [90] developed a test for melting behavior based on the tendency of a viscoelastic material to climb up a rotating rod, known as the Weissenberg effect. A modified version of this method, in which the rod was replaced by a helical screw, was reported [98].

2.2.4. Capillary Rheometry

Capillary rheometry measures apparent viscosity (resistance to flow) over a broad range of shear rates and at varied temperatures. The data is commonly used to determine processing parameters, for lot-to-lot quality control, to measure processing degradation, which could reduce physical properties, and to study thermal stability.

Smith et al. [104] used capillary rheometry to study the flow properties of Mozzarella, and American process cheeses at various temperatures. The standard protocol and corrections, which were developed originally for plastic polymers, were applicable to melted Mozzarella cheese but not to the other types, in which slippage due to fat separation and other artifacts played a dominant role. Therefore, though capillary rheometry appears to have some value for testing the viscosity of Mozzarella, appropriate correction factors must be applied. Determination of such correction factors is time-consuming and tedious, as it requires multiple tests using capillaries of different diameters. These difficulties and the expensive test instrumentation necessary deterred further investigations of cheese using the capillary rheometer [42].

2.2.5. Helical viscometry

It has been applied to study viscosity of cheese and other food materials. Test apparatus consisted of a digital Brookfield viscometer mounted on a helipath stand and coupled to a strip chart recorder. The viscometer T-bar spindle was positioned near the bottom of a column of grated cheese and the cheese was melted at 60°C under controlled conditions. Resistance exerted by the melted cheese on the rotating spindle as it was raised through the melted cheese column was recorded continuously by strip chart recorder [42].

Kindstedt et al [62] proposed an instrumental method to measure meltability of Mozzarella cheese, known as Helical viscometry, a rotational viscometer (e.g., Brookfield). A T-bare spindle is attached to the rotating part instead of a cylindrical spindle as used in a typical rotational viscometry. The T-bar is lowered inside a ground mass of Mozzarella held at 60°C in glass baker. The torque to rotate the spindle at a certain speed (1 rpm) as the T-bar spindle is raised through the melted cheese is recorded.

Lee et al. [71] used rotational viscometry to investigate the effect of temperature on viscosity of various cheeses, including Mozzarella cheese. Melting curve of each cheese was derived from viscosity data. The melting behavior of Mozzarella cheese has also been characterized with the Brabendre farinograph, an instrument used to measure the rheological properties of bread dough [98].

2.2.6. Rapid Visco Analyser

The Rapid Visco Analyzer (RVA) is a computer-integrated instrument developed to determine the viscous properties of cooked starch, grain, batter and other foods. This instrument continuously measures apparent viscosity under variable conditions of shear and temperature. Recently, the RVA utilized to measure apparent viscosity in several dairy related applications including cheese melt analysis, rennet casein hydration, and process cheese manufacture. For cheese melt analysis was used to characterize the apparent viscosity of process cheese and the apparent viscosity vs. time curve obtained was used as an index of melt properties. This method was designed to facilitate a rapid and inexpensive assessment of how various formulations and processing conditions affect process cheese characteristics. Not only could this system be used to analyze different cooking profiles, but it could facilitate selection of a manufacturing profile that best suits a particular cooker design and produce process cheese with the targeted functional properties.

Kapoor et al. [54] performed a study wherein they manufactured pasteurized process cheese (PC) and pasteurized process cheese food (PCF) in the RVA (25-g batches) and compared the final functional properties to process cheese manufactured on a pilot-scale twin-screw process cheese cooker (4.5-kg batches). In the methodology described by Kapoor et al. [54] after the process cheese was manufactured in the RVA, the molten cheese was transferred into copper cylinders (20 mm diameter x 30 mm height) and cooled. The cheese was then removed from the cylinders and the unmelted textural properties of the cheese were measured with texture profile analysis (TPA).

Prow [95] and Prow and Metzger [96] used the RVA to evaluate the melted textural properties of process cheese and process cheese spreads. Prow [95] performed melt analysis on 18 commercial samples of block process cheese using various melt tests including the RVA and showed that the RVA melting properties of the process cheeses (hot apparent viscosity and time at 5000cP) showed a high correlation with process cheese melt temperature as determined by dynamic stress rheometry (0.82 and 0.85, respectively), and also showed a good correlation with the Schreiber melt test (0.60 and 0.65, respectively).

Further study has shown that the use of a computer vision system to measure the area of melted cheese samples can provide a meltability index in terms of area before and after heating which is rapid, objective, reliable and straightforward [112,114]. Melting characteristics and oiling-off properties of cheese have also been analysed using computer vision techniques.

Meltability of different brands of Cheddar and Mozzarella cheeses was determined with a novel computer vision method as well as with 2 traditional methods, that is, the Arnott and Schreiber tests. Correlation between the results of these methods was analysed and it showed that the meltability determined with a computer vision system was significantly ($P < 0.0001$) interrelated with the Arnott ($R^2 = 0.69$) and Schreiber ($R^2 = 0.88$) meltabilities. The computer vision method provided an accurate quantitative account of the physical changes in cheese during melting, and thus was capable of revealing meltability differences of cheese that were difficult to distinguish by the traditional methods. The new approach was also applicable to a wide range of cheeses [112].

2.2.7. UW meltmeter

The UW Meltmeter is one of the first instrumental methods to be made available in a long time, measure the melt/flow behavior of cheeses at different temperatures, consisting of a temperature-controlled heater, an LVDT (linear variable differential transformer), and a personal computer with a data acquistion system. This device operates in the lubricated squeeze-flow tests and can be used either a constant force or at a constant deformation rate by removing the LVDT and circular plate and bringing the test platen of a uniaxial testing machine in contact with the sample and flow platform at the beginning of a test. [66,67,109].
Under constant force, once the sample reached to the desired temperature test (60°C), the lever arm is lowered to bring the annulus down. Simultaneously, the sample is subjected to lubricated axial compression due to the weight of the circular plate and LVDT core. This

causes the cheese to flow. Additional weight may be added or a lighter plate can be used, as required, to change force causing flow. The sample height vs. time of low data is continuously recorded [105].

Under constant deformation rate, once the sample reached to the desired temperature test (60°C), the lever arm is lowered and crosshead of the uniaxial testing machine is activated simultaneously to deform the sample at a constant rate [42].

The UW Meltmeter, operated on squeeze flow configuration for evaluating melt and flow characteristics of cheese was also used to determine the softening point of cheeses. The softening point of cheese increased with reduction in fat content of cheese and increased oven temperature. In general, the softening point of cheese decreased with age of cheese [42].

2.2.8. Creep test

Creep test is one of the fundamental measurements used to characterize the properties of viscoelastic materials. A creep test is performed by measuring the strain or deformation as a function of time when a constant stress is applied. Results are expressed in terms of a parameter called the compliance J=strain/stress, because the stress remains constant. The changes in strain of a material can also often be measured when the stress is removed, i.e. creep recovery.

Kuo et al [70] modified the UW Meltmeter to operate in a constant stress mode instead of the previously described constant force mode. The only modification necessary was making the diameter of the circular plate connected to the LVDT the same as the diameter of the sample at the beginning of the test. By so doing, the UW Meltmeter test readily becomes a creep test, apply an instantaneous and constant stress and record the strain with time. In order to stay within the linear viscoelastic range, however, the test temperature of 40°C and a constant stress of 1119.5 Pa were selected (cheese rheology and texture). The generalized Kelvin model was considered for representing creep data.

Ma et al. [75] studied the viscoelastic properties of cheeses by using oscillatory dynamic experiments and creep tests. The creep test data was used to identify the differences in viscoelastic properties of cheeses due to fat reduction and addition of lecithin.

2.2.9. UW Profiler

The UW Melt Profiler [83], which is based on the principle of squeeze flow rheometry, determines softening point that compare reasonably well with the temperature at crossover modulus, which is measured rheologically [41]. At first, the sample was heated from 10 to 100°C and then cooled down to 10°C at a rate of 5°C/min the strain amplitude of 0.05% was employed at a frequency of 1 Hz.

Muthukumarappan et al [82] adapted the UW Meltmeter to perform a transient temperature test. They used two objective test methods: constant temperature test and transient temperatures test (range of 60°C-80°C) for determining the softening point (Ts) of cheeses, accounting for both rheological and thermal characteristics. An apparatus operated on modified squeeze-flow platform, along with the LVDT deformation sensor, is placed inside an oven. The cheese sample (30-mm-diameter,7-mm-thickdisk) height is recorded continuously as the sample is heated by the oven set at a constant temperature. This apparatus is similar to the UW Meltmeter without the sample heating and moving cylinder sections, and is called the UW Melt Profiler. Tests with Mozzarella, and pizza cheeses of different composition and age showed that Ts values increase with reduction in cheese fat content and decrease with cheese aging.

2.2.10. Fluorescence and infrared Spectroscopy

Fluorescence spectroscopy is a rapid, nondestructive analytical technique with high sensitivity and specificity. The potential of using fluorescence in food research has increased during the last few years with the propagated application of chemometrics and with technical and optical developments of spectrofluorometers. Fluorescence spectroscopy applied on dairy products [59] has previously been investigated to monitor structural changes in milk proteins and their physico-chemical environment during milk heating [29], milk coagulation [31,46,47], cheese manufacture and ripening [28,30,78] cheese melting and in the evaluation of oxidative changes in processed cheese during storage [20].

Karoui and Dufour [55] prediction of the rheology parameters of ripened semi-hard cheeses using fluorescence spectra in the UV and visible ranges recorded at a young stage. Storage modulus (G'), loss modulus (G''), strain, tan (δ) and complex viscosity (η*) of 20 semi-hard cheeses were measured by dynamic oscillatory analysis after 2, 30 and 60 days of ripening. On the same cheeses and at the same ages, tryptophan and riboflavin fluorescence spectra were recorded. They demonstrated that front-face fluorescence spectroscopy, coupled with chemometric tools, could be used as a non-invaise technique to predict the rheological properties of ripened cheeses (60days) recorded at 80°C. It is important for the dairy industry to be able to predict at a young stage the melting properties of ripened cheeses such as Raclette. Front-face fluorescence spectroscopy has the potential to dramatically reduce analytical time and cost of rheology parameter measurements. As riboflavin fluorescence is located in the visible range, its measurement may be a valuable and cheap method based on marketed laptop spectrofluorimeter running in the visible range for the prediction of rheology properties of ripened cheeses from spectra recorded at a young stage.

Karoui et al. [57] showed that dynamic testing rheology measurements and front face fluorescence spectroscopy can be useful for monitoring the change in the texture and for the determination of the melting point of cheeses fats and cheese matrix during a temperature increase. On the other hand, The tryptophan and vitamin A fluorescence spectra of cheeses were fingerprints that allowed identification of the investigated cheeses (Comté, Emmental and Raclette). Rheological parameters such as the storage modulus (G'), loss modulus (G''), tan δ and the strain could be used to explain structural characteristics and changes in the 5–

60°C temperature range. Applying canonical correlation analysis (CCA) to the rheological data and fluorescence spectra can provide different information related to the physical state of the fats, the structure of the cheese matrix and the change in the texture of cheeses during an increase in the temperature.

A study by Garimella, et al. [37] evaluate the feasibility of front-face fluorescence spectroscopy (FFFS) to predict the meltability of process cheese spreads or products. Twenty-seven commercial samples from 3 manufacturers were used in this study. Each sample was analysed using dynamic stress rheometry, which was used to calculate the meltability index (temperature at tan δ=1). Additionally, fluorescence spectra of tryptophan (excitation: 290 nm; emission: 305 to 400 nm) were collected on each sample at 20°C using a front-face accessory. Fluorescence spectrum for each sample consisted of an average of 36 scans (6 scans performed on 6 replicates). The spectral data set consisted of normalized and mean-centered spectra from all the samples. Multivariate statistical analysis was used to correlate spectral data with cheese meltability index as measured by dynamic stress rheometry. A prediction model was developed using partial least square regression and was calibrated using a cross-validation method. A correlation coefficient of 0.93 was obtained between fluorescence spectra and cheese meltability. The regions 335 to 350 nm and 385 to 400 nm had the highest correlation to cheese meltability. A negative correlation between the peak height of tryptophan (335 to 350 nm) and cheese meltability index was observed. This correlation may be due to presence of tryptophan residues in a more hydrophobic environment in stronger emulsions as compared with a more polar environment in weak emulsions. These results indicate that the melt properties of process cheese spreads or products are related to molecular structure that can be measured using FFFS. Hence, FFFS can be used as an analysis technique to predict process cheese meltability.

Fagan et al**.** [34] demonstrates that mid-infrared spectroscopy has potential application for the prediction of instrumental textural and meltability attributes of process cheese.

3. MEASURING CHEESE STRETCHABILITY

Stretch is the ability of the casein network to maintain its integrity (not break) when a continuous stress is applied to the cheese. From the viewpoint of the consumer eating pizza, stretch probably means the ability of cheese to form fibers of reasonable tension when a slice is lifted up from the rest of the pizza. Typical tests to measure stretchability can be summarized as the fork test, imitative tensile stretch technique, instrumental vertical elongation method, and 3-pronged hook probe test.

3.1. EMPIRICAL METHODS

Fork test

Fork test [80] is the most popular technique used by cheese manufacturers and pizza companies, in which cheese is baked on a pizza and then tested for how far it will stretch [109]. In this test, shredded cheese (240 to 360 g) is placed on a pizza crust (20 to 36 cm in diameter) with sauce (60 to 150 g) and baked (4 to 6 min at 160°C) and after pizza is baked, a fork is inserted into the melted cheese and raised vertically until all the cheese strands break. Strand length at the time the strands break is used as measure of stretchability. Various types of tensile tests of melted cheese have been developed as possible replacements for the fork test [3,5,23].

3.2. OBJECTIVE METHODS

Stretchability (ease and extent of stretch) is a uniaxial property (compared to meltability, a biaxial property). The objective test is account for the applied force or stress (ease to stretch) and the failure deformation or strain (the extent of stretch). Attempts have also been made to use objective instrumental techniques to measure cheese stretchability.

3.2.1. Helical viscometry

Helical viscometry was suggested as a way to measure stretchability of cheese [63,64,87]. The standard Brookfield viscometer is used in conjunction with the Brookfield Helipath stand. This stand is designed to raise and lower a Brookfield viscometer slowly so that its rotating shearing element describes a helical path through a test sample [43]. However, helical viscometry melt profiles indicates it primarily gives a measure of apparent viscosity of melted cheese plus some qualitative information on stretch characteristics that could be obtained from the area of the melt profile after the T-bar rises above the cheese surface. Measurements by helical viscometry had been shown to be inversely related to the melt test of [89].

The methods of Addeo & Masi [1] and Cavella et al. [23] involved measurement of the force required to break a string (~1 mm thick) of melted cheese, extruded using a piston type capillary rheometer, and the percentage elongation of the string at fracture.

Alison and Micheal [8] evaluated the suitability of helical viscometry for assessing the functional performance of molten Mozzarella cheese. The results of this study indicate that helical viscometry is not suitable for assessing the functional properties of unripened Mozzarella cheese, but is suitable for cheese that has matured for at least 2 weeks.

3.2.2. Vertical elongation

It is the most popular tensile test configuration that vertically strain a cheese sample until failure. It based on uniaxial extension of heated cheese samples (e.g. slices, cylinders) of given dimensions using a Universal Instron-type instrument or load-cell [2,5,91]. The resulting force-displacement curves were used to determine the force required to achieve a certain displacement (i.e. stretch), the force and displacement at fracture and/or the elastic modulus and viscosity of the extended sample.

The vertical elongation method [44] in which shredded cheese in a Petri dish is placed in a water bath that provides temperature control. A T-bar attached to the crosshead of a uniaxial testing machine (an instron) is immersed inside the cheese shreds during melting. As the T-bar is raised, strands are formed and the cheese is stretched at a constant deformation rate. The resultant force-deformation curve is termed the "stretch profile". The stretch profile is recorded to analyze the peak force (a measure of ease of stretch), failure strain (a measure of extant of stretch), and toughness (area under the force time- deformation-curve

Apostolopoulos [7] proposed a test based on how consumers would assess the stretchability of cheese on a pizza. A circular plate (165-mm-diameter Prespex plate) was used as a template to hold a pizza crust, and a smaller circular piece (with a vertical rod attached) was cut out, allowing the center of the template to be raised independently of the edge. A similarly cut pizza crust was placed on the template and a standard weight of cheese sprinkled on top of the crust. The complete apparatus was heated in a microwave oven for 15 s to melt the cheese. On removal from the oven, the vertical rod was attached to the head of a tensile testing machine and pulled vertically at 25mm/s, stretchability is measured as the distance through which the center piece could be lifted until all the strands failed. This method was modified by Guinee and O'Callaghan [39] such that the two halves of a cheese-covered pizza were pulled horizontally, rather than vertically. The distance between the two helves until complete strand failure is taken as an index of cheese stretchability.

In a recent modified tensile test method [36] a test for measuring the stretchability of cheese was developed by adapting a texture-profile analyzer to pull strands of cheese upwards from a reservoir of melted cheese. Seven different cheeses were analyzed using the Utah State University stretch. The cheeses were also analyzed for apparent viscosity with a helical viscometer, for meltability using a tube melt test, and for stretch using the pizza-fork test. Cheese was placed into a stainless steel cup and tempered in a water bath at 60, 70, 80, or

90°C for 30 min before analysis. The cup was then placed in a water-jacketed holder mounted on the base of the instrument. A three-pronged hook-shaped probe was lowered into the melted cheese and then pulled vertically until all cheese strands broke or 30 cm was reached. This produced a stretch profile as the probe was lifted through the reservoir of melted cheese and then pulled strands of cheese upwards. Three parameters were defined to characterize the stretchability of the cheese. The maximum load, obtained as the probe was lifted through the cheese, was defined as melt strength (F_M). The distance to which cheese strands were lifted was defined as stretch length (SL). The load exerted on the probe as the strands of cheese were being stretched was defined as stretch quality (SQ). There was a correlation between F_M and apparent viscosity. There was also some correlation between SL measured by the fork test and SL when the cheese was tested at 90°C, but no correlation occurred at lower temperatures.

3.2.3. Ring-and-Ball method

Hicsasmaz, et al**.** [48] used an objective method to evaluate stretchability of cheese based on the principle of the Ring-and-Ball method used to measure the softening point of polymers. This technique, which controls temperature and moisture loss, was used to quantify the stretchability of Mozzarella cheese. Average stretch length varied between 4 to 9 cm between the youngest and the oldest cheese samples. The method was found to be sensitive enough to discriminate between cheeses of different ages. The results showed that the technique is reproducible and gives reliable stretch length and stretch length vs. time data, which was further used to estimate extensional viscosity of the test sample.

3.2.4. Fiber-spinning technique

One of the objective methods proposed to measure cheese stretchability is the fiber-spinning technique [23], originally developed to assess the spinnability of polymeric melts in which the strength of a thin string of melted cheese is measured as it is extruded through a capillary. The best operating conditions for Mozzarella cheese are an extrusion speed of 1.24

cm/s and a rate of increase in pick-up speed of 38.5 cm/s. Although the method is capable of measuring the failure stress and strain values directly in a temperature- and moisture-controlled medium, it is hard to adopt in an industrial setting [43].

4. MEASURING CHEESE OILING-OFF

The oiling-off property of cheese, also called free oil (FO) formation or fat leakage, is the separation of liquid fat from the melted cheese body into oil pockets, particularly at the cheese surface [61]. Heating of natural cheese is accompanied by an increase in the degree of clumping and coalescence of fat globules at the microstuctural level. Free oil contributes positively to functionality by lubricating the surfaces of adjoining layers of the para-casein matrix and reducing dehydration of the cheese during baking.

4.1. Traditional methods

Traditionally, FO formation was measured by melting cheese on filter paper to achieve an oil ring. The resulting ring size related to the amount of free oil in the cheese product [17]. Kindstedt and Rippe [66] developed a rapid, simple quantitative test for measuring FO in melted Mozzarella cheese using standard Babcock equipment. Eighteen grams of whole milk or part skim Mozzarella were weighed into 50 or 20% Paley-Babcock bottles, respectively. Bottles were immersed in boiling water for 4.0 min to melt cheese. Distilled water (20 ml at 57.5°C) was then added and the bottles were centrifuged hot (ca. 57.5°C) for 10 min. A portion of 1:l distilled water:methanol (21°C) was added to a final level in the upper region of the calibrated neck and then centrifuged for 2 min. Bottles were then rocked by hand for 10s, centrifuged for 2 min, and a second time rocked by hand for 10s and centrifuged for 2 min. Finally, bottles were tempered in 57.5°C water for 5 min and the fat column then measured with glymol. This procedure gave a clear, defined fat column. Free oil was expressed as percentage in cheese and percentage in cheese fat. A modified Gerber test was also devised for European counterparts [67].

4.2. Computer vision

Wang and Sun [114] investigated the evaluation of cheese oiling-off affected by baking conditions and sample dimensions using computer vision. For Mozzarella cheese, obvious oiling-off was observed at all temperature from 70 to 200°C, while the maximum oiling-off occurred at 160°C. Little free oil was formed for the Mozzarella cheese below 100°C and the highest value of percentage oil area was observed at 130°C. The oiling-off property of cheese was significantly influenced by sample dimensions. Regardless of sample dimension, the maximum oiling-off Mozzarella cheese occurred at 1min of cooking and little free oil was observed after cooking for 4 min. Free oil formation was limited for Mozzarella cheese in spite of a wide range of heating temperature and sample dimensions applied.

5. MEASURING CHEESE BROWNING

Browning is typically a desirable property for baked cheese. The browning of cheeses like Mozzarella during baking is due to the Maillard reaction [106], a heat-induced reaction between sugars and protein. The intensity of the browning depends on the lactose and galactose content of the cheese and the ability of the free-amino groups to remain hydrated during baking. However, excessive browning creates a problem for cheeses like Mozzarella, Pizza cheese or Parmesan [94].

5.1. Sensory analysis

Visual descriptive analysis of Mozzarella cheese on pizza has been used by several researchers to study browning properties [83]. More attempts have been made to analyse browning of cheese during baking or during storage time to improve the quality of cheese used as toppings. Researchers have investigated the influence of different starter cultures on the residual sugar in cheese and thus on cheese browning.

Matzorf et al. [78] investigated the browning of Mozzarella cheese during high temperature pizza baking. Their results show that both browned and low browned pizza were acceptable by the panelists, indicating that browning of Mozzarella cheese on pizza was not an undesirable property. This is consistent with the opinion of Johnson and Olson [53] and Alvarez [6] that different pizza manufactures and consumers have different expectations for the browning of cheese.

For the evaluation of cheese color, in addition to qualitative sensory evaluation [102], instrumental methods involving colorimeters or spectrophotometers are employed. Bley et al. [13] developed a predictive test to assess the tendency of Mozzarella cheese to turn brown when heated and after processing. They first heated cheese in a boiling water bath for 1h, and then placed the cheese in an incubator at 70°C for 48h. The three colour indexes, namely, L*, *a** and *b**, of cheese samples were measured using a Hunterlab colorimeter. Three readings of each colour index were taken from each sample by rotating the cheese surface by 120° C. The modified versions of this method have been subsequently employed by the most researchers for the determination of cheese colour [11,87].

5.2. Computer vision

Wang and Sun [113] developed a computer vision method to determine the colour changes during heating. The browning properties of both Cheddar and Mozzarella cheeses were analysed and compared. The influence of baking temperature (70–200°C) and time (0–20 min) on the browning property of cheese was significant for both cheeses. However, the time and temperature dependencies of a specially defined browning factor (BF) were different for Cheddar and Mozzarella cheeses. Cooking for 2–4 min, the BF of Mozzarella cheese increased almost linearly with baking temperature. As for the Mozzarella cheese, after heating for 8–12 min, a linear relationship between the BF and baking temperature was observed from 70 to 130°C. Further browning hardly occurred when cooking temperature increased from 130 to 160°C, probably because of depletion of reducing sugars and amino groups in cheese that are necessary for the browning reaction. When heating at temperatures above 160°C, the colour of Mozzarella cheese darkened dramatically due to scorching. The results also show that the computer vision method developed provides an objective and efficient approach for assessing the browning of cheese.

6. SPECTROSCOPIC TECHNIQUES FOR CHEESE STUCTURE AND TEXTURE DETERMINATIONS.

Texture is an imprtant parameter in the evaluation of cheese quality. Cheese structure is heterogeneous with several of its constituents present as a soild matrix (paracasein), some as a liquid phase. Rheological characterisation of cheese is important as a means to determine textural characteristics of a cheese. A descripation of the structural characteristics of cheeses can also achieved using fluoresence spectroscopy [30,47] and infrared spectroscopy [24].

Karoui and Dufour [55] investigated differences in ripened soft cheeses (of surface to center) by dynamic testing rheology and fluorescence spectroscopy. The surface to center responses of three different retailed soft cheeses (M1, M2 and M3) were characterized by dynamic rheology and front-face fluorescence (tryptophan and vitamin A) spectra. The storage modulus (G') and the loss modulus (G') values of the samples increased from the surface to the inner part of the cheeses, while strain and tan δ decreased. Protein tryptophan (excitation: 290 nm; emission: 305–400 nm) and vitamin A (emission: 410 nm; excitation: 250–350 nm) spectra were recorded at 20°C in samples cut from the surface to the centre. For each cheese, the data sets containing fluorescence spectra and rheology data were evaluated using multidimensional statistical methods. In addition, the three cheeses were well discriminated by their spectra by applying factorial discriminant analysis. From the tryptophan fluorescence data sets, 94% and 87.7% good classifications were observed for calibration and validation groups, respectively. A better classification (100% and 96% for principal and test samples) was obtained from the vitamin A spectra. Canonical correlation analysis was performed on the rheology and tryptophan fluorescence spectral data sets, and on the rheology and vitamin A fluorescence spectra data sets. The two groups of variables were found to be highly correlated since the squared canonical coefficients for canonical variates 1, 2, 3, 4 were higher than 0.98. These high correlations indicate that cheese rheology is a reflection of its structure at the molecular level.

Karoui and Dufour [56] evaluated the feasibility of using fluorescence spectroscopy as a non-destructive technique to predict rheological characteristics of 20 semi-hard cheeses determined at 80°C by dynamic oscillatory analysis on 60-days-old cheeses from tryptophan and riboflavin fluorescence spectra recorded at 20°C on 2 days-old cheeses. Using partial least square, tryptophan fluorescence spectra recorded at 20°C on 2-days-old cheeses predicted G', G'', strain, tan (δ) and η* measured at 80°C on the 60-days-old cheeses with correlation coefficients (R) of 0.98, 0.97, 0.98, 0.98 and 0.97, respectively. Riboflavin fluorescence spectra gave slightly lower correlation coefficients of 0.88, 0.88, 0.92, 0.87 and 0.88, respectively. Dependent only on visible light, the riboflavin fluorescence spectra potentially provide viable and economic prediction of the rheology of ripe cheese.

Kulmyrzaev et al. [68] investigated changes at molecular and macroscopic levels of young and ripened soft-cheeses using uniaxial compression (1000N load) at constant displacement rate (50mm min^{-1}), infrared spectroscopy (between 3000 and 900 cm^{-1}at a resolution of 4 cm^{-1}), front-face fluorescence spectroscopy and components and specific weights analysis (CCSWA) was applied to the rheology data, infrared spectra and fluorescence spectra to demonstrated the relationship between molecular structure and manufacturing processes of cheeses. Twelve traditional and 12 stabilised experimental soft cheeses were made according to a factorial design to two levels of dry matter (44% and 48%) and two fat on dry matter ratios (51% and 55%). Common components and specific weights analysis showed that the common component 1discriminating young and ripened cheeses explained 95%, 92%, 73% of the inertia of the 900–1500, 2800–3000 and 1500–1700 cm^{-1}

infrared regions, respectively, and 51% of the rheology data. Common component 2 discriminating cheeses as a function of the technology explained 88%, 23% and 11% of the inertia of vitamin A spectra, chemical data and rheology data, respectively. They concluded that, fluorescence and infrared data are spectra that allow us to derive information on the molecular structure and interactions of the cheese matrix. Moreover, it is also suggested that the phenomena observed at molecular (fluorescence, infrared) and macroscopic (rheology) levels are related to the texture of cheeses.

6. RELATIONSHIPS BETWEEN INSTRUMENTAL AND SENSORY ATTRIBUTES

Consumer preference for a food product is principally determined by its sensory characteristics. Texture clearly plays a role in consumer acceptance of cheeses. Consumers expect high quality shredded cheese starting from package appearance to acceptable performance during baking. The functionality of heated Mozzarella cheese depends on several factors: meltability, stretchability, free oil formation, and browning. These properties play heavily on consumer perceptions of cheese quality [62,95,100]. Correlations between sensory and rheological data have been used to differentiate and define different cheese varieties. The instrumental mechanical measurements correlated well with the mechanical sensory perceptions, instrumentation could not accurately profile the entire sensory textural experience. Table (2) demonstrated some examples of studies on the relation between instrumental measure and sensory attributes.

6.1. Rheological and sensory data

Rheological and sensory methods can be used for complet characterization of cheese. Instrumental texture profile analysis (TPA), is an imitative texture test that has been used extensively [5,93]. It is generally conducted by uniaxial compression of a sample between 2 plates at a chosen cross-head velocity for a chosen level of deformation. Force, deformation and work (area under the force-deformation curve) measurments are used to calculate texture parameters of facturability, hardenss, cohesiveness, adhesiveness, springiness, gumminess and chewiness. In a typical experiment, samples are evaluated by sensory analysis and instrumental texture profile analysis and then correlations are determined.The correlation between sensory texture and instrumental textural profile analysis properties of cheeses varies among investigations [19,25,26].

Cheese is a time-dependent material in that the response of the cheese to an applied stress depends upon the speed in which the stress is applied making such methods appropriate. These methods are used to define both the elastic and the viscous nature of cheese. Results from viscoelastic characterization have provided a way to characterize and differentiate cheese varieties [86,108]. Small strain dynamic rheological methods are non-destructive; conducted within the linear viscoelastic region and determine the elastic and viscous nature of cheese. Large strain rheological methods occur outside of the linear viscoelastic region and characterize the non-linear and fracture properties of the material. These types of methods correlate well with sensory properties since mastication is a large strain action; results from such studies have been used to show the impact of changes in formulation and processing practices on the texture of cheese [16,35, 51,108,109]. Such methods have also been used to characterize certain varieties of cheese grouped and distinguished French cheeses based upon instrumental and sensory measurements of texture [4,9].

Lee et al. [71] attempted to correlate compressive measurements and data from a trained descriptive sensory panel for cheeses having a wide range of textures. They found sensory hardness correlated with instrumental measurements of compressive force, work ratio, adhesive force, and force at the inflection point. Sensory springiness related to elastic recovery and force at the inflection point, and sensory adhesiveness was related to adhesive force and inversely related to force at the inflection point.

Jack et al. [52] attempted to discriminate nineteen different types of Mozzarella cheese each having previously shown different perceived textures. They wanted to be able to suggest types of instrumental and sensory methods that could be used to predict texture using the foretold analytical relationships. The Mozzarella showed significant differences rheologically, compositionally, and sensorally, however, there was limited correlation between the sensory and the instrumental methods. Though all types of measurements did differentiate the cheeses, each method used different characteristics of the cheeses to differentiate.

Drake et al. [26] used sensory descriptive methods, small strain rheological testing, and compressive instrumental methods to draw correlations between instrumental and sensory data and to differentiate natural and process cheeses. They showed that the compressive measurements alone could predict mouth and hand firmness. Additionally, they found that frequency sweep and creep recovery data together could predict the sensory attributes of mouth smoothness, hand rubberiness, and hand brittleness, however, these fundamental measurements alone were poor predictors of sensory attributes. They concluded that the compressive measurements were better able to predict sensory texture than the fundamental rheological tests. Rheological and sensory methods can be used for complete characterization of cheeses, which has great implications in product development.

Brown et al. [18] investigated the sensory and rheological properties of young cheeses in order to better understand perceived cheese texture. Mozzarella and Monterey Jacks were tested at 4, 10, 17, and 38 days of age; process cheese was tested at 4 days. Rheological methods were used to determine the linear and non-linear viscoelastic and fracture properties. A trained sensory panel developed a descriptive language and reference scales to evaluate cheese texture. All methods differentiated the cheeses by variety. Principal component analysis of sensory texture revealed that three principal components explained 96.1% of the total variation in the cheeses. The perception of firmness decreased as the cheeses aged, whereas the perception of springiness increased. Principal component analysis of the rheological parameters (three principal components: 87.9% of the variance) showed that the cheeses, solid-like response (storage modulus and fracture modulus) decreased during aging, while phase angle, maximum compliance, and retardation time increased. Analysis of the instrumental and sensory parameters (three principal components: 82.1% of the variance) revealed groupings of parameters according to cheese rigidity, resiliency, and chewdown texture. Rheological properties were highly associated with rigidity and resiliency, but less so with chewdown texture.

Kealy [60] examined cream cheese using TPA, one of the main instrumental techniques for texture measurement, and compared the results with those of a trained taste panel. Although a reasonably strong correlation was found between the taste panel results and TPA-derived hardness and adhesiveness parameters, the correlation for cohesiveness was not straightforward. Everard [33] also investigated the prediction of sensory attributes of processed cheese from instrumental texture attributes derived from TPA, a compression test, and a 3-point bend test. He could predict the texture attributes of firmness, rubbery, creamy, chewy, fragmentable, and mass-forming with a good level of accuracy.

6.2. Mid, Near-infrared spectroscopy and sensory data

Spectroscopic analysis in combination with predictive mathematical models [58], developed using multivariate data analysis techniques such as partial least squares (PLS) regression, have potential use in controlling and monitoring the quality of raw materials through to the final product in food processing. In particular, infrared spectroscopy has been applied as an objective and non-destructive technique to provide a rapid and real-time analysis of both composition and quality. Blazquez et al**.** [12] modelled the sensory attributes of processed cheese using near-infrared reflectance spectroscopy with predictive mathematical models, developed using multivariate data analysis techniques such as partial least squares

(PLS) regression. They found that it was possible to model a number of attributes including firmness, melting, rubbery, and creamy.

Downey et al.[24] using near-infrared (NIR) reflectance spectroscopy (750-2498nm) to predict cheese maturity (age) and several sensory attributes in Cheddar cheese (crumbly, rubbery, chewy, mouthcoating and massforming). Also Sørensen and Jepsen [103] successfully demonstrated that near-infrared spectroscopy in conjunction with PLS regression can be used to predict several sensory attributes of Danbo cheese.

Karoui et al. [59] demonstrated the feasibility of near–infrared (NIR) spectroscopy to predict some sensory attributes (adhesivity, elasticity, firmness, aroma, odour intensity, bitterness, saltiness, acidity sweetness) of Emmental cheeses originating from different European regions. Calibration models between sensory attributes and NIR spectra were developed using partial least squares (PLS) regression. The squared correlation coefficient (R^2° were greater than 0.5 for adhesivity, elasticity, firmness, aroma, bitterness, saltiness, acidity and sweetness. In addition, a good correlation between sensory attributes and NIR spectra was found using canonical correlation analysis (CCA).

Fagan et al. [34] investigated the potential application of mid-infred spectroscopy in conjunction with partial least squares (PLS) regression for determination of selected sensory attributes (firmness, rubbery, creamy, chewy, mouth-coating, fragmentable, melting, mass-forming) in a range of experminatal manufactured processed cheese samples. They concluded that mid-infrared spectropscopy could be successfully used for the nonodestructive and objective assessment of processed cheese sensory quality. Mid-inrared spectropscopy produced results similar to or bettere than the models previously developed using instrumental texture attributes.

6.3. Flurescence spectroscopy and sensory data

Dufour et al. [27] delineation of the structure of soft cheeses at the molecular level by fluorescence spectroscopy relationship with texture. Tryptophan fluorescence spectra of eight different soft cheeses were recorded directly on cheese samples using front-face fluorescence spectroscopy. Discriminant ability of the data was investigated by discriminant analysis. A correct classification was observed for 95% and 92% of the calibration and validation samples, respectively. It was concluded that tryptophan fluorescence spectra enable the identity of individual cheeses to be finger-printed. Canonical correlation analysis was applied to soft-cheese sensory profile data and fluorescence spectral collection in order to measure the link between the two groups of variables measured on the same samples. The two groups of variables were found highly correlated since the squared canonical coefficients for canonical variates 1 and 2 were 0.93 and 0.80, respectively. A subset of four soft cheeses was investigated closely in order to establish a molecular basis of the discrimination. It was shown that molecular level information may be derived from the fluorescence spectra.

Lebecque et al. [72] investigated the texture of Salers cheese (special semi-hard cheese produced in the center of France) by sensory analysis, rheological measurements and fluorescence spectroscopy. They found that sensory analysis, as well as fluorescence spectroscopy and rheology measurements may be valuable tools to define quality, identity and authenticity of Salers cheese. The fluorescence spectra recorded directly on cheeses were fingerprints allowing their identification. Principal Component Analysis (PCR) and Canonical Correlation Analysis (CCA) demonstrated there are strong correlations between instrumental data and sensory attributes of cheese texture. Using PCR, 4 sensory attributes, over the 8, can be predicted with a good accuracy from the vitamin A fluorescence spectra.

8. CONCLUSION

Functional properties of cheese is guidance critical for successuful use of cheese as an ingredient. Although significant progress has been made on the measurement of cheese functional properties, the available testing techniques still have some limitations. In general, there are rheological-based methods that provide critical and accurate data, but they require expensive equipment and are timeconsuming to perform. In contrast, the available empirical-based methods provide crude results but are simple to perform and do not require expensive equipment. Consequently, there is still an unmet need for accurate, rapid, and cost-effective techniques for measuring cheese functional properties. A logical way to develop non-subjective methods to characterize cheese texture is to correlate its rheological properties with its sensory properties and such data could be useful in predicting the quality of final product as cheese is used as an ingredient into other food products.

9. REFERENCES

[1] Addeo F., and Masi P., Production of Pasta cheese, In 3rd Cheese Symposium, National Dairy Products Research Center, Moorepark, ed. T.M., Cogan Teagasc, Fermoy, Co. Cork, 1992, pp. 31-40.

[2] Ak M.M., and Gunasekaran S., Measuring elongational properties of Mozzarella cheese, J. Texture Stud. 26(1995)147–160.

[3] Ak M.M., and Gunasekaran S., Evaluating rheological properties of Mozzarella cheese by the squeezing flow method, J. Texture Stud. 26 (1995)695–711.

[4] Ak M.M., and Gunasekaran S., Anisotropy in tensile properties of Mozzarella cheese, J. Food Sci. 62(1997)1031-1033.

[5] Ak M.M., Bogenrief D., Gunasekaran S., and Olson N.F., Rheological evaluation of Mozzarella cheese by uniaxial horizontal extension, J. Text. Stud. 24 (1993) 437–453.

[6] Alvarez R.J., Expectations of Italian cheese in the pizza industry, Proc. 23rd Annu. Marschall Invit. Ital. Cheese Sem., Madison, WI, 1986.

[7] Apostolopoulos C., Simple empirical and fundamental methods to determine objectively the stretchability of Mozzarella cheese, J. Dairy Res. 61 (1994) 405–413.

[8] Alison A.S., and Michael W.A.M., Evaluation of helical viscometry for assessing the functional properties of Mozzarella cheese, International J. Dairy Tech. 53 (2000) 57-62.

[9] Antoniou K.D., Petridis R., Raphaelides S., Ben Omar Z., and Kesteloot R., Texture assessment of French cheeses, J. Food Sci. 65 (2000) 168-172.

[10] Arnott D.R., Morris H.A., and Combs W.B., Effect of certain chemical factors on the melting quality of process cheese, J. Dairy Sci. 40 (1957) 957-963.

[11] Barbano D.M., Yun J.J., and Kindstedt P.S., Mozzarella cheese making by a stirred curd, no brine procedure, J. Dairy Sci., 77 (1994) 2687-2694.

[12] Blazquez C., Downey G., O'Callaghan D., Howard, V., Delahunty C., Sheehan E., and O'Donnell C.P., Modelling of sensory and instrumental texture parameters in processed cheese by near infrared reflectance spectroscopy, J. Dairy Res. 73 (2006) 58–69.

[13] Bley M., Johnson M.E., and Olsson N.F., Predictive test for the tendency of Mozzarella cheese to brown after processing, J. Dairy Sci. 68 (1985) 2517-2520.

[14] Blumenthal A., Weymuth H., and Hansen W., Flow and drip points of Raclette cheese. Schweiz. Milchztg 102 (1976) 391–395.

[15] Bourne M.C., Texture profile analysis, Food Technol. 32 (1978) 62–66.

[16] Bowland E.L., and Foegeding E.A., Factors determining large-strain (fracture) rheological properties of model processed cheese, J. Dairy Sci. 82 (1999) 1851-1859.

[17] Breene W.A., Price W.V., and Ernstrom C.A., Manufacture of Pizza cheese without starter, J. Dairy Sci. 47 (1964) 1173-1180.

[18] Brown J. A., Foegeding E.A., Daubert C.R., Drake M.A., and Gumpertz M., Relationships among rheological and sensorial properties of young cheeses, J. Dairy Sci. 86 (2003) 3054-3067.

[19] Bryant A., Ustunol Z., and Steffe J., Texture of Mozzarella cheese as influenced by fat reduction, J. Food Sci. 60 (1995) 1216–1219.

[20] Christensen J., Povlsen V.T., and Sørensen J., Application of fluorescence spectroscopy and chemometrics in the evaluation of the stability of processed cheese, J. Dairy Sci. 86 (2003)1101-1107.

[21] Campanella O.H., Popplewell L.M., Rosenau J.R., and Peleg M., Elongational viscosity measurements of melting American process cheese, J. Food Sci. 52 (1987) 1249–1251.

[22] Casiraghi E.M., Bagley E.B., and Christianson D.D., Behaviour of Mozzarella, and processed cheese spread in lubricated and bonded uniaxial compression, J. Texture Stud. 16(1985) 281–301.

[23] Cavella S., Chemin S., and Masi P., Objective measurement of the stretchability of Mozzarella cheese, J. Texture Stud. 23 (1992)185–194.

[24] Downey G., Sheehan E., Delahunty C., O'Callaghan D., Guinee T., and Howard V., Prediction of maturity and sensory attributes of Cheddar cheese using near-infrared spectroscopy, Int. Dairy J. 15 (2005) 701–709.

[25] Drake M.A., Boylston T.D., and Swanson B.G., Fat mimetics in low fat cheeses, J. Food Sci. 61 (1996) 1267–1270.

[26] Drake M.A., Gerard P.D., Truong V.D., and Daubert C.R., Relationship between instrumental and sensory measurements of cheese texture, J. Text Stud. 30 (1999) 451-476.

[27] Dufour E., Devaux M.F., Fortier P., Herbert s., Delineation of the structure of soft cheeses at the molecular level by fluorescence spectroscopy relationship with texture, Int Dairy J. 0(2001) 1–9.

[28] Dufour E., Mazerolles G., Devaux M.F., Duboz G., Duployer M.H., Mouhous Riou N., Phase transition of triglycerides during semi-hard cheese ripening, Int Dairy J. 10 (2000) 87–99.

[29] Dufour E., Riaublanc A., Potentiality of spectroscopic methods for characteristics of dairy products: 1. Font-Face Fluorescence study of raw, heated and homogenized milks, Lait 77 (1997) 657-670.

[30] Dufour E., Devaux M.F., Fortier P., and Herbert S., Delineation of the structure of soft cheeses at the molecular level by fluorescence spectroscopy—relationship with texture, Int. Dairy J. 11 (2001) 465–473.

[31] Dufour E., Lopez C., Riaublanc A., Mouhous Riou N., La spectroscopie de fluorescence frontale : une approche non invasive de la structure et des interactions entre les constituants des aliments, Agroral 10(1998) 209-215.

[32] Eberhard P., Moor U., and Ruegg M., Objective measurement of the softening and dropping point of Raclette cheese, Schweiz. Milchwirtsch. Forsch 15 (1986) 93–95.

[33] Everard C.D., Determination of quality characteristics of process and Mozzarella cheeses using rheological, sensory and dielectric measurement techniques, Ph.D. Diss. University College Dublin, Dublin, Ireland, 2005.

[34] Fagan C.C., Everard C., O'Donnell C.P., Downey, G. and Sheehan E.M., Evaluating mid-infrared spectroscopy as a new technique for prediction sensory texture attributes of processed cheese, J. Dairy Sci. 90 (2007)1122-1132.

[35] Fenelon M.A., and Guinee, T.P., Primary proteolysis and textural changes during ripening in Mozzarella cheeses manufactured to different fat contents, Int. Dairy J.10 (2000) 151-158.

[36] Fife R.L., McMahon D.J., and Oberg, C.J., Test for measuring the stretchability of melted cheese. J. Dairy Sci. 85 (2002)3549–3556.

[37] Garimella S.K.P, Prow L.A., and Metzger L.E., Utilization of Front-Face Fluorescence Spectroscopy for Analysis of Process Cheese Functionality, J. Dairy Sci. 88 (2005) 470-477.

[38] Guinee T.P., The functionality of cheese as an ingredient: a review, Aust. J. Dairy Technol. 57 (2002) 79-91.

[39] Guinee T.P., and O'Callaghan D.J., The use of a simple empirical method for objective quantification of the stretchability of cheese on cooked, J. Food Engineering 31 (1997) 147-161.

[40] Guinee T.P., Harrington D., O'Cororan M., Mullholland E.O., and Mullins C., The compositional and functional properties of commercial Mozzarella, and analogue pizza cheeses, Int. J. Dairy Technol. 53 (2000) 51-56.

[41] Gunasekaran S., Hwang C.H., and KO S., Cheese melt/flow measurement methods-recent developments, Aust. J. Dairy Technol. 57 (2002) 128-133.

[42] Gunasekaran S., and Ak M.M., Measuring cheese melt and flow properties, in Cheese Rheology and Texture, CRC Press, Boca Raton, FL, 2003, Pages 331–375.

[43] Gunasekaran S., and Ak M.M., Measuring cheese stretchability, in Cheese Rheology and Texture. CRC Press, Boca Raton, FL , 2003, Pages 377–397.

[44] Gwartney E.A., Foegeding E.A., and Larick D.K., The texture of commercial full fat and reduced fat cheeses, J. Food Sci. 87 (2002) 812-816.

[45] Hennequin D., Hardy J., Évaluation instrumentale et sensorielle de fromages à pâte molle, Int Dairy J. 3 (1993) 635–647.

[46] Herbert S., Caractérisation de la structure moléculaire et microscopique de fromages à pâte molle, Analyse multivariée des données structurales en relation avec la texture, Thèse, École Doctorale Chimie Biologie de l'Université de Nantes, France, 1999, page 118.

[47] Herbert S., Riaublanc A., Bouchet B., Gallant D.J. and Dufour E., Fluorescence spectroscopy investigation of acid-and rennet-induced coagulations of milk, J. Dairy Sci. 82 (1999) 2056–2062.

[48] Hicsasmaz Z., Shippelt L., and. Rizvi S.S.H, Evaluation of Mozzarella Cheese Stretchability by the Ring-and-Ball Method, J. Dairy Sci. 87 (2004) 1993-1998

[49] Hokes J.C., Mangino M.E., and Hansen P.M.T., A model system for curd formation and melting properties of calcium caseinates, J. Food Sci. 47 (1982)1235-1249.

[50] Horne D.S., Banks J.M., Leaver J., Law A.J.R., Dynamic mechanical spectroscopy of Mozzarella cheese: in cheese yield and factors affecting its control, Special issue 9402, Int. Dairy Fed., Brussels, Belgium, 1994, pp. 507–512.

[51] Hsieh Y.L., Yun J.J., and. Rao M.A., Rheological properties of Mozzarella cheese filled with dairy, egg, soy proteins, and gelatin, J. Food Sci. 58 (1993) 1001-1004.

[52] Jack F.R., Piggott, J.R., and Paterson A., Relationships between rheology and composition of Mozzarella cheeses and texture as perceived by consumers, Int. J. Food Sci. Technol.28 (1993) 293-302.

[53] Johnson M.E., and Olson N.F., Nonenzymatic browning of Mozzarella cheese, J. Dairy Sci. 68 (1985)3143.

[54] Kapoor R., Lehtola P., and Metzger L.E., Comparison of pilot-scale and rapid visco analyser process cheese manufacture, J. Dairy Sci. 87 (2004) 2813-2821.

[55] Karoui R., and Dufour E., Dynamic testing rheology and fluorescence spectroscopy investigation of surface to centre differences in ripened soft cheeses, International Dairy Journal 13 (2003) 973–985.

[56] Karoui R., and Dufour E., Prediction of the rheology parameters of ripened semi-hard cheeses using fluorescence spectra in the UV and visible ranges recorded at a young stage, International Dairy Journal 16 (2006) 1490–1497.

[57] Karoui R., Laguet A., and Dufour E., Fluorescence spectroscopy: A tool for the investigation of cheese melting–correlation with rheological characteristics, Lait 83 (2003) 251–264.

[58] Karoui R., Mazerolles G., and Dufour E., Spectroscopic techniques coupled with chemometric tools for structure and texture determination in dairy products, Review, International Dairy Journal 13 (2003) 607–620.

[59] Karoui R., Pillonel L., Schaller E., Bosset J.-O, Baerdemaeker J.De, predication of sensory attributes of European Emmental cheese using near-infrared spectroscopy: A feasibility study, Food chemistry, 101 (2006) 1121-1129.

[60] Kealy T., Application of liquid and solid rheological technologies to the textural characterisation of semi-solid foods, Food Res. Intern. 39 (2006)265–276.

[61] Kindstedt P.S., Effect of manufacturing factors, composition, and analysis on the functional characteristics of Mozzarella cheese, Crit. Rev. Food Sci. Nutr. 33 (1993)167-187.

[62] Kindstedt P.S., Factors affecting the functional characteristics of unmelted and melted Mozzarella cheese: in Chemistry of Structure-Function Relationships in Cheese, Malin E.L., and Tunick M.H., ed. Plenum Press, New York, NY, 1995, Pages 27–41.

[63] Kindstedt P.S., Rippe, J. K., and Duthie, C.M., Application of helical viscometry to study commercial Mozzarella cheese melting properties, J. Dairy Sci. 72 (1989) 3123–3128.

[64] Kindstedt P.S., Rippe, J.K., and Duthie C.M., Measurement of Mozzarella cheese melting properties by helical viscometry, J. Dairy Sci. 72 (1989)3117–3122.

[65] Kindstedt P.S., and Fox P.F., Modify Gerber test for free oil in Mozzarella cheese, J. Dairy Sci. 56 (1990) 1115-1116.

[66] Kindstedt P.S., and Rippe J.K., Raid quantitive test for free oil (oiling-off) in melted Mozzarella cheese, J. Dairy Sci. 73 (1990) 867-873.

[67] Kosikowski F., Cheese and fermented milk food. 2nd ed. New York: F.V. Kosikowski and Associates, 1977, pp. 404-406.

[68] Kulmyrzaev A., Dufour E., Noel Y., Hanafi M., Karoui R., Quannari E.M., Mazeroles G., investigation at the molecular level of soft cheese quality and ripening by infrared and fluorescence spectroscopies and chemometrics-relationships with rheology properties, Inter. Dairy J., 15 (2005)669-678.

[69] Kuo M.I., Wang Y.C. and Gunasekaran S., A viscoelasticity index for cheese meltability evaluation, J. Dairy Sci. 83 (2000) 412-417.

[70] Kuo M.I., Wang Y.C., Gunasekaran S., and Olson N.F., Effect of heat treatment on the meltability of cheeses, J. Dairy Sci. 84 (2001) 1937-1943.

[71] Lee C., Imoto E.M., and Rha C., Evaluation of cheese texture, J. Food Sci.43 (1978)160.

[72] Lebecque A., Laguet A., Devaux M.F., and Dufour E., Delineation of the texture of Salers cheese by sensory analysis and physical methods, Lait 18 (2001) 609-623.

[73] Lefevere I.., Dewettinck K., and Huyghebaert A., Cheese fat as driving force in cheese flow upon melting, Milchwissenschaft, 55 (2000) 563-565.

[74] Lucey J.A., Some perspectives on the use of cheese as a food ingredient, Dairy Sci. Technol. (2008) 1-22.

[75] Ma L., Drake M.A., Barbosa-Canovas G.V., and Swanson B.G., Viscoelastic properties of reduced-fat and full-fat Mozzarella cheeses, J. Food Sci. 61 (1996) 821–823.

[76] Marshall R.J., Combined instrumental and sensory measurement of the role of fat in food texture, Food Quality Preference 2 (1991) 117–124.

[77] Masi P., Characterization of history-dependent strees-realaxtion behaviour of cheeses, J. Texture Stud. 19 (1989) 373-388.

[78] Matzdorf B., Cuppett S. L, Keeler L., and Hutkins R.W., Browning of Mozzarella cheese during high temperature pizza baking, J. Dairy Sci.77 (1994) 2850–2853.

[79] Mazerolles G., Devaux M.F., Duboz G., Duployer M.H, Mouhous Riou N., and Dufour E., Infrared and fluorescence spectroscopy for monitoring protein structure and interaction during cheese ripening, Lait 81 (2001) 509–527.

[80] McMahon D.J., Measuring Stretch of Mozzarella Cheese, in Proc.12th Biennial Cheese Ind. Conf., Utah State University, Logan, 1996, Page 19.

[81] Mounsey J.S., and O'Riordan E.D., Empirical and dynamic rheological data correlation to characterize melt characteristics of imitation cheese, J. Food Sci. 64 (1999) 701-703.

[82] Muthukumarappan K., Wang Y.-C., and Gunasekaran S., Short communication: modified Schreiber test for evaluation of Mozzarella cheese meltability, J. Dairy Sci. 82 (1999) 1068–1071.

[83] Muthukumarappan K.Y., Wang Y.-C., and Gunasekaran, S., Estimating Softening Point of Cheeses, J. Dairy Sci. 82 (1999) 2280-2286.

[84] Noël Y., Zannoni M., Hunter E.A., Texture of Parmigiano Reggiano cheese: statistical relationships between rheological and sensory varieties, Lait 76 (1996) 243–254.

[85] Noël Y., Ardö Y., Pochet S., Hunter E.A., Lavanchy P., Luginbuhl W., Le Bars D., Polychroniadou A., Pellegrino L., Characterisation of protected denomination of origin cheeses: relationships between sensory texture and instrumental data, Lait 78 (1998) 569–588.

[86] Nolan E. J., Holsinger V.H., and Shieh J.J., Dynamic rheological properties of natural and imitation Mozzarella cheese, J. Texture Stud. 20 (1989)179–189.

[87] Oberg C.J., Merrill R.K., Moyes L.V., Brown R.J., and Richardson G.H., Effects of *Lactobacillus helveticus* culture on physical properties of Mozzarella cheese, J. Dairy Sci. 74 (1991) 4101–4107.

[88] Olson N.F., Gunasekaran S., and Bogenrief D.D., Chemical and physical properties of cheese and their interactions, Neth. Milk Dairy J. 50 (1996) 279-294.

[89] Olson N.F., and Price W.V., A melting test for pasteurized process cheese spread, J. Dairy Sci. 41 (1958) 999–1000.

[90] Olson N.F., and Nelson D.L., A new method to test the stretchability of Mozzarella cheese on pizza, Proc. 17th Ann. Marschall Invit. Ital., Cheese Sem, 1980.

[91] Pagliarini E., and Beatrice N., Sensory and rheological properties of low-fat filled pasta filata cheese, J. Dairy Res. 61 (1994) 299-304.

[92] Park J., Rosenau J.R., and Peleg M., Comparison of four procedures of cheese meltability evaluation, J. Food Sci. 49 (1984) 158–1162.

[93] Peleg M., Texture profile analysis parameters obtained by an Instron universal testing machine, Journal of Food Sci. 41 (1976) 721-722.

[94] Pilcher S.W., and Kindstedt, P.S., Survey of Mozzarella cheese quality *at* restaurant end use, J. Dairy Sci. 73 (1990) 1644.

[95] Prow L.A., Development of a melt test for process cheese spread and process cheese product using the rapid visco analyzer, M.S. Thesis, University of Minnesota, St. Paul , 2004.

[96] Prow L.A., and Metzger L.E., Melt analysis of process cheese spread or product using a rapid visco analyser, J. Dairy Sci. 88 (2005)1277–1287

[97] Rayan A.A., Kalab M., and Enstrom C.A., Microstructure of process cheese. Scan. Electron micros 111(1980) 635-643.

[98] Rippe J.K., and Kindstedt P.S., Preliminary evaluation of objective methods for measuring rheological properties of melted Mozzarella cheese, J. Dairy Sci. 71 (1988) 69.

[99] Rowney M., Roupas P., Hicky M.W., and Everett D.W., Factors affecting the functionality of Mozzarella cheese, Aust. J. Dairy Technol 54 (1999) 94-102.

[100] Ruegg M., Eberhard P., Popplewell L.M., and Peleg M., Melting properties of cheese. Bull. 268, Int. Dairy Fed., Brussels, Belgium, pages 36–43(1991).

[101] Ruegg M., and Moor U., Softening and dropping point temperatures of semi-hard and hard cheese varieties, Schweiz. Milchwirtsch. Forsch 17 (1988)69–71.

[102] Ruden M.A., Barbano D.M., A model of Mozzarella cheese melting and browning during pizza baking, J. Dairy science, 81 (1998) 2312-2319.

[103] Sørensen L.K., and Jepsen R., Assessment of sensory properties of cheese by near-infrared spectroscopy, Int. Dairy J. 8 (1998) 863–871.

[104] Smith C.E., Rosenau J.R., and Peleg M., Evaluation of the flowability of melted Mozzarella cheese by capillary rheometry, J. Food Sci. 45 (1980)1142–1145.

[105] Sutheerawattananonda M., and Bastian E.D., Monitoring process cheese meltability using dynamic stress rheometry, J. of Texture Studies 29 (1998) 169-183.

[106] Thomas M.A., Browning reaction in Mozzarella cheese, Australian journal of Dairy Technology, 24 (1969) 49-53.

[107] Tunick M.H., Nolan E.J., Shieh J.J., Basch J.J., Thompson M.P., Maleeff B.E., and Holsinger V.H., Mozzarella and Cheshire cheese rheology, J. Dairy Sci. 73 (1990)1671–1675.

[108] Tunick M.H., Mackey K.L., Smith P.W., and Holsinger V.H., Effects of composition and storage on the texture of Mozzarella cheese, Neth. Milk Dairy J. 45 (1991)117-125.

[109] Tunick M.H., Malin E.L., Smith P.W., Shieh J.J., Sullivan B.C., Mackey K.L., and Holsinger V.H., Proteolysis and rheology of low-fat and full-fat Mozzarella cheeses prepared from homogenized milk, J. Dairy Sci. 76 (1993) 3621-3628.

[110] Ustunol Z., Kawachi K., and Steffe J., Arnott test correlates with dynamic rheological properties for determining Mozzarella cheese meltability, J. Food Sci. 59 (1994) 970–971.

[111] Wang Y.-C., Muthukumarappan K., Ak M.M., and Gunasekaran S., A device for evaluating melt/flow characteristics of cheeses, J. Text. Stud 29 (1998) 43–55.

[112]Wang H.H., and Sun D.W., Correlation between cheese meltability determined with a computer vision method and with Arnott and Schreiber tests, J. Food Sci, 67(2002) 745-749.

[113] Wang H.H., and Sun D.W., Assessment of cheese browning affected by baking condition using computer vision, J. Food Engineering, 56 (2003) 339-345.

[114] Wang H.H., and Sun D.W., Evaluation of the oiling-off property of cheese with computer vision: influence of cooking conditions and sample dimensions, J. Food Engineering, 61 (2004) 57-66.

[115] Zhou N., and Mulvaney S.J., The effect of milk, fat the ratio of casein to water and temperature on the viscoelastic properties of rennet casein gels, J. Dairy Sci. 81 (1998) 2561-2571.

Table 1 Major functional attributes of heated cheese used as an ingredient.

	Definition	*Application*	**Examples of cheese-based ingredient**	*Measurement techniques*
Meltability	Tendency of cheese to soften on heating.	All cooked dishes Pizza pie. Toasted sandwiches Pasta sauces	Mozzarella Cheddar Raclette	**Empirical**: Measuring dimensional changes (diameter, height, melt area, distance of flow) in the cheese samples upon heating. Such as Arnott test and Schreiber test. **Objective:** Measuring magnitude changes in Storage modulus (G) or loss modulus (G") on heating that means measure viscosity, viscoelasticity, dropping and softening points such as Dynamic stress rheometry, Melt profile analysis, Rapid visco analyzer .
Stretchability	Tendency of heated cheese to form strings when extended uniaxially.	Pizza pie Pasta dishes.	Mozzarella Cheddar Provolone Swiss-type	**Empirical:** Measuring the length of strings of heated cheese at failure when applied a force. Such as fork test **Objective**: Measuring the applied force or stress and the failure deformation such as Helical viscometry and vertical elongation.
Oiling-off	Tendency of heated cheese to exude oil on heating.	Cheesecake Home-made desserts.	Most cheeses apart from low-fat types or those made from homogenised milks.	**Empirical:** Measuring quantity of separation of oil from melted cheese body into oil pockets. **Objective:** Free oil area by computer vision.
Browning	Tendency of cheese to becomes darker in colour during processing or baking.	All cooked dishes Pizza pie Macaroni cheese sauce	All natural cheeses.	Visual descriptive analysis**,** colorimeters methods, computer vision

Table 2 Examples of studies on the relation between instrumental and sensory data.

Instrumental type	Cheese type	Sensory attributes	Correlation type	Reference
Compressive tests (TPA)	Cheese of different texture	Hardness, springiness, adhesiveness	Good correlation	Lee et al. [69]
Texture Profile Analyzer (TPA)	Cream cheese	Hardness,cohesiveness adhesiveness	Good relationships for hardness, adhesiveness, less satisfactory for cohesiveness	Kealy [58]
Compressive, small strain rheological tests	Processed cheese	Mouth and hand firmness, smoothness, rubberiness, brittlenes	Compressive test were better able to predict sensory texture than rheological tests.	Drake et al. [26]
fluorescence spectroscopy, Rheological tests	Semi-hard cheese	Firmness/elasticity, microstructure/humidity, firmness/humidity, microstructure/adhesivity, adhesivity/humidity	Strong correlations	Lebecque et al. [70]
Near-infrared spectroscopy	Emmental cheeses	Adhesivity, elasticity, firmness, aroma, odour intensity, bitterness, saltiness, acidity sweetness	High correlation	Karoui et al. [58]
Near-infrared spectroscopy	Experimental Cheddar cheeses	Crumbly, rubbery,chewy, melting, greasy/oily,mouthcoating massforming.	Satisfactory relationship	Downey et al. [24]
Mid-infred spectroscopy	Experminatal processed cheeses	firmness, rubbery, creamy, chewy, mouth-coating,fragmentable, melting, mass-forming)	Good correlation.	Fagan et al. [34]

METHODOLOGIE, RESULTATS

DISCUSSION

I. Caractérisation des 4 catégories de fromages à pâte persillée

Ce paragraphe est présenté sous forme d'un article « Characterization of physicochemical, textural, sensorial properties of different Blue-veined cheeses » Khaled ABBAS, Cécile BORD, Shaïmaa OTHMAN, Abderrahmane AÏT KADDOUR, Annick LEBECQUE (Article n°2 non publié). L'article englobe une partie des résultats présentés ci-dessous.

I.1. Objectifs et méthodologie

I.1.1. Objectifs

Ce chapitre présente les caractéristiques physicochimiques et sensorielles des fromages à pâte persillée étudiés et certaines propriétés de texture à froid. L'objectif de ce chapitre est de définir les propriétés à froid de ces fromages dans la perspective de les comparer à celles mesurées après un traitement thermique. Toutefois certaines propriétés fonctionnelles à chaud sont également présentées dans ce chapitre telles que les capacités à chaud de gratiner, d'exsudation et d'étalement ainsi que les températures de ramollissement et de goutte.
Les quatre types de fromages AOP à base de lait de vache ont été choisis pour leurs diversités organoleptiques et de fabrication (voir revue bibliographique). Notre choix s'est porté sur les fromages à pâte persillée car très peu d'informations dans la littérature scientifique existent bien que les pâtes persillées soient utilisées comme ingrédient par les industriels.

I.1.2. Echantillons de fromages

Quatre types différents de fromages à pâte persillée du commerce ont été étudiés. Ces fromages ont été sélectionnés pour couvrir une large gamme de structure et de texture des fromages à pâte persillée. Ils représentent différents types de procédés de fabrication. Les caractéristiques technologiques et les codes pour les différents types de fromage sont présentés dans le Tableau n°9. Ces types de fromages répondent à un cahier des charges précisant certaines de leurs caractéristiques.

Pour cette étude exploratoire, 20 fromages (tableau n°9) ont été prélevés au stade de la commercialisation, ils sont issus de dix entreprises différentes (8 fourmes d'Ambert, 4 fourmes de Montbrison, 4 bleus des Causses et 4 bleus d'Auvergne). Ces fromages présentent une large diversité de fabrication tant au niveau du traitement du lait (cru, thermisé, pasteurisé) qu'à celui du temps d'affinage (30 à 45 jours, au-delà des temps d'affinage préconisés par les cahiers des charges AOP et à des temps de conservation variés). Les échantillons d'une même référence sont issus du même lot. Les échantillons ont été conditionnés, surgelés immédiatement puis stockés à -20°C et conservés jusqu'à leur analyse. Les échantillons ont été décongelés en chambre froide à +3°C (environ 13h, fonction de la taille de l'échantillon).

I.1.3. Méthodes

I.1.3.1. Analyses physico-chimiques

La détermination de la composition physico-chimique du fromage à pâte persillée a été faite suivant les normes internationales ou françaises ou selon des méthodes internes au laboratoire. Toutes les analyses ont été faites en triple.

Les analyses qui ont été réalisées sont la matière sèche (NF EN ISO 5534), la matière grasse (NF V04-287), l'azote total et soluble (NF EN ISO 8968-1), les minéraux totaux (cendres), les teneurs en sodium et calcium (NF ISO 8070), la teneur en chlorure (NF EN ISO 5943), la teneur en phosphore (protocole interne). Le pH a été mesuré à l'aide d'un pH mètre (Schott CG840, Paris, France) après avoir dispersé 10 g de fromage râpé dans 50 ml d'eau distillée à 20°C. L'ensemble des résultats pour les 20 fromages est présenté dans le tableau n°10.

I.1.3.2. Analyses sensorielles des fromages à froid

Panel : Un groupe de 11 sujets qualifiés (11 femmes âgées de 30 à 70 ans) a été sélectionné et entraîné selon la norme ISO 8586-1 pour l'évaluation des descripteurs sensoriels des échantillons des fromages à pâtes persillées à froid. La mise en place de la grille sensorielle et l'entraînement des sujets ont été conduits selon les recommandations de la norme ISO 11035. La performance du panel a été vérifiée avec les échantillons expérimentaux sur 3 répétitions. Les panélistes ont évalué au maximum 8 échantillons de fromages par session (une heure et demi), chaque fromage a été évalué 3 fois.

Préparation des échantillons : Les échantillons de fromage ont été coupés en morceaux d'environ 20 g (5x2x3cm) et répartis dans des assiettes codées avec trois chiffres aléatoires. Ce code a été modifié entre chaque séance d'évaluation. Les échantillons coupés ont été maintenus à température de dégustation (15 ± 1°C) et présentés aux panélistes selon un plan d'expérience préétablis avec une présentation monadique séquentielle des échantillons.

Modalités d'évaluation : Les membres du panel évaluent les fromages pour leurs propriétés sensorielles en utilisant une échelle continue de 10 points. Des références ont été données aux panélistes lors des entrainements afin de minimiser la variabilité entre individus (tableau n°11).
Les évaluations ont été effectuées dans un laboratoire sensoriel (VetAgro Sup de Clermont) avec des cabines standardisées, informatisées. Les données ont été enregistrées et stockées en utilisant le logiciel Tastel (ABT Informatique, Paris, France).

I.1.3.3. Rhéologie des fromages à froid : test de compression dynamique

Les propriétés de texture des fromages à froid ont été mesurées à l'aide d'un rhéomètre (CP 20, TA Instrument, Guyancourt, France) équipé d'une plaque de 20 mm de diamètre et d'un système à effet Peltier. Les mesures ont été pratiquées sur 3 échantillons par référence (2x20mm) à une température contrôlée de 20°C. Les composants G', G'', tan δ ont été déterminés dans une région de viscosité linéaire en appliquant une force constante de 0.1N et une fréquence de 1HZ. Toutes les analyses ont été répétées 3 fois. (tableau n°12)

I.1.3.4. Point de ramollissement et point de goutte

Les points de ramollissement et de goutte des différents fromages ont été mesurés de façon automatique (Mettler-Toledo FP 800). Le protocole suivi est celui proposé par P. Eberhard (1986). Le point de ramollissement est la température à laquelle l'échantillon chauffé lentement se ramollit jusqu'à s'écouler sur une longueur de 20 mm de l'orifice de la cupule dont le diamètre est de 6.35 mm. Le point de goutte est la température à laquelle une première goutte d'échantillon tombe de l'orifice de 2.8 mm de diamètre de la cupule normalisée, dite « point de goutte », dans laquelle l'échantillon est lentement chauffé. La température s'élève de 30°C à 100°C à une vitesse de 2°C/mn. 3 mesures ont été effectuées sur chaque référence de fromage (tableau n°13).

I.1.3.5. Test de Schreiber : étalement du fromage et exsudation de la matière grasse

Le test de Schreiber est une méthode empirique qui a pour principe d'évaluer, à l'aide de la mesure du diamètre ou de la surface, l'étalement du fromage et l'exsudation de la matière grasse d'un échantillon circulaire de fromage posé sur un support et chauffé.
Pour cela, des cylindres de 20 mm de hauteur et de 20 mm de diamètre ont été découpés dans les fromages puis déposés sur des patrons comprenant 4 séries de cercles concentriques distants de 10 mm les uns des autres. Ces cercles sont séparés en 6 axes gradués permettant la mesure de l'étalement et de l'exsudation de matière grasse. La moyenne des 6 axes est prise en compte. Les mesures ont été faites dans un four à chaleur sèche dans 3 conditions de chauffage : 80°C/15mn, 120°C/7mn, 250°C/4mn. La température de 80°C est légèrement supérieure à celle du point de ramollissement de la matrice, la température de 250°C est celle qui est couramment préconisée dans la préparation des plats cuisinés tels que les pizzas, 120°C est une température intermédiaire.

I.1.3.6. Analyse de la couleur par colorimétrie : gratinage

Le gratinage n'a pas été mesuré sur les fromages râpés tel que les pâtes pressées sont couramment utilisées. Le gratinage a été mesuré sur des échantillons de fromages entiers, non râpées pour se rapprocher de la perception du consommateur lors d'utilisation de ces fromages non déstructurés sur des plats préparés tels que les pizzas (les morceaux peuvent être de tailles différentes dans le commerce). La couleur a été mesurée sur les pâtes persillées à chaud selon 3 températures de chauffage différentes : 80-120-250°C. La colorimétrie est l'une des méthodes utilisées pour quantifier les couleurs et les exprimer par le biais de données numériques conformes aux normes internationales (R. Richoux, 2001). L'espace de couleur* L*a*b* est actuellement l'un des plus utilisé pour mesurer la couleur des objets dans pratiquement tous les domaines. Dans cet espace couleur, L* indique la clarté, tandis que a* et b* sont les données de chromacité : +a* va vers le rouge et -a* va vers le vert ; +b* va vers le jaune et –b* va vers le bleu. Le chromamètre Minolta CR-400 est un analyseur de couleur composé d'une tête de mesure qui fournit des résultats moyens sur une zone circulaire de 50mm de diamètre. La lumière réfléchie par l'objet passe devant des capteurs munis de filtres reproduisant une perception des couleurs voisine de celle de l'œil humain. Les données fournies par les capteurs sont ensuite analysées par un processeur qui calcule les valeurs tristimuli. Des tranches de fromage de 1cm d'épaisseur ont été découpées et disposées dans des moules à tartelette de 10 cm de diamètre. Après chauffage aux 3 conditions définies, 5 mesures colorimétriques par tranche sont nécessaires pour être représentatives de la surface de la tranche avec une variabilité acceptable.

I.2. Résultats, discussion

I.2.1. Composition physicochimique des fromages

Les échantillons se différencient significativement sur les taux de matière grasse, d'azote soluble, de cendre et de minéraux (tableau n°3, article n°2). Les Bleus se distinguent nettement de la catégorie « fourme » par leur rapport azote soluble/azote total : 66,6% et 62,2% contre 44,0% et 34,1% pour respectivement les bleu d'Auvergne, bleu des Causses, Fourme d'Ambert, fourme de Montbrison.

Une analyse en composante principale (figure n°1 article n°2) indique que ces 4 catégories de fromages se différencient principalement sur l'axe 1 (61,4% de la variation totale) sur les teneurs en protéine et calcium en opposition avec les valeurs de pH, azote soluble, sodium. L'axe 2 (34,51% de la variation totale) est un axe explicatif de la variabilité en matière grasse et humidité qui sont opposées. Cependant les 4 catégories se distinguent bien les unes des autres avec toutefois une proximité de composition entre la fourme d'Ambert et le Bleu d'Auvergne.

La fourme de Montbrison se distingue nettement des autres par son taux de protéine plus élevé et une quantité d'azote soluble faible. Son rapport N soluble/N total est de loin le plus faible (34,1%). Les taux de calcium et de phosphore sont les plus élevés des 4 catégories. Ces éléments laissent penser que ces fourmes de Montbrison sont moins protéolysées que les autres, certainement dû à une action moindre des souches de Penicillium et en raison du type de fabrication par léger pressage donnant une pâte peu oxygénée. Le taux de sodium des FM est le plus bas des 4 catégories. Ces fromages se distinguent nettement des autres sur l'ACP et particulièrement sur l'axe 1 expliquant les différences dues aux valeurs en protéines, calcium et à l'opposé les faibles valeurs en sodium, chlorure, azote soluble et pH.

Le Bleu des Causses se distingue par son fort taux de matière grasse ce qui le différencie nettement sur l'axe2 de l'ACP, il possède également des taux élevés d'azote soluble, de cendres et de sodium mais son taux d'humidité est le plus faible bien que pas significativement différent des autres.

Le Bleu d'Auvergne et la Fourme d'Ambert sont identifiables par une humidité plus élevée que les autres. Ils se différencient entre eux par un taux d'azote soluble, de matière grasse et de phosphore plus élevé pour le Bleu d'Auvergne. La Fourme d'Ambert a le taux de matière grasse le plus faible de tous les fromages.

I.2.2. Caractéristiques sensorielles des fromages à froid

26 descripteurs sensoriels ont été générés par le jury pour décrire les 20 fromages (tableau n°15) dont :

- *aspect* : 6, couleur de la pâte, humidité, brouille, quantité de persillage, répartition du persillage, quantités de cavités
- *Odeur* : 4, intensité globale, ammoniac, lactique, moisi
- *Saveur* : 3, salé, acide, amer
- *Arôme* : 6, intensité globale, lactique, moisi, ammoniac, piquant, persistance aromatique
- *Texture* : 7, ferme au doigt et en bouche, crémeux, crayeux, granuleux, collant, résidus

Une analyse de variance à deux facteurs réalisée sur les données a permis de mettre en évidence des différences entre les fromages (tableau n°15). Les produits sont significativement différents pour tous les descripteurs de la grille d'analyse. Une comparaison de moyenne 2 à 2 (test de fischer) a permis de préciser la nature des différences entre produit (tableau non présenté).

L'analyse en composantes principales confirme l'opposition entre les fourmes et les bleus (figure n°15). L'axe 1 (42% de la variance totale) représente un axe de flaveur, il se définit par les descripteurs suivants : amère, salée, intensité de l'arôme, arôme d'ammoniaque. L'axe 2 (13% de la variance totale) est un axe relié à la texture. Les descripteurs qui contribuent à cet axe sont les suivants : ferme au doigt et en bouche, crayeuse, granuleuse, collant et présence de résidus. Quant au troisième axe, il est spécifique à l'acidité (saveur acide, flaveur lactique) mais également à l'aspect (couleur de la pâte, quantité et répartition du persillage).

Globalement, la texture et les flaveurs semblent être les caractéristiques principales de différenciation entre les bleus et les fourmes. Toutefois, au sein de la catégorie des bleus des Causses, un produit semble se détacher, le BC 014. Il se démarque par une flaveur très prononcée, marquée par une flaveur d'ammoniaque, une sensation piquante et une flaveur persistante. Il en est de même pour le fromage bleu d'Auvergne BA 011, qui se définit particulièrement par son acidité.

Par ailleurs, au sein de la catégorie des fourmes, il semble également exister une diversité sensorielle. En effet, les fourmes FA 12, FA 013, FA 01 et FA 017 sont fermes au doigt et en bouche, sont granuleuses, crayeuses et présentent des résidus. Par contre, les fourmes FA 18, FA 02, FA 16 et FA 15 sont plus persillées.

Les 20 fromages semblent très différents entre eux avec une disparité plus prononcée pour les fourmes d'Ambert que pour les « bleus ».

Globalement les « bleus » sont des fromages avec une puissance aromatique et des propriétés de flaveur plus intenses que les fourmes : notes ammoniaquées, saveur salée et amère, texture crémeuse. En revanche, les fourmes se démarquent plus particulièrement par leur texture ferme et crayeuse en bouche avec des arômes et des saveurs moins prononcés.

L'article n°2 analyse de façon plus précise l'évaluation de la texture de ces fromages (figure n°4, article n°2). Les bleus se distinguent par leur texture crémeuse surtout pour le bleu des Causses et leur texture collante en bouche. A l'opposé se trouve la fourme de Montbrison avec sa texture crayeuse, granuleuse et ferme. La fourme d'Ambert a une texture intermédiaire mais avec une tendance un peu plus collante que la fourme de Montbrison.

I.2.3. Rhéologie des fromages à froid

Les paramètres rhéologiques sont présentés dans le tableau n°4, article n°2. Le module G' est un paramètre important pour un échantillon de prédominance élastique ou fortement structuré. La valeur de G'' est importante pour un échantillon à prédominance visqueuse. Les bleus ont un G' nettement plus important que les fourmes, ceux sont des fromages avec une pâte plutôt à tendance élastique avec une composante visqueuse non négligeable (G'') et une viscoélasticité plus faible que les fourmes.

Les pâtes de la catégorie des bleus sont très peu élastiques et plus coulantes que celles de fourme. L'analyse en composantes principales sur les moyennes positionne les fromages les uns par rapport aux autres. Les bleus des Causses se distinguent nettement des Fourmes qui ont un comportement textural assez proche à froid.

I.2.4. Points de ramollissement et de goutte

Ces types de résultats (tableau n°4, article n°2) nous donnent des indications sur la résistance de la matrice fromagère à la chaleur et la fonte de la matière grasse. La fourme de Montbrison se différencie significativement des autres fromages avec un temps de ramollissement de la matrice fromagère et de goutte de la matière grasse plus élevé que les autres. Par ailleurs, on observe très peu de différences sur les températures de ces 2 mesures qui tournent autour de 65°C. Les points de ramollissement de la matrice des BA, FA, BC ne sont pas significativement différents et oscillent entre environ 49,6 et 53,4°C en accord avec les observations de Lucey (2008) qui précise que le point de ramollissement de la plupart des fromages se situe au-delà de 40°C.

La fermeté de la fourme de Montbrison pourrait s'expliquer par son taux en protéine et calcium plus élevé, un pH plus bas que les autres, par une pâte et un réseau plus ferme dû probablement aux étapes de fabrication (petit pressage). Les autres fromages ont un réseau beaucoup plus lâche dû au type de fabrication mais aussi à des taux d'azote soluble plus élevés. Cependant, la température de ramollissement la plus base est celle des bleus d'Auvergne (49,6°C ± 2,3). Par comparaison, la fonte de la matrice d'emmental se fait entre 60 et 70°C (Famelard, 2002).

I.2.5. Etalement de la matrice fromagère et fonte des matières grasses

Le test de Schreiber permet d'observer les capacités des fromages à s'étaler au cours de la montée en température et de visualiser également l'exsudation de matière grasse qui peut être un facteur protecteur face à la dessiccation mais également un point négatif aux yeux des consommateurs si elle est en excès (tableaux N°16, 17, figures n°16, 17). Les coefficients de variation montrent une très grande variabilité dans les résultats qui est due à la diversité des échantillons mais également à la faible répétabilité du test. Une analyse de variance à deux facteurs a été réalisée sur les 2 variables, elle montre une différence significative entre les 4 catégories : BC>FA>FM>BA (non présentée). Au terme du chauffage (250°C), l'ensemble des fromages double au moins leur rayon initial (10mm) en étalement hormis les BA qui présentent un rayon d'atélément plus faible (2/3 du rayon initial en moyenne). Les distances d'étalement à 250°C varient de 6,67 mm à 12,75 mm. En comparaison avec les travaux de Reparet (2000) sur différents fromages à pâtes pressées cuites et non cuites, les distances d'étalement sont de l'ordre de 8 mm pour le Cantal, 13 mm pour l'Emmental et 18 mm pour le Beaufort. Le facteur multiplication de l'exsudation des matières grasses est de 1,5 à 2 par rapport à leur rayon initial hormis pour les BA qui ne font que doubler le rayon initial du fromage.

Si l'on décrit chaque fromage, on peut observer que :

- le BA s'étale peu et exsude peu même à des hautes températures,
- la FA s'étale dès 80°C et amplifie son étalement au-delà de 120°C pour rejoindre un étalement supérieur à la FM. De la même façon, elle exsude dès 80°C avec une amplification nette à des températures supérieures à 120°C mais pas autant que le BC,
- la FM s'étale et exsude dès les basses températures inférieures à 80°C comme le BC mais son étalement et son exsudation n'évoluent quasiment pas avec l'élévation de température,
- le BC s'étale largement dès les basses températures avec un développement d'étalement très important au-delà de 120°C. Il exsude dès les basses températures et continue son exsudation jusqu'à 250°C, c'est le plus « coulant » des fromages

En conclusion, dans tous les cas, l'exsudation de gras est plus importante que l'étalement de la matrice fromagère, ce qui signifie que ces fromages seront protégés du dessèchement dans les conditions appliquées mais il y a de forte chance que ces fromages ne gratinent pas.

Par ailleurs, le BC est le fromage qui a le meilleur étalement quelle que soit la température mais il libère également une grande quantité de gras. La FM est un fromage qui s'étale et exsude également bien dès les basses températures mais ces 2 paramètres n'évoluent plus vraiment aux températures supérieures. La FA est un fromage qui nécessite d'appliquer des températures supérieures à 120°C pour développer un étalement et une exsudation supérieures à celles de la FM mais inférieures au BC. Les 2 fourmes atteignent des valeurs d'étalement et d'exsudation légèrement différentes mais c'est surtout le comportement de la matrice fromagère lors des variations de température qui différencie ces 2 catégories de fromages. Le BA n'a pas une forte propension à s'étaler et ni même à exsuder.

I.2.6. Développement de la couleur au cours du chauffage

Les résultats révèlent pour l'indice L*, une différence de luminosité en fonction des températures (figure n°18). En effet, à 80°C les fromages sont plus lumineux qu'à 120 °C et 250°C, du au dessèchement lors du chauffage. On observe également des différences entre les catégories de fromages : la FM et le BC ont tendance à être moins lumineux que la FA et le BA. Cependant on ne peut pas parler de brunissement après cuisson. Contrairement à l'emmental, qui gratine à partir d'une certaine température, chez les pâtes persillées, il n'y a pas de réaction de Maillard produite (Richoux, 2001). En effet les pâtes persillées libèrent une forte quantité de matière grasse lors de leur cuisson, celle-ci joue un rôle protecteur pour le fromage en limitant le gratinage. Les Bleus ne pourront donc pas être cuisinés dans le but d'être gratinés.

En ce qui concerne la chromacité, la FA a plus tendance que les autres à aller vers le -a*, c'est-à-dire vers le vert. La couleur évolue également selon les températures, à 250°C la FM, le BA et le BC ont des valeurs pour a* et b* proches, ce qui traduit une uniformisation du persillage du fromage à cette température. Les FM à froid ayant tendance à être de couleur jaune, on aurait pu supposer que leur b* serait le plus élevé, mais ce n'est pas le cas.

Une ACP sur ces données (non présentée), a permis d'observer des différences entre les fromages. L'un d'eux se distingue fortement : il s'agit de la fourme de Montbrison fermière au lait cru, qui possède un persillage très prononcé. La diversité des fromages a pu être mise en évidence. Elle est due essentiellement à la quantité et à la répartition du persillage qui peut dépendre notamment de la souche utilisée de *Penicillium roqueforti*.

I.2.7. Corrélation entre les données à froid

L'ensemble des corrélations entre les données ont été étudiées dans l'article n°2. Les mesures rhéologiques Tan δ de viscoélasticité sont corrélées positivement avec l'azote soluble et négativement avec les taux de protéines.

Les mesures sensorielles de texture des fromages sont corrélées positivement avec les teneurs en matières grasses, protéines, calcium, phosphore et plus particulièrement le crémeux avec le pH, la matière grasse et l'azote soluble. Ces éléments confirment que certaines perceptions telles que le crémeux ne sont pas liées uniquement à la matière grasse mais bien à un ensemble de composants qui peuvent relever de la technologie de fabrication mais également de l'affinage de ces fromages.

De même, la fermeté au doigt, en bouche et le crayeux sont positivement corrélés aux paramètres rhéologiques tels que G' et G'' et négativement avec le crémeux.

Par contre, les attributs sensoriels « granuleux, collant, résidu » ne sont corrélés à aucun paramètre rhéologique.

Les données physicochimiques et rhéologiques sont donc insuffisantes pour décrire la perception des consommateurs des fromages à froid. Elles peuvent permettre de faire un tri préalable sur certaines propriétés mécaniques (fermeté, viscosité, collant) mais pas sur des propriétés géométriques (granuleux).

I.3. Conclusion

Ces premiers éléments ont permis de caractériser les 4 catégories de fromages.

Les *fourmes de Montbrison* étudiées sont des fromages qui ont un taux important de protéine mais peu d'azote soluble, un taux de calcium et de phosphore élevé, c'est le fromage le moins salé. Ces éléments laissent à penser que ce type de fromage est moins protéolysé que les autres. Sa texture est ferme, granuleuse, crayeuse. Les mesures rhéologiques à froid montrent que les FM ont une texture plutôt à tendance élastique.

Du point de vue de ses qualités à chaud, c'est un fromage qui s'étale et exsude très tôt lors des traitements thermiques à des températures inférieures à 80°C mais pas de façon excessive. Ces fromages ne gratinent pas.

Les *fourmes d'Ambert* étudiées se caractérisent par son taux d'humidité plus élevé que les autres mais c'est également la catégorie la moins grasse. Comme toutes les fourmes sont rapport N soluble/protéine est plus bas que celui des Bleus. Les taux de calcium et de phosphore sont élevés comparés aux autres. Sa texture ressemble à celle des FM : ferme, crayeuse, granuleuse avec une tendance plus collante. Cette catégorie possède une texture à tendance élastique avec toutefois une composante visqueuse plus importante que les FM.

Du point de vue de ses qualités à chaud, c'est un fromage qui s'étale et exsude très tôt lors des traitements thermiques à des températures inférieures à 80°C mais ce phénomène s'amplifie dès 120°C. Ces fromages ne gratinent pas.

Les *Bleus d'Auvergne* étudiés se caractérisent par un fort taux d'azote soluble soit un rapport N soluble/protéine le plus élevé. Sa texture est crémeuse et collante en bouche. Du point de vue rhéologique, cette catégorie est plus marquée par sa tendance visqueuse comparée aux fourmes.

Du point de vue de ses qualités à chaud, les BA ne s'étalent quasiment pas et exsudent peu par rapport aux autres catégories même à des hautes températures. Ces fromages ne gratinent pas.

Les *Bleus des causses* sont atypiques. Ils se distinguent par un fort taux de matière grasse, un taux élevé d'azote soluble, de cendres et de sodium. Leur texture est crémeuse et collante en bouche. Leur viscoélasticité est la plus importante de toutes les catégories, les autres composantes rhéologiques sont les plus basses.

Du point de vue de ses qualités à chaud, c'est une catégorie de fromage qui s'étale et exsude très tôt lors des traitements thermiques à des températures inférieures à 80°C et ce phénomène s'amplifie dès 120°C. Son exsudation en matières grasses est très importante ce qui peut devenir un défaut aux yeux des consommateurs. Ces fromages ne gratinent pas.

L'ensemble de ces analyses est complémentaire. L'analyse sensorielle décrit les perceptions des consommateurs et permet de comparer les fromages entre eux. La rhéologie décrit bien des descripteurs de texture tels que la fermeté, le crayeux, le crémeux. Bien que des corrélations ont été mises en évidence, certains attributs sensoriels tels que « granuleux, collant, résidu » ne sont pas corrélés aux mesures instrumentales étudiées.

ARTICLE N°2: CHARACTERIZATION OF PHYSICOCHEMICAL, TEXTURAL AND SENSORIAL PROPERTIES OF DIFFERENT BLUE-VEINED CHEESES

Khaled ABBAS, Cécile BORD, Shaimaa OTHMAN, Abderrahmane AIT KADDOUR, Annick LEBECQUE*

U.R CALITYSS, VetAgro Sup, Campus agronomique de Clermont,

89 av. de l'Europe -BP 35- 63370 Lempdes, France.

*Corresponding author: annick.lebecque@vetagro-sup.fr

Tel: 0033.(0)4.73.98.13.41

INTRODUCTION

Cheese quality is measured either by instrumental analysis (physical, chemical) or by sensory analysis. The correlations between sensory and instrumental analysis are necessary to determine which compounds are responsible for sensory characteristics, and show how the differences in the gross composition could modify the texture and the structure of cheese. Thus, it is useful to relate chemical or physical data and sensory analysis to efficiently understand the quality of cheese and to provide guidance to the cheese manufacturers. The textural properties of cheese are widely recognized as a determinant of overall quality and consumer acceptance of cheeses and texture is an important characteristic used to differentiate many cheese varieties.

France is ranked third in the world for cheese production (approximately 1850,000 t/yr; Barry & Tamine, 2010). It exists a very large diversity of traditional cheeses, 42 of which have a Protected Denomination of Origin (PDO) status. Traditional cheeses represent a cultural heritage and the result of accumulated empirical knowledge passed from generation to generation. Protected Denomination of Origin (PDO) cheeses often represent a large variety of textures and tastes. Thus, there is an increasing need for characterization of PDO cheeses, including cheese composition, and physical properties. For this reason, some research groups have carried out studies on chemical, physical, and sensory characteristics of different cheese varieties (Bertola *et al.*, 1992; Lebecque *et al.*, 2001; Romain *et al.*, 2001; Pillonel *et al.*, 2002; Pinho *et al.*, 2004).

Blue cheese is a PDO veined-cheese of the Massif Central area of France and is manufactured from raw cow's milk, according to the specifications of its Denomination of Origin Regulatory Board. Although Blue-veined cheeses are growing in popularity, no research has examined what are the physical (texture and meltability), chemical (pH value, fat, protein, moisture, ash, Na, P, and Ca contents), and sensorial textural characteristics of such cheeses. For this reason, it is interesting to study its characteristics, especially sensory textural attributes.

Therefore, this investigation aimed: **1)** to characterize chemical (pH value, dry matter, protein, fat, Ca, Na, P contents), physical (texture, meltability) and sensorial texture characteristics of different Blue-veined cheeses **2)** to put in evidence the relationships between physicochemical and sensorial texture characteristics of the cheeses investigated. By determination of these relationships, it was hoped to provide a broad understanding of the compositional differences between cheeses, and of how these differences are reflected in specific sensory texture attributes.

MATERIALS AND METHODS

Cheese Blue Samples

Four categories of Blue-veined cheeses were purchased from supermarket located in the Massif Central area of France. Cheeses were selected for their representativeness of the different cheese technologies and represent a wide range of texture. A total 13 cheeses were selected; Fourme d'Ambert (n = 4), Fourme de Montbrison (n= 2), Bleu d'Auvergne (n=3), and Bleu des Causses (n=4) and were made from pasteurized milk. Representative samples were taken from the middle of the cheese height for chemical, physical and sensory analysis.

Physicochemical Analysis

Grated cheese sample were analyzed in triplicate for protein, fat, ash, moisture, Ca, Na, P and water-soluble nitrogen content according to French standards (AFNOR, 2004). The percentage of protein (Total Nitrogen x 6.38) was measured by Kjeldahl method (NF EN ISO 8668-1), fat using a Gerber method (NF V04-287), ash using a dry ash method (NF ISO 8070) and moisture by drying 3 g cheese at 105°C in oven for 24 h (NF EN ISO 5534). Total calcium and sodium in cheeses were measured by using an atomic absorption spectroscopy procedure (NF ISO 8070). Total phosphorus in cheeses was determined colorimetrically (AOAC, 1995). The pH was measured at 20°C by introducing an Ingold electrode (Ingold, France) into some ground cheese (10 g).

Texture Measurement

The evaluation of the cheeses' textural properties (Table 1) was carried out using 3 samples (2 mm thick and 20 mm diameter) by using a rheometer (CP 20, TA Instrument, Guyancourt, France) equipped with plate geometry of 20 mm diameter and a Peltier plate that provided very accurate and rapid temperature control. The elastic component G' (storage modulus), the viscous component G" (loss modulus), Tan δ (G"/G'), and complex viscosity (η*) were determined in the linear viscoelastic region by applying a constant force of 0.1 N and a constant frequency of 1 Hz (force sweep tests indicated that this was within the linear viscoelastic region, results not shown) at 20°C. All analyses were made in triplicate.

Meltability by softening and dropping point

Softening and dropping points were measured in triplicate by the automatic Mettler thermosystem FP 800 system (Mettler–Toledo, Inc (France) which was operated at 30-100°C in 2°C/min according to Eberhard *et al.* (1986). The dropping point corresponds to the temperature at which the first drop of melted sample falls off a nipple with an orifice diameter of 2.8 mm. Softening point of cheese was defined as the temperature at which the first drop started to flow out of a nipple with an orifice diameter of 6.4 mm (Table 1).

Sensory evaluation

Panel and sensory texture attributes

Eleven panelists experienced (females) in the evaluation of dairy products were chosen for the assessment of the 7 descriptors in texture attributes of cheese samples (Table 2). They were asked to evaluate and describe the cheese texture by hand (hand firmness) and mouth (6 descriptors). Panelists were trained for 1 week in 4 sessions of 1.5 h in order to determine the terminology and the definition of the 7 descriptors. Panelists evaluated four cheeses per session and each cheese was evaluated in triplicate. Standard references were given to panelists during evaluations to minimize variability.

Sample preparation

Samples of cheese were cut into pieces about (3 x 3 x 2 cm in size, 15-20g) and placed on white plates coded with three-digit random numbers. The cut samples were tempered by holding at ambient temperature (20 ± 2°C) and then presented to the panelists in a random order for testing. Panel members evaluated cheese for texture (table 2) using a 10-point scale, with 1 being the worst and 10 the best quality. A round-table discussion was then conducted to reach an agreement among the panelists.

Evaluations were conducted in a sensory laboratory (Vetagro-sup) with isolated booths and using normal lighting. Data were recorded and stored using the Stragraphics 3.1

(Statistical Graphics Vorp., Rockville, USA) and Tastel (ABT Informatique, Paris, France) statistical packages. All samples were analysed in triplicate.

Statistical Analysis

Analysis of variance (one-way ANOVA) was performed on the different results obtained to assess the significance of the chemical, rheological, and sensory differences among the samples. The means were compared using Fisher's least significant difference (LSD) test and the statistical significance was determined at $P < 0.05$. Later, the data sets were subsequently analyzed using Principle Components Analysis (PCA). It was performed separately for each group of data (chemical, rheology and sensory). Principal component analysis was used to make visual comparisons of how the data differentiated the cheeses (Bertrand & Scotter, 1992). Finally, the relationships between chemical, rheological and sensory data were also evaluated by CCA (Saporta, 1990). All statistical analyses were performed using XLSTAT software version 2007 (Addinsoft, Paris, France).

Table 1: Definitions of rheological characteristic as measured using dynamic test rheology and indicators of softening and melting characteristics.

Term	Symbol	Information provided
Elastic modulus, storage modulus	G'	Energy stored per deformation storage modulus cycle; solid-like or elastic behavior
Viscous modulus, loss modulus	G"	Energy dissipated per loss modulus deformation cycle; fluid-like or viscous behavior
Complex viscosity	η^*	Viscoelastic flow
Phase angle	Tan δ	Viscoelasticity index
Dropping point	T_{dp}	Defined as temperature at which first drop of melted sample (oil) falls from orifice of a sample holder under defined conditions; used to determine the meltability characteristics
Softening point	T_{Sp}	Defined as temperature at which the changes from a semisolid to an almost free-flowing liquid; used to determine the softening characteristics

Table 2: Definition and terms used in evaluating sensory texture attributes of Blue-veined cheeses investigated.

Texture attributes	Definition	Cheese standards
Hand firmness	The amount of force required for deform the product using a finger	Cantal
First bite firmness (in mouth)	The amount of force required to completely bite through the sample (deformation of the cheese)	Cantal
Chalkiness (crayeuse)	The amount of particulates on tongue after biting	
Creaminess (crémeuse)	Combined perception of fat, smoothness, and viscosity	Boursault
Graininess (Granuluse)	The feeling of coarse particles in the mouth during mastication	Cantal
Adhesiveness (Collant)	The degree to which the chewed mass sticks to mouth surfaces	Vache qui rit
Residues at the end of mastication (Résidus)	The degree of smoothness felt in the mouth after expectorating the sample	Fourme d'Ambert

RESULTS AND DISCUSSION

Physicochemical characteristics of Blue-veined cheeses

Cheese composition (protein, fat, moisture, WSN, pH value, and mineral content) play an important role in determining the rheological, textural and functional properties of cheese (Fox *et al.*, 2000).

Results of the chemical analyses of different Blue-veined cheeses are summarized in Table 3. There was variability in the gross composition of cheeses belonging to the same category. However, there were no significant differences in the pH, moisture and protein content between all the four cheeses investigated. Fat, WSN, ash and mineral salt contents (Na, P, and Ca) were significantly different among the cheese samples.

Moisture content

Moisture is the major component of cheese which acts as a lubricant or plasticizer in the protein matrix thereby making it less elastic and more susceptible to fracture upon compression (Fox *et al.*, 2000). The result with respect to moisture content depicted in Table 3 revealed that no significant differences were observed among the cheese samples.

Protein content

The proteins in cheese are the most important constituent for the texture of the cheese. The mean and standard deviation regarding the protein content of different blue-veined cheeses are shown in Table 3. Insignificant difference existed in the protein content of the investigated cheeses.

Fat content

Fat is known to play an essential role in cheese texture formation due to its plasticizing effect and its ability to inhibit cross-links between the casein chains. It also contributes to the overall flavor quality of cheese and functionality of the cheese. The mean and standard deviation for the fat content of different blue-veined cheese are depicted in Table 3. The fat content of Blue de Causses cheese was significantly (P<0.05) higher (31.23%) than the other cheeses investigated. A significant existence of variation in fat content in cheese might be due to high fat content in the milk used in the manufacture of Blue de Causses. This may be contributed towards higher level of the fat in cheese.

Ash content

The ash content in the foodstuff represents the inorganic matters remaining after the organic matters have been burnt. The results pertaining to analysis of variance for total ash contents are given in Table (3) which indicated that the ash content of Blue cheeses were significantly (P <0.05) higher (4.62%) than the other cheeses.

pH value of cheese

The pH significantly affects the texture and structure of cheese because of its influence on calcium and phosphate solubilization, which results in changes in the protein network. The mean regarding the pH of different cheese are given in Table (3). The statistical results further explicated that pH value did not differ significantly among different cheese samples.

Proteolysis in Blue cheese (WSN %)

Water-Soluble nitrogen is an indicator of the integrity of the protein matrix and the difference in the extent of proteolysis between cheeses probably reflects the easiness of structural degradation of protein matrix. Proteolysis impacts positively upon meltability of cheese. The result in Table (3) indicated that water soluble nitrogen content differed significantly in FA and FM while BA and BC differed no-significantly. This difference may be due the wide variation in the proteolytic activity of the different starter culture at strain level used in manufacture this cheeses.

Table 3: Mean (± standard deviation) percentage of physicochemical characteristics of different Blue-veined cheeses (FA: Fourme d'Ambert, FM: Fourme de Montbrison, BA: Bleu d'Auvergne; BC: Bleu des Causses).

Cheese	**Chemical characteristics of Blue-veined cheeses**			
	FA	**FM**	**BA**	**BC**
pH value	5.96 (±0.28)^{a}	5.68 (±0.16)^{a}	5.97 (±0.43)^{a}	6.10 (±0.08)^{a}
Moisture (%)	46.61 (±0.91)^{a}	45.33 (±0.45)^{a}	46.69 (±1.33)^{a}	44.69 (±2.05)^{a}
Fat (%)	28.52 (±1.03)^{b}	29.75 (±0.00)^{ab}	29.17 (±1.00)^{b}	31.23 (±1.28)^{a}
Protein (%)	20.32 (±0.40)^{a}	20.99 (±0.00)^{a}	20.08 (±0.10)^{a}	20.07 (±1.02)^{a}
Water-soluble nitrogen (%)	0.78 (±0.04)^{b}	0.55 (±0.12)^{c}	0.97 (±0.11)^{a}	1.03 (±0.06)^{a}
Ash (%)	4.07 (±0.20)^{b}	3.56 (±0.32)^{b}	4.14 (±0.39)^{ab}	4.62 (±0.37)^{a}
Total P (µg/g)	3.34 (±0.15)^{c}	4.13 (±0.57)^{a}	3.74 (±0.23)^{b}	4.04 (±0.25)^{a}
Total Ca (%mg/g)	0.54 (±0.02)^{c}	0.76 (±0.16)^{a}	0.58 (±0.03)^{bc}	0.62 (±0.04)^{b}
Na (%mg/g)	1.21 (±0.09)^{a}	0.82 (±0.05)^{b}	1.19 (±0.16)^{a}	1.34 (±0.12)^{a}

^{a,b,c} Values within a column with different superscript letters are significantly different at *P* <0 .05.

Mineral content in Blue cheese

Mineral salts play a key role in coagulation of milk during cheese manufacturing and the structure and texture development of cheese and also affect the functional and rheological properties (McSweeney, 2007).

Cheese matrix is essentially Ca-P paracasein matrix linked together by various interactions between paracaseins and fat globules, moisture and dissolved substances, and enzymes exist in the pores of this matrix. A dynamic equilibrium exists for the concentration of calcium and phosphate between the paracasein matrix and cheese serum. This equilibrium is influenced by pH and other factors, such as the concentration of sodium ions in the serum (Fox *et al.*, 2000). Controlling these physicochemical conditions during cheese manufacturing is essential for a good quality product. The results of minerals like sodium, calcium and phosphorus contents are discussed here.

Sodium content

The salt content in cheese significantly affects cheese structure by increasing the level of protein hydration and results in a swelling of the protein matrix (solubilization of caseins). This swelling also affects the functional properties of cheese. Increased protein hydration results in decreased protein-protein interactions and increased protein-water interactions. The statistical results for sodium content of cheese presented in Table 3 and figure 1 revealed that sodium content in Fourme de Montbrison cheese was significantly lower than the other blue cheeses.

Calcium content

Calcium plays an important role in micelle structure as well as cheese texture by increase cross-linking between protein-protein interactions within the cheese matrix which resulted in decreased hydration of casein. The results pertaining to analysis of variance for calcium content of different cheese are depicted in Table 3 showed that the total calcium content of 4 cheeses was significantly differed among the cheese samples.

Phosphorus content

The phosphate has an important role in the rennet coagulation of milk and in the structure development. The results pertaining to analysis of variance for phosphorus content of different Blue cheese are given in Table 3. The results indicated that there was significant differences existed in P content between the cheese samples.

Principal component analysis of the physicochemical results indicated that 95.91% of the total variance among the samples could be explained by two principal components (PC), PC_1 and PC_2 (Figure1). PC_1 explained 61.40 % of this variance and was positively driven by protein, Ca and in a less extent by P contents and negatively driven by Ash, pH, WSN and Na. FM cheeses presented positive scores while BC, BA, and FA cheeses presented negative scores according to PC1. PC_2 explained 34.51% of the total variance and was positively driven by Fat and P contents and negatively driven by moisture content. According to PC2 BC and FM cheeses presented positive scores while BA and FA cheeses presented negative scores.

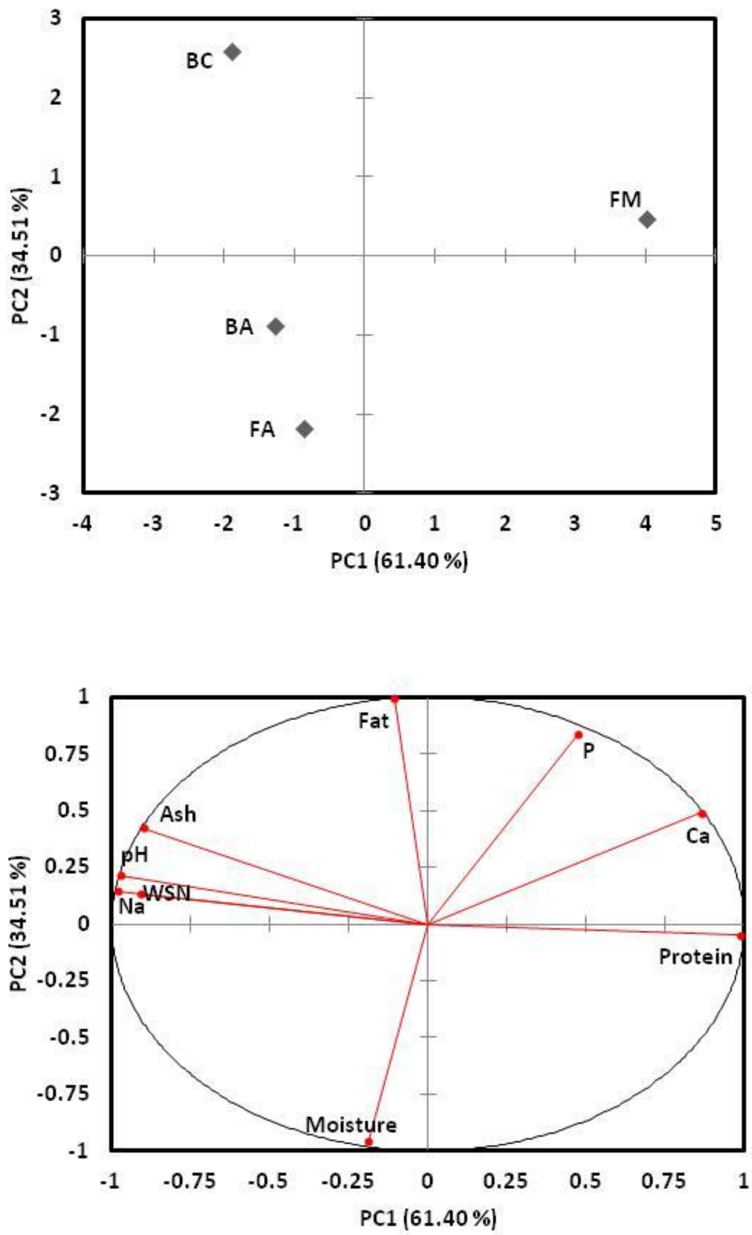


Figure 1: PC_1 and PC_2 similarity map determined by principal components 1 (61.40%) and 2 (34.51%) and correlation circle associated obtained after principal components analysis of physico-chemical characteritics of Blue-veined cheeses (FA: Fourme d'Ambert, FM: Fourme de Montbrison, BA: Bleu d'Auvergne; BC: Bleu des Causses). (WSN : Water-Soluble Nitrogen; Na: Sodium; Ca: Calcium; P: phosphate).

Textural characteristics of Blue-veined cheeses

Cheese has a complex structure that causes differences, even within the same variety of cheese, which depend on compositional factors, their changes during ripening and manufacturing process. Table 1 defines the rheological parameters used in statistical analysis.

The parameters of G’, G”, Tan δ and η* were used for comparing the texture and rheology of 4 blue cheeses and its results are summarized in Table 4. Cheese rheology is affected by structural heterogeneity. Storage modulus values ($G'_{20°C}$) were used as an indication of the number and strength of interactions present at 20°C. Analyses of variance revealed that, there were significant differences in $G'_{20°C}$ between cheeses. Fourme cheeses (FA, FM) had significantly greater G´20°c than the other Blue cheese (BA, BC). This variety of cheese (FA, FM) was more rigid (G') and solid-like (G''), less viscoelastic (Tan δ) and had more resistance to flow (η*) than the other blue cheeses. This differences is may be attributed to submicelle size and distribution of the casein submicelle could explain some of the textural differences between the cheese (Tunick, 1997).

Principal components analysis of rheological data showed that the first two principal components were able to explain 99.89% of the total variance seen between cheese samples. Figure 2 shows PC_1 and PC_2 similarity map and the associated correlation circle. PC_1 and PC_2 explained 95.51% and 4.38% of the total variance respectively. PC_1 is positively driven by G', G'' and η* and negatively driven by tan δ. According to PC_1 the fourme cheeses (FM and FA) presented positive scores while blue cheeses (BA and BC) presented negative scores. FA and FM cheeses presented a less marked liquid like behavior compared to the BA and BC cheeses. Indeed, the FA and FM cheeses exhibited the highest values of G' and G'' and η* and the BA and BC cheeses the lowest ones. These differences may be explained by the characteristics of the gross composition of the cheeses and the organization of the protein network. It was previously reported that the factors such pH, salt concentration, moisture and protein breakdown markedly affect the viscoelastic properties of cheeses.

Table 4: Viscoelastic properties (G', G'', tan δ, and η*), dropping and softening points of different Blue-veined cheeses (FA: Fourme d'Ambert, FM: Fourme de Montbrison, BA: Bleu d'Auvergne; BC: Bleu des Causses).

	Cheeses			
	FA	**FM**	**BA**	**BC**
G' (KPa)	3.83 (±1.19)[a]	3.30 (±0.58)[a]	2.29 (±1.09)[ab]	1.13 (±0.69)[b]
G'' (KPa)	1.26 (±0.36)[a]	1.09 (± 0.21)[ab]	1.02 (±0.04)[ab]	0.52 (±0.25)[b]
Tan δ (G''/G')	0.33 (±0.01)[b]	0.33 (±0.01)[b]	0.45 (±0.02)[a]	0.48 (±0.07)[a]
η*(KPa.s)	0.64 (±0.20)[a]	0.55 (±0.09)[a]	0.40 (±0.18)[ab]	0.20 (±0.11)[b]
Dropping point (°C)	56.05 (±2.42)[b]	65.40 (±2.97)[a]	53.23 (±2.66)[b]	52.85 (±4.46)[b]
Softening point (°C)	53.43 (±4.16)[b]	65.00 (±2.97)[a]	49.60 (±2.29)[b]	51.78 (±4.99)[b]

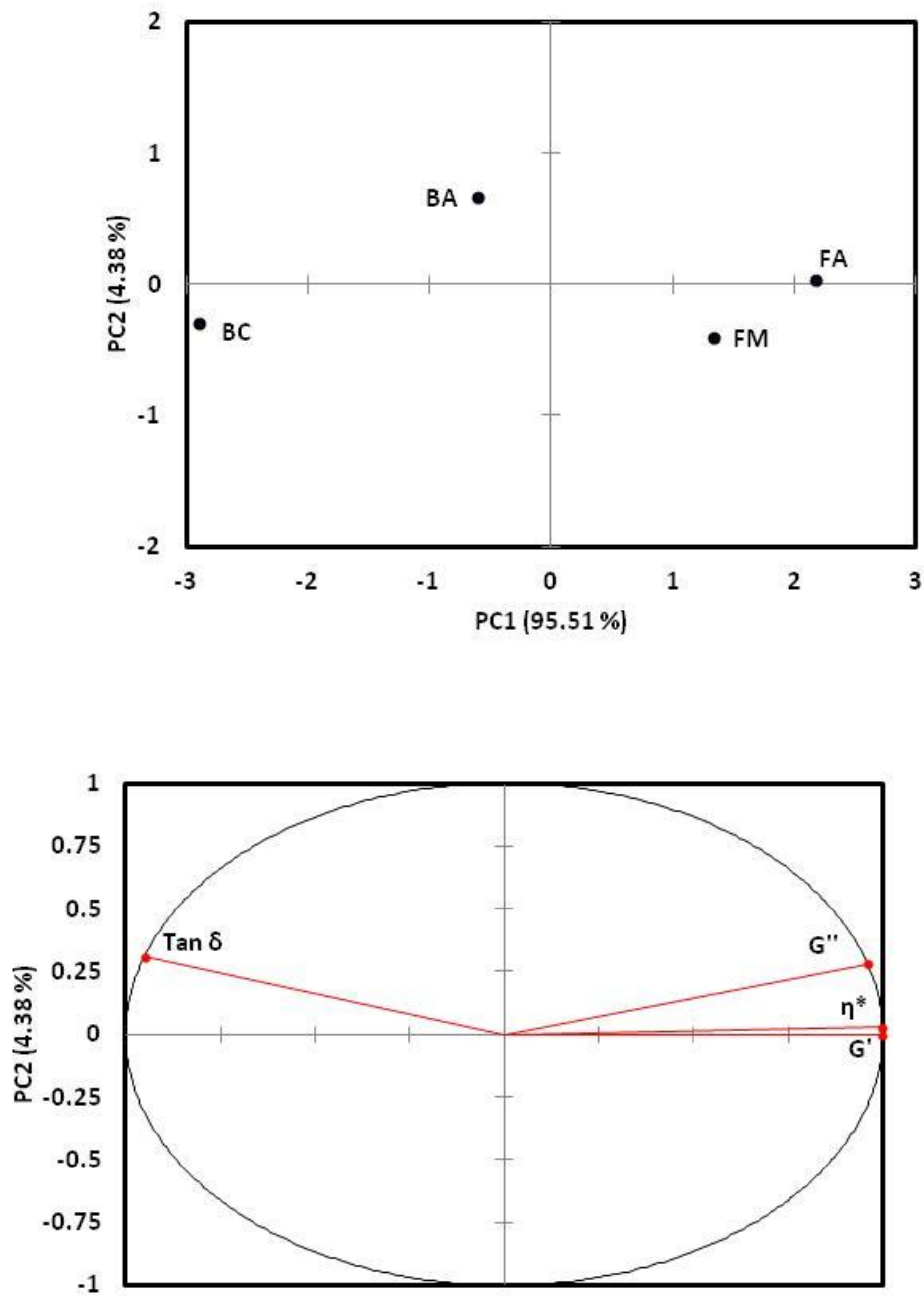


Figure 2: PC_1 and PC_2 similarity map determined by principal components 1 (95.51%) and 2 (4.38%) and correlation circle associated obtained after principal components analysis of rheological attributes (G', G", tan δ, and η*) measured on Blue-veined cheeses (FA: Fourme d'Ambert, FM: Fourme de Montbrison, BA: Bleu d'Auvergne; BC: Bleu des Causses).

Melting properties of Blue-veined cheeses

The melting of cheese is one of the most important functional properties. Cheese meltability is mainly related to heat transfer through cheese during heating and how semi solid cheese mass flows and its transformation from elastic solid to viscous liquid. Fat content in cheese is responsible for the melting during heating, which undergoes changes form solid state to liquid state (Lucey *et al.*, 2003). Casein and serum proteins are other important components present in cheese that doesn’t melt, but interactions between them change by loss of integrity of individual cheese fibers that exhibit the melting characteristics of cheese.

Various empirical methods to measure meltability have been suggested by Arnott et al. (Arnott *et al.*, 1957),Olson and Price (Olson & Price, 1958), and Schreiber test by Kosikowski (Kosikowski, 1982). Softening and dropping points are useful indicators of melting and softening characteristics. For example, softening point (T_{sp}) corresponds to the temperature at which the cheese changes from a semisolid to an almost free flowing liquid. For most cheeses, these changes occur at temperature > 40°C (Lucey, 2008). Softening happens in all cheese, the extent depends on composition, age and pH. Softening is caused by a reduction in the strength of the casein interaction with increasing temperature and contraction of the network (Guinee *et al.*, 1999).

The softening and dropping points for four blue-veined cheeses are reported in Table (4). The results revealed significant differences ($P < 0.05$) among different types of Blue-veined cheeses. Fourme de Montbrison cheese exhibited the highest values on T_{Sp} and T_{Dp} than other Blue cheeses. This can be explained by the reduction in the elastic moduli which would results in the structural properties of cheese matrix. We can assume that the Fourme de Montbrison cheese has very specific structural properties. This could be related to the structural properties of the cheese matrix, protein-protein interactions and water-protein interactions and thus to the cheese-making conditions. During aging, there was increase in soluble calcium as compared to colloidal calcium which increased plasticization of the cheese thereby increasing the functional properties such as melt (Guinee *et al.*, 2000).

Sensory evaluation of cheese texture

Cheese texture is defined as all the physical, rheological and structural attributes of the product perceptible by means of mechanical, tactile and where appropriate, visual and auditory receptors. Texture attribute of food play a major role in consumer appeal, during food buying and consumption. Cheese texture is a sensory characteristic and therefore is directly measurable only by sensory mean. For some foods, texture is more important to consumers than flavor and color (Szczesniak & Kleyn, 1963).

The cheese samples were evaluated for some sensory textural characteristics by a panel of trained assessors. The mean texture attribute scores and associated standard deviations for Blue-veined cheeses are listed in Table 5.

Firmness (by hand)

Hardness is the force required to attain a given deformation. In sensory terms, it is the force required to compress the cheese with the fingers. Based on the firmness, food materials are classified into soft, semi-soft, hard, semi-hard, very hard and fresh cheese.

Hand firmness is the force required to compress the cheese with the fingers (Hort & Le Grys, 2000). The mean (± standard deviation) values for hand firmness of blue cheese are given in Table (5). It is evident from the statistical results that hand firmness of cheese was significantly differences between cheese samples. The fourme cheeses are more firm as compared to the Blue cheeses. Although there are no major differences in the protein and fat content of the cheese but it may be the extent of proteolysis and also Ca, P contents that might be responsible for this difference. This trend is in line with the rheological data.

First bite firmness (in mouth**)**

First bite firmness is a force required to compress cheese between the molars teeth. Even though the perception of texture may perhaps occur during the entire masticatory process, the first bite and its force are the keys to the estimation of hardness, which, in turn, depends on the magnitude of the applied force and the extent to which the food is deformed during the first bite (Peyron *et al.*, 1994). The statistical results for scores assigned to first bite firmness for the cheese samples given in the Table 5 showed that the mouth firmness of cheese was significantly differences between cheese samples. The observed differences in mouth firmness may be related to differences in cheese bound Ca and P and proteolysis among the cheeses. Moreover, fourme cheeses exhibit higher apparent viscosity, low fat content, tougher and minimal breakdown during chewing than the other blue cheese.

Chalkiness (crayeuse)

The mean (± standard deviation) values for chalkiness of blue cheese are given in Table 5. It is evident from the statistical results that the difference in chalkiness scores of cheese observed was non significant.

Creaminess

It is evident from results depicted in Table 5 that fourme cheeses exhibited significantly lower creaminess character than the blue cheeses which gave higher values for creaminess character. This difference may be ascribed to the higher fat content in Blue cheese than the fourme cheeses. Moreover, the creaminess is affected by the average size of fat globules, distance between fat globules and the variation in the size of globules (Richardson & Booth, 1993). So it is significantly affected due to variation in the fat level of milk used for different cheese.

Graininess

It is obvious from the results reported in Table 5 that there was no significant difference in graininess between the cheese samples.

Adhesiveness

Adhesiveness is the work required to overcome the attractive force between the cheese and the contact surfaces of the universal testing machine (Tunick, 2000). In sensory terms, it is the force required to remove the material that adheres to the mouth, particularly the palate, during the normal eating process. Based on the adhesiveness, food materials are classified into sticky, tacky and gooey categories. The Table 5 indicates that there was no significant difference in adhesiveness between the cheese samples.

Residues

The Table 5 indicates that the amount of residues at the end of the mastication did not differ significantly between the cheeses.

It is concluded from sensory results that the fourme cheese (FA and FM) was more firm and less creaminess than the Blue cheeses. Since, the highest scores were given to hand firmness, first-bite firmness and creaminess. All the sensory parameter such as chalkiness, graininess, adhesiveness and residues did not differ significantly for different cheese sensory parameters. Physicochemical properties of foods can be related to firmness or creaminess of the cheese because the extent to which moisture plasticizes the protein matrix of cheese determines its firmness, that is, the amount of work required to fracture the cheese (Marshall, 1993).

Principal component analysis was applied on 7 texture attributes. Principal component analysis of the sensory results indicated that 90.47 % of the total variation among the samples could be explained by two principal components (PC), PC_1 and PC_2 (Figure 3). Principal component one explained 72.25 % of this variation and was positively driven by adhesiveness and creaminess while the other texture attributes had a negative loading. Principal component analysis of sensory results distinguished the samples into two different groups on the basis of the texture attributes. The first group included samples that showed positive scores in PC_1 and were characterized by an adhesive texture. Blue cheeses (BA and BC) had characteristics similar to those cheeses from Group 1. Cheeses in Group 2 had negative scores in PC_I because of their low adhesiveness and low creaminess values. This category included most fourme cheese samples (FA and FM).

Table 5: Mean texture attributes scores (± standard deviation) of different Blue-veined cheeses (FA: Fourme d'Ambert, FM: Fourme de Montbrison, BA: Bleu d'Auvergne; BC: Bleu des Causses).

Cheese	**Sensory texture evaluation of Blue-veined cheeses**			
	FA	**FM**	**BA**	**BC**
Firmness (by hand)	5.31 (±0.95)^{a}	6.34 (±0.41)^{a}	3.60 (±0.88)^{b}	3.62 (±0.74)^{b}
First bite firmness (in mouth)	4.81 (±0.99)^{a}	5.92 (±0.79)^{a}	2.80 (±1.14)^{b}	2.81 (±0.74)^{b}
Chalkiness (crayeuse)	1.47(±1.26)^{a}	2.04(±0.42)^{a}	1.50(±0.74)^{a}	1.19(±0.77)^{a}
Creaminess (crémuse)	3.15 (±1.61)^{bc}	1.19 (±0.52)^{c}	4.91 (±1.44)^{ab}	5.51 (±0.95)^{a}
Graininess (Granuluse)	1.47 (±1.26)^{a}	1.67 (±0.21)^{a}	1.21 (0.51)^{a}	1.46 (±0.88)^{a}
Adhesiveness (Collant)	3.84 (±0.95)^{a}	2.50 (±0.39)^{a}	3.21 (±0.64)^{a}	2.99 (±0.70)^{a}
Residues (Résidus)	2.50(±0.45)^{a}	2.73 (±0.20)^{a}	2.38 (±0.37)^{a}	2.66 (±1.14)^{a}

a,b,c,Means in the same row with no common superscript differ ($P < 0.05$).

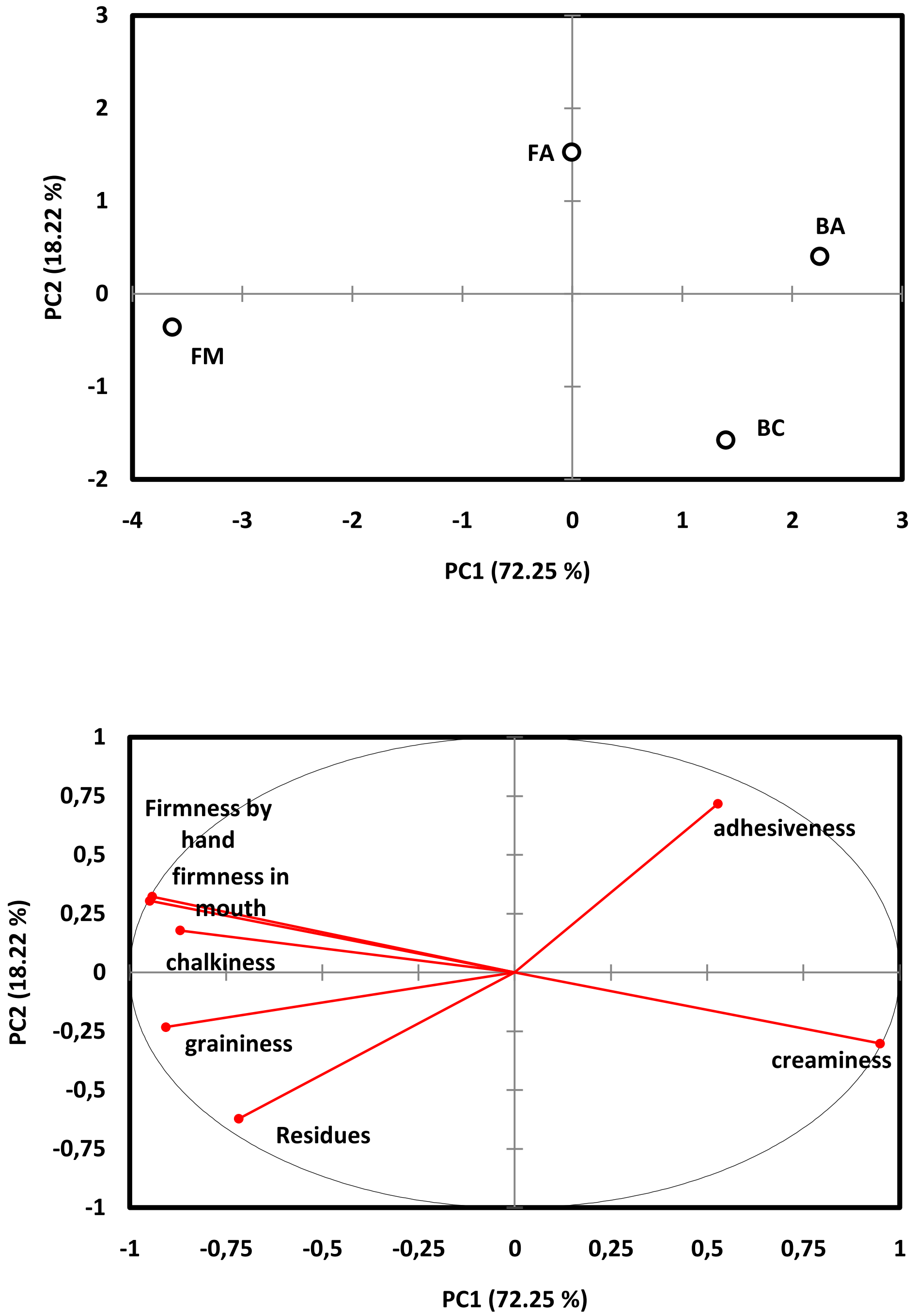


Figure 3: Principal components plot of sensory texture attributes used to differentiate of four Blue-veined cheeses (FA: Fourme d'Ambert, FM: Fourme de Montbrison, BA: Bleu d'Auvergne; BC: Bleu des Causses).

Correlation analysis

In this context, canonical correlation analysis may be applied when the same samples have been characterized by two different techniques. This method provides both global measure of the link between the group of variables and a graphical representation of the correlation revealed. This method make it possible to assess new variables, called canonical variates, as linear combinations of the variables of each data set so that these new variables exhibit the highest correlation that may be found between the two groups of data.

Physicochemical-Rheological data correlations

The matrix of canonical correlation coefficient between physico-chemical characteristics and rheological properties of blue-veined cheeses is presented in Table (6).

For the CCA analysis, the physicochemical and the rheological data sets for all cheeses were correlated. The first 4 pairs of canonical variates were assessed to describe this relation. The physicochemical and the rheological data were correlated with squared canonical correlation coefficients equal to 1.00, 0.96, 0.73 and 0.45 (Table 6). It can be seen from CCA biplot of the physicochemical and the rheological data (Figure 4) that the G', G" and η* variables were positively correlated to protein which are located at the negative side according to the first canonical variate. With regard to Tan δ, it was positively correlated to WSN and ash content, while it was negatively correlated to protein content according to CV_1.

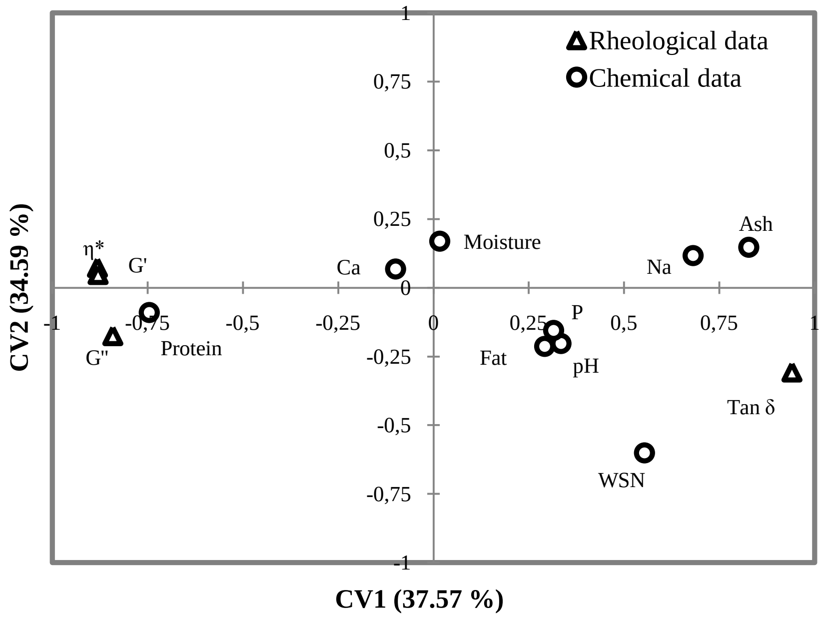


Figure 4: Similarity map of CCA applied to the physico-chemical data and the rheological data sets of Blue veined cheeses.

Physicochemical-melting data correlations

The matrix of canonical correlation coefficient between physico-chemical data and meltability of cheese is presented in Table (6). For the CCA analysis, the physicochemical and the meltability data sets for all cheeses were correlated. The first 2 pairs of canonical variates were assessed to describe this relation. The physicochemical and the meltability data were correlated with squared canonical correlation coefficients equal to 0.98, and 0.93 (Table 6). It is obvious from CCA biplot that the physicochemical and the functional data (Figure 6) that softening and dropping point had negative correlation with WSN, ash and fat content, but it was positively correlated with protein, moisture, P and Ca content according to CV_2. These results indicated that increase in protein, P, and Ca can result in increase in softening and melting characteristics. On the other hand decrease in fat content may increase of softening and dropping point.

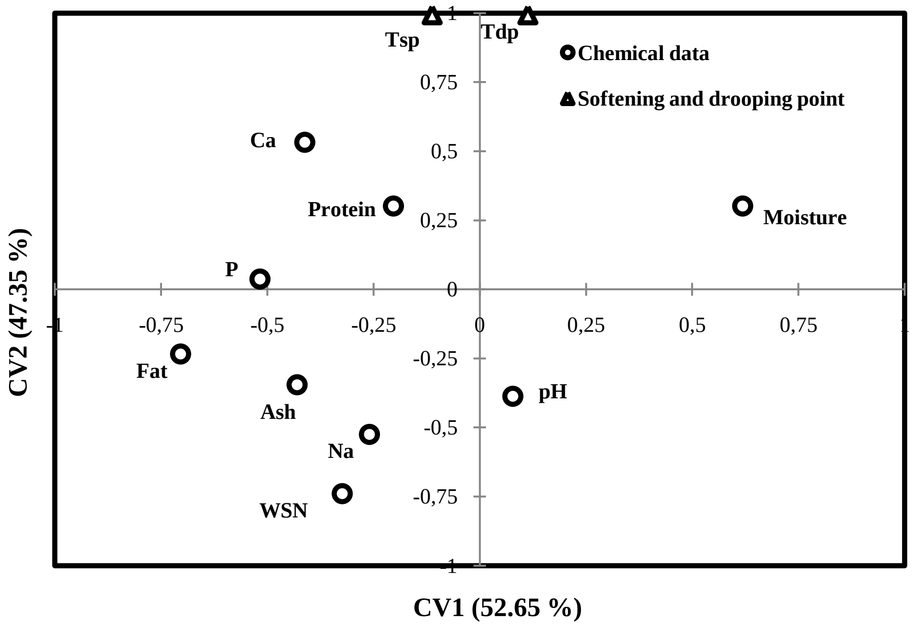


Figure 5: Similarity map of CCA applied to the physico-chemical data and the meltability data sets of Blue veined cheeses.

Table 6: Squared canonical correlation coefficient between physico-chemical data (X-matrix) and the instrumental data (Y-matrix) of blue-veined cheeses.

Data	Canonical variates						
	1	2	3	4	5	6	7
Rheology	0.99	0,96	0,73	0,45	--	--	--
Softening and Dropping point	0,98	0,93	--	--	--	--	--
Sensory texture attributes	1,00	1,00	1,00	1,00	0,96	0,86	0,62

Physicochemical-Sensorial data Correlations

The matrix of canonical correlation coefficient between physico-chemical characteristics and sensory texture attributes of blue cheese is presented in Table (6). For the CCA analysis, the physicochemical and the sensorial data sets for all cheeses were correlated. The first 7 pairs of canonical variates were assessed to describe this relation. The physicochemical and the sensorial data were correlated with squared canonical correlation coefficients equal to 1.00, 1.00, 1.00, 1.00, 0.96, 0.86 and 0.62 (see Table 6). The result of CCA biplot depicted that all the sensory texture attributes positively correlated with fat, protein, Ca and P contents with except of creaminess which showed positive correlation with the pH, fat, and WSN contents (Figure 7).

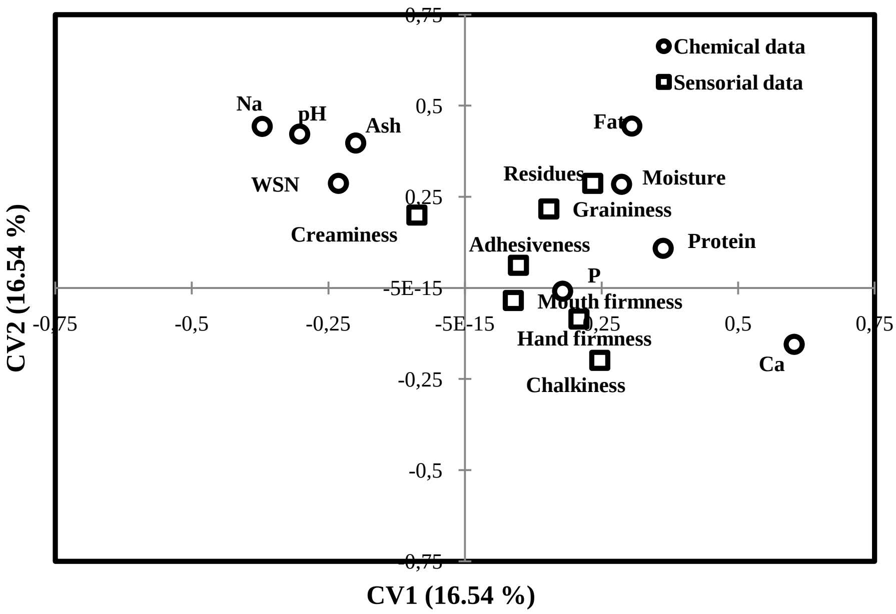


Figure 6: Similarity map of CCA applied to the physico-chemical data and the sensory texture data sets of Blue veined cheeses.

Rheological-Sensorial data Correlations

The matrix of canonical correlation coefficient between the rheological characteristics and sensory texture attributes is presented in Table (7). The first 4 pairs of canonical variates were assessed to describe this correlation. The rheological and the sensorial data were correlated with squared canonical correlation coefficients equal to 0.94, 0.69, 0.65 and 0.56. The result of CCA biplot depicted that the G', G" and η* variables were positively correlated with hand firmness, mouth firmness, chalkiness but negatively correlated with creaminess character (figure 8). The creaminess possessed positive correlation with the Tan δ. The rheological variables did not correlated with the other sensory texture attributes (graininess, adhesiveness and residues) (see Table 8).

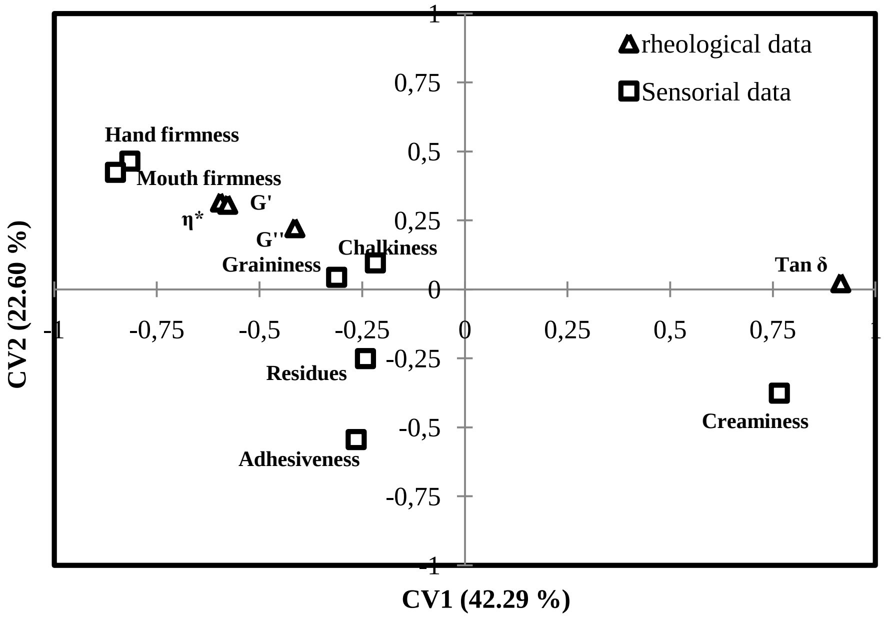


Figure 7: Similarity map of CCA applied to the rheological data and the sensory texture data sets of Blue veined cheeses.

Rheological-meltability data Correlations

The matrix of canonical correlation coefficient between the rheological characteristics and the meltability data (softening and dropping points) is presented in Table (7). The first 2 pairs of canonical variates were assessed to describe this correlation. The rheological and the meltability data were correlated with squared canonical correlation coefficients equal to 0.59 and 0.36 (Table 7). The result of CCA biplot depicted that the G', G" and η* variables were negatively correlated with softening and drooping point. Tan δ variable did not correlated with the softening and dropping point (Figure 9).

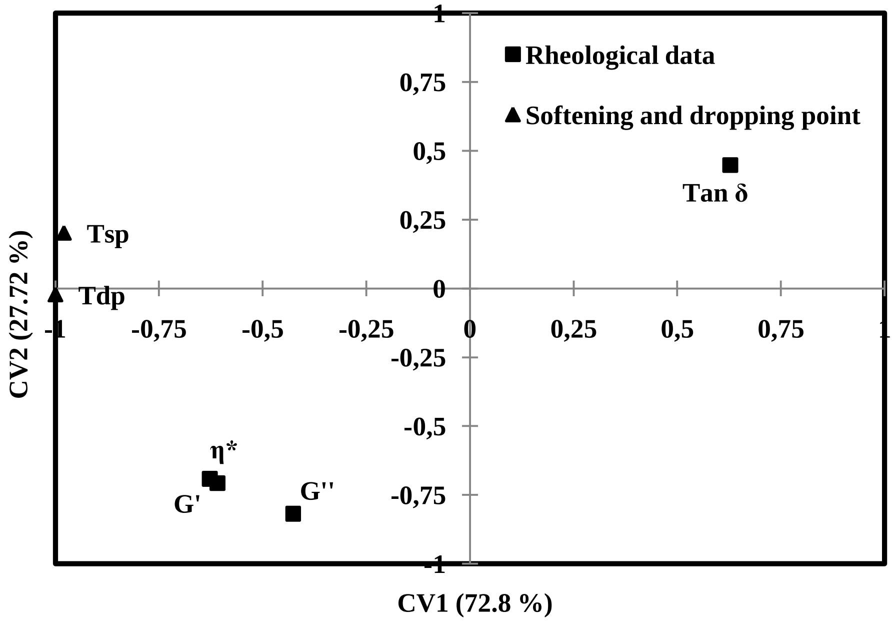


Figure 8: Similarity map of CCA applied to the rheological and the meltability data sets of Blue veined cheeses.

Sensorial-meltability data Correlations

The matrix of canonical correlation coefficient between the sensorial data and the meltability data (softening and dropping points) is shown in figure (10). The first 2 pairs of canonical variates were assessed to describe this correlation. The sensorial and the meltability data sets were correlated with squared canonical correlation coefficients equal to 0.95 and 0.83. The result of CCA biplot depicted that the hand and mouth firmness were positively correlated with softening and drooping point. Creaminess was negatively correlated with the softening and dropping point.

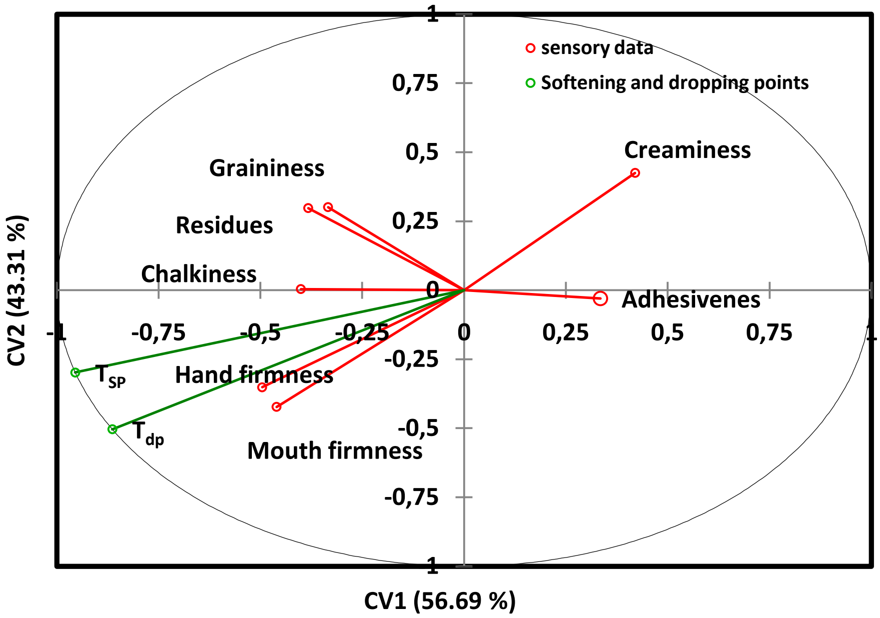


Figure 9: Similarity map of CCA applied to the sensorial and the meltability data sets of Blue veined cheeses.

Table 7: Squared canonical correlation coefficient between rheological data (X-matrix) and sensory texture attributes and meltability properties (Y-matrix) of blue-veined cheeses.

Data	Canonical variates			
	1	2	3	4
Sensory texture attributes	0,94	0,69	0,65	0,56
meltability data	0,59	0,37	--	--

Table 8: Correlation coefficients from the CCA analysis between sensory data (Y-matrix) and instrumental data (X-matrix) used to define cheese texture attributes.

R^2						
	Independent variables (instrumental)					
Dependent variables (sensory)	T_{sp}	T_{dp}	G'	G"	Tan δ	η *
Hand Firmness	0,540	0,558	0,701	0,555	-0,777	0,688
Mouth Firmness	0,526	0,558	0,708	0,564	-0,799	0,695
Chalkiness	0,365	0,329	0,494	0,519	-0,371	0,498
Creaminess	-0,489	-0,526	-0,748	-0,641	0,776	-0,739
Graininess	0,230	0,149	0,391	0,384	-0,365	0,391
Adhesiveness	-0,299	-0,264	-0,125	-0,184	-0,181	-0,132
Residues	0,275	0,191	0,224	0,237	-0,274	0,226

CONCLUSION

Results showed that the most significant components among the cheeses were: fat, WSN and mineral contents (Ca, P, and Na) among the cheeses. Many cheese texture attributes were similar, but Fourme cheeses (FA and FM) were perceived as firm texture and less creamy than the Blue cheeses (BA and BC). Rheological results supported that the Fourme cheeses were firmer and less elasticity than the Blue cheeses. It was found that the chemical composition was highly correlated with sensory texture attributes and rheological parameters. We were also found that the rheological data was much better than compositional data in relation to sensory texture. This study provided a useful baseline of the physical, chemical and sensory properties for researchers interested in the study of Blue cheese and also the cheese manufacturers. But there is limited understanding of the molecular level interactions in cheese and how these interactions are related to the texture and structure cheese.

ACKNOWLEDGEMENTS

This research was funded by Fayoum University (Egypt) and “Syndicat des Fromages Française". Special thanks to Mm. Delphin (Vetagro-sup, Clermont) for her help in chemical analysis of the cheeses and to all the panelists who participated in the study.

REFERENCES

AFNOR (2004) Association Française de Normalisation. Chemical analysis.

AOAC (1995) Chapter 33,Dairy products. In:Official methods of analysis of Association of Official Analytical Chemistry. 16th ed. Virginia: AOAC. p 60–1.

Arnott, D.R., Morris, H.A. & Combs, W.B. (1957) Effect of Certain Chemical Factors on the Melting Quality of Process Cheese. Journal of dairy Science 40(8), 957-963.

Barry, A.L. & Tamine, A.Y. (2010) Technology of cheesemaking. London, UK.

Bertola, N.C., Bevilacqua, A.E. & Zaritzky, N.E. (1992) Proteolytic and Rheological Evaluation of Maturation of Tybo Argentino Cheese. J. Dairy Sci. 75(12), 3273-3281.

Bertrand, D. & Scotter, C.N.G. (1992) Application of Multivariate Analyses to NIR Spectra of Gelatinized Starch Applied Spectroscopy 46, 1420–1425.

Eberhard, P., Moor, U., Ruegg, M. & Fluckiger, E. (1986) Objective measurement of the softening and dropping point of Raclette cheese Schweiz Milchwirtschaftliche Forsch 15, 93-96.

Fox, P.F., Guinee, T.P., T.M., C. & McSweeney, P.H. (2000) Fundamentals of cheese science. ASPEN Publishers, Inc., Gaithersburg, Maryland.

Guinee, T.P., Aut, M.A.E. & Fenelon, M.A. (2000) The effect of fat on heat rheology, microstructure and heat induced functional characteristics of cheddar cheese. Int. Dairy J. 10: 277-288.

Guinee, T.P., Auty, M.A.E. & Mullins, C. (1999) Observations on the microstructure and heat-induced changes in the viscoelasticity of commercial cheeses. Australian J. Dairy Technol. 54, 84 - 89.

Hort, J. & Le Grys, G. (2000) Rheological models of Cheddar cheese texture and their application to maturation. J. Texture Stud. 31, 1-24.

Kosikowski, F. (1982) Process cheese and related type. in cheese and fermented milk foods, 2nd ed., p 405-406. Edwards Brothers, Inc., Ann Arbor, MI.

Lebecque, A., Laguet, A., Devaux, M.F. & Dufour, E. (2001) Delineation of the texture of Salers cheese by sensory analysis and physical methods. Le Lait 81, 609-623.

Lucey, J.A. (2008) Some perspectives on the use of cheese as a food ingredient. Dairy Sci. Technol., 1-22.

Lucey, J.A., Johnson, M.E. & Horne, D.S. (2003) Perspectives on the basis of the rheology and texture properties of cheese. Journal of dairy science 86(9), 2725-2743.

Marshall, R. (1993) Chemical and physical methods. In: Standard methods for the examination of dairy products. 16th ed.Washington, D.C.: American Public Health Assn. p 4333–529.

McSweeney, P.L.H. (2007) Cheese problems solved. Woodhead Publishing Limited, Cambridge, England. p.18.

Olson, N.F. & Price, W.V. (1958) A Melting Test for Pasteurized Process Cheese Spreads. J. Dairy Sci. 41(7), 999-1000.

Peyron, M.A., Mioche, L. & Culioli, J. (1994) Bite force and sample deformation during hardness assessment of viscoelastic models of foods. J. Text. Stud. 25, 59-76.

Pillonel, L., Badertscher, R., Bütikofer, U., Casey, M., M., D.T., Lavanchy, P., Meyer, J., Tabacchi, R. & Bosset, J.O. (2002) Analytical methods for the determination of the geographic origin of Emmentaler cheese. Main framework of the project; chemical, biochemical, microbiological, colour and sensory analyses. European Food Research and Technology 215(3), 260-267.

Pinho, O., Mendes, E., Alves, M.M. & Ferreira, I.M.P.L.V.O. (2004) Chemical, Physical, and Sensorial Characteristics of "Terrincho" Ewe Cheese: Changes During Ripening and Intravarietal Comparison. J. Dairy Sci. 87(2), 249-257.

Richardson, N.J. & Booth, D.A. (1993) Multiple physical patterns in judgements of the creamy texture of milks and creams. Acta Psychol. 84: 93-101.

Romain, R., Grégory, R., Marie-Hélène, F. & Jean-René, K. (2001) Diversité de quelques propriétés fonctionnelles à chaud de l'Emmental français. Lait 81, 547-559.

Saporta, G. (1990) Probabilités – Analyse des données statistique, Technip édn., Paris.

Szczesniak, A.S. & Kleyn, D.H. (1963) Consumer awareness of texture and other food.

TTunick, M.H., P.H. Cooke, E.L. Malin, P.W. Smith and V.H. Holsinger (1997) Reorganization of casein submicelles in Mozzarella cheese during storage. Int. Dairy J. 7:149-155.

Tunick, M.H. (2000) Rheology of Dairy Foods that Gel, Stretch, and Fracture. J. Dairy Sci. 83(8), 1892-1898.

II. Utilisation de la spectroscopie de fluorescence pour la caractérisation physicochimique des pâtes persillées

Ce paragraphe a fait l'objet d'un article « Application of synchronous fluorescence spectroscopy for the determination of some chemical parameters in PDO French blue cheeses" Khaled Abbas, Romdhane Karoui, Abderrahmane Aït-Kaddour (Article n°3 accepté le 16 décembre 2011 dans European Food Research and Technology).

II.1. Objectifs et méthodologie

II.1.1. Objectifs

Dans les industries agroalimentaires, une grande partie du contrôle de la qualité des denrées en cours de transformation et des produits finis repose sur leur analyse biochimique. Les produits transformés doivent respecter des spécifications qui portent en particulier sur leur composition (teneur en eau, en protéines, en glucides…). Les analyses chimiques de référence sont en général longues, nécessitent parfois l'utilisation de réactifs onéreux et polluants et ne sont applicables que par des opérateurs qualifiés (Bertrand et Dufour 2006). L'industrie laitière, comme l'ensemble des industries agroalimentaires, est dans l'obligation de fournir des produits de qualité élevée et constante afin de répondre aux exigences des consommateurs. Ces industries sont très sensibles aux nouvelles méthodes d'analyse rapide, peu couteuses permettant de mesurer en ligne ou hors ligne la qualité de leurs produits alimentaires. La rapidité d'une analyse est primordiale dans le contrôle de la production. En effet, le dérèglement éventuel d'une installation ne peut être détecté que lorsque les résultats des analyses chimiques est connu. Aujourd'hui, il y a un besoin pour l'industrie de la transformation fromagère de disposer d'outils lui permettant de piloter en temps réel les lignes de production afin de vérifier la conformité des produits lors du procédé de transformation.
La spectroscopie de fluorescence est une méthode d'analyse sensible, rapide et non-invasive qui peut être utilisée selon deux modes, le mode frontal et le mode synchrone. Le fromage et particulièrement les fromages à pâte persillée présente une matrice complexe et anisotrope avec différentes propriétés physico-chimiques. L'utilisation de seulement certaines longueurs d'onde d'excitation ou d'émission (fluorescence frontale) des acides aminés aromatiques et des

acides nucléiques tels que le tryptophane, la vitamine A et riboflavine pourrait limiter la capacité de cette technique à prédire efficacement certains paramètres physico-chimiques dans les fromages. Pour pallier cette lacune, le mode synchrone, par la variation de la longueur d'onde d'excitation et d'émission permet la détermination simultanée des composés fluorescents présents dans la matrice. Cette méthode présente donc l'avantage de donner une emprunte spectrale du produit étudié.

L'objectif de cette étude est d'étudier le potentiel de la spectroscopie de fluorescence synchrone couplée à des analyses statistiques multivariées pour la détermination de certains paramètres chimiques de fromages à pâte persillée.

II.1.2. Méthodes

II.1.2.1. Analyses physicochimiques

La détermination de la composition physico-chimique des fromages à pâte persillée a été faite suivant les normes internationales ou françaises ou selon des méthodes internes au laboratoire. Toutes les analyses ont été réalisées en triple.

Les analyses qui ont été réalisées sont la matière sèche (NF EN ISO 5534), la matière grasse (NF V04-287), l'azote total et soluble (NF EN ISO 8968-1), et les minéraux totaux (cendres). Le pH a été mesuré à l'aide d'un pH mètre (Schott CG840, Paris, France) après avoir dispersé 10 g de fromage râpé dans 50 ml d'eau distillée à 20°C. L'ensemble des résultats pour les 20 fromages est présenté dans les tableaux n°1 et 3.

II.1.2.2. Analyses chimiométriques

La méthode de régression par les moindres carrés partiels a été utilisée pour construire trois modèles afin d'évaluer la performance de cette méthode spectrale :

- Le premier modèle comprenant la catégorie des Fourmes (Fourme d'Ambert et Fourme de Montbrison),
- Le second modèle comprenant la catégorie des Bleus (Bleu d'Auvergne et Bleu des Causses)
- Le troisième modèle comprenant les quatre fromages à pâte persillée (Fourme d'Ambert, Fourme de Montbrison, Bleu d'Auvergne et Bleu des Causses).

De plus les vecteurs propres associés aux composantes principales nous ont permis une interprétation au niveau moléculaire des données spectrales et ainsi d'identifier les longueurs d'onde les plus intéressantes pour la prédiction d'un paramètre physicochimique donné.

II.2. Résultats et Discussion

Cette étude préliminaire montre le potentiel de la spectroscopie de fluorescence synchrone pour prédire certains paramètres physico-chimiques de matrices fromagères hétérogène et anisotrope (les fromages à pâte persillée). En effet, cette étude donne des résultats encourageants pour prédire la teneur en cendres et en protéines des fromages à pâte persillée lorsque l'on analyse les deux classes de fromages séparément (Fourmes et Bleus). Lorsque les deux groupes ont été analysés conjointement (Fourmes + Bleus), la spectroscopie de fluorescence synchrone a échoué dans la prédiction de l'ensemble des paramètres physico-chimiques. L'analyse des coefficients de régression a permis d'identifier les longueurs d'onde les plus intéressantes impliquées dans la prédiction des paramètres physicochimiques et permettant de différencier les deux groupes de fromages à pâte persillée (Fourme et Bleus).

ARTICLE N°3: APPLICATION OF SYNCHRONOUS FLUORESCENCE SPECTROSCOPY FOR THE DETERMINATION OF SOME CHEMICAL PARAMETERS IN PDO FRENCH BLUE CHEESES

Khaled Abbas[a], Romdhane Karoui[b], Abderrahmane Aït-Kaddour[a]*

[a]Clermont Université, F-63 000, Clermont-Ferrand, VetAgro Sup, Campus agronomique, 89 avenue de l'Europe -BP 35- 63370 Lempdes, France. U.R CALITYSS

[b]Artois University, Faculty of Sciences Jean-Perrin, Rue Jean Souvraz, 62300 Lens, France

*Corresponding author: a.aitkaddour@vetagro-sup.fr

Tel: 0033(0)4 73 98 13 78

Fax: 0033 (0)4 73 98 13 90

Introduction

Nowadays, the dairy industry has come under increasing pressure to deliver products of high and constant quality into the market place [1]. Thus, a large number of analyses during the manufacturing and storage of foodstuffs, such as cheeses, is needed. However, food quality can be difficult to assess, because the complex composition of food complicates quantitative and qualitative determination of the individual constituents [2].

For example, the physico-chemical characterization of dairy products (e.g. milk, butter, cheese, etc.) is based on determining pH value, fat and mineral contents, nitrogen fractions, etc. When these parameters are determined, isolation of the compound of interest is necessary allowing to the destruction of the food. In addition, the destruction step is problematic, since it: (i) is tedious and laborious; (ii) and required a huge quantity of chemical reagents as well as skilled operators. Moreover, the information contained in initial matrix can be lost when these components are removed from their context [2]. These methods are not effective enough to cover the growing demand of listing that industry requires [3].

Taking this into account, there is a continuing demand for new, rapid and relatively cheaper methods for direct rapid determination of the quality measurements in food and food ingredients of food products. Spectroscopic techniques such as near infrared, mid infrared, fluorescence, etc. are fast, relatively of low-cost and provide a great deal of information with only one test measurement. They are considered as sensitive, non-destructive, rapid, environmentally friendly and non-invasive, which make these methods suitable for on-line or in-line process control to determine several quality parameters.

Fluorescence spectroscopy has been widely utilized as a technique allowing the characterization of food quality of several food products. It has been used to authenticate milk [4], cheese [5,6], yoghurt [7], honey [8,9], cereal [10], egg [11-13], and fish [14-16] samples. This technique has demonstrated its ability to determine the cheese melting point [17,18] of semi-hard and hard cheeses as well as some chemical parameters (e.g. moisture, fat, total nitrogen, water soluble nitrogen, pH) of: (i) Emmental cheeses produced during summer and autumn periods [19] and (ii) soft cheeses at both the external and central zones [20].

However, all the above mentioned studies on fluorescence spectroscopy have applied classic front face fluorescence spectroscopy after fixing the excitation or the emission wavelengths of some intrinsic probes. In addition to the best of our knowledge, no study has been performed on PDO and industrial blue veined cheeses. Considering that cheese is a complex and anisotropic matrix presenting different physico-chemical properties, the use of only some excitation or emission wavelengths for the excitation of aromatic amino acids and nucleic acids, tryptophan, vitamin A and riboflavin could limit the ability of this technique to predict efficiently some physico-chemical parameters in cheeses. To comply with this requirement, the variation in the excitation and emission wavelengths allows simultaneous determination of compounds in several foodstuffs. Therefore, it would be interesting to use for each food product different excitation wavelengths simultaneously. This could be realized by using synchronous fluorescence spectroscopy (SFS) which presents two interesting advantages since: it (1) allows the consideration of the whole fluorescence landscape, i.e. spectra recorded at different offsets; and (2) retains information related to several fluorophores compared to a classical emission spectrum, which is mainly specific to a sole fluorophore.

The present study was aimed at investigating the potential of using SFS coupled with multivariate statistical analyses for the determination of some chemical parameters (pH, fat, dry matter (DM), Protein and water soluble nitrogen (WSN)) of French bleu veined cheeses belonging to four brands. Three Partial Least square regression (PLS-R) models were considered the first one including the “Fourme cheeses” (*Fourme d’Ambert* and *Fourme de Montbrison*), the second one including the “Bleu cheeses” (*Bleu d’Auvergne* and *Bleu des Causses*) and the last one including the 4 Bleu veined cheeses (*Fourme d’Ambert*, *Fourme de Montbrison*, *Bleu d’Auvergne* and *Bleu des Causses*).

Materials and methods

French bleu cheeses

This study was performed on 20 French bleu veined cheeses produced from raw, thermized or pasteurized milk and belonging to four denominations: 8 *Fourme d’Ambert* (FA), 4 *Fourme de Montbrison* (FM), 4 *Bleu des Causses* (BC) and 4 *Bleu d’Auvergne* (BA) obtained from different companies and supermarkets located in the Auvergne region. The FA cheeses investigated presented different ripening times (30 and 45 days), while the ripening times of the other bleu cheeses were unknown. Upon arrival to our laboratory, each bleu veined cheese was frozen rapidly to -20°C and kept until analysis.

Physicochemical analysis

The physicochemical analyses performed on cheese samples were dry matter (DM), fat, protein and water-soluble nitrogen (WSN) contents using standards NF EN ISO 5534, NF V04-287, and NF EN ISO 8668-1, respectively (AFNOR, 2002). The pH was measured by a pH meter (Schott, CG840, Paris, France) after grating 10 g of cheese and dispersing it in 50 ml of ionized water. Each analysis was performed in triplicate.

Synchronous fluorescence spectroscopy

Cheese SF (Synchronous fluorescence) spectra were recorded using a FluoroMax-2 spectrofluorimeter (Spex-Jobin Yvon, Longjumeau, France). The broad nature of conventional fluorescence spectrum and spectral overlap can be overcome and enhanced selectivity by using this technique. In SFS, the excitation wavelength (λ_{ex}) and emission wavelength (λ_{em}) are scanned simultaneously (synchronously), usually maintaining a constant wavelength interval, $\Delta\lambda$, between λ_{ex} and λ_{em}. In this study a $\Delta\lambda$ = 80 nm was identified as being the most suited value after analyzing spectral shape obtained at different $\Delta\lambda$ = 20, 40, 80, 100, 120 and 140 nm (data not shown). Before acquisition of spectra, cheeses were sliced into thin samples (2 cm x 1 cm x 0.3 cm) and placed in a quartz cell which was then mounted on a front face accessory, such that the incidence angle of the excitation radiation was set at 56°. SF spectra were recorded between 250 and 500 nm at 20°C. The spectrofluorimeter was equipped with a thermostatically controlled cell-holder and the temperature was controlled by a Haake temperature controller (Haake, Champlan, France). The SF spectra were recorded five times per sample.

Principal component analysis

Principal component analysis (PCA) is a modeling method that gives an interpretable overview of the main information in a multidimensional data table. It produces a new smaller set of variables called principal components (PCs). By plotting the PCs, it is possible to distinguish interrelationships between different variables, and detect and interpret sample patterns, groupings, similarities or differences.

Partial least squares regression

Several statistical modeling techniques can be adopted for proper calibration performance, such as linear and nonlinear multiple regression analyses, principal component regression and partial least square regression (PLS-R). PLS-R is a recent technique that generalizes and combines features from PCA and multiple regressions. Its goal is to predict or analyze a set of dependent variables from a set of independent variables or predictors. This prediction is achieved by extracting from the predictors a set of factors called latent variables that realize a compromise between the variance explanation and the prediction of the response. In the present study PLS-R was used in order to predict physico-chemical parameters from SF spectra recorded on four cheese brands. As the number of observations was small the regression models were validated by leave-one-out cross-validation. The performance of the PLS-R models can be evaluated by analyzing different factors (e.g. the root mean square error of cross validation - RMSECV, the determination coefficient of cross-validation - R^2 and the ratio of standard deviation to root mean square error of cross-validation - RPD). Williams [21] defines 7 levels of model accuracy based on the R^2 values obtained for prediction. If the R^2 value is less than 0.25, the calibration is considered not usable; between 0.26 and 0.49 the correlation is very poor and the reasons should be investigated; from 0.50 to 0.64 the calibration is acceptable for rough screening because more than 50% of the Y variance is accounted for by the X variance; between 0.66 and 0.81 the calibration can be used for screening and approximate predictions; from 0.83 to 0.90 it is usable for most applications, including research; between 0.92 and 0.96 the calibration is considered usable in most applications, including quality assurance; and above 0.98 the calibration would be usable in any application. The R^2 provides good information about the calibration quality, but gives no direct information about the prediction accuracy. In this research five levels of prediction accuracy for a heterogeneous material based on the RPD were also considered. An RPD value below 1.5 indicates that the prediction accuracy has not been substantially increased by the sensor compared to using the mean value for all samples, thus the calibration is not usable. An RPD value between 1.5 and 2.0 reveals that the prediction error has decreased to half the original one, which can be interpreted as a possibility to distinguish between high and low values. When the RPD value lies between 2.0 and 2.5 approximate quantitative predictions for the manure composition can be made. For RPD values between 2.5 and 3.0, and above 3.0, the prediction accuracy will be classified as good and excellent, respectively, because an on-line sensor with this accuracy could promote better manure management than one based on mean compositions for a complete storage facility. The interpretation of the prediction accuracy based on the R^2 and RPD values is interesting for comparison of the different models prediction accuracy.

Results and discussion

The results for fat, DM, pH, protein and WSN of the investigated cheeses are reported in **Tables 1 and 2**. These results are in agreement with previous research on bleu cheeses (http://www.afssa.fr/TableCIQUAL/). These researches presented the physicochemical composition (e.g. DM, Fat, and proteins) for the FA and the BA cheeses. The FA cheeses presented values of 53.9%, 19.8%, and 28.5% for DM, protein and Fat, respectively. While the BA presented values of 54.8%, 29.7%, and 19.9% for DM, Protein and Fat, respectively. No physicochemical data were presented for the BC and the FM cheeses. In order to identify the difference between physicochemical characteristics of the blue veined cheeses an ANOVA test ($\alpha = 0.5\%$) was applied to the different data sets.

Table 1 Physicochemical composition of the different Fourme cheeses (*Fourme d'Ambert*: FA; *Fourme de Montbrison*: FM) used for the partial least square regression models.

Physicochemical parameter	Cheese	Min	Max	Mean	SD*	CV**
DM (%)	FA	51.75	56.46	53.39	1.56	2.9
	FM	54.21	55.05	54.64	0.45	0.8
Fat (%)	FA	26.75	30.58	28.46	1.37	4.8
	FM	28.5	30.25	29.23	0.75	2.6
Ash (%)	FA	3.88	4.99	4.35	0.38	8.8
	FM	2.94	4.02	3.52	0.48	13.6
pH	FA	5.55	6.45	5.92	0.32	5.4
	FM	5.2	5.8	5.58	0.27	4.9
Protein (%)	FA	19.15	20.16	19.72	0.38	1.9
	FM	20.33	21.29	20.81	0.53	2.5
WSN (%)	FA	0.93	1.63	1.36	0.24	17.7
	FM	0.91	1.28	1.10	0.15	13.9

*SD: Standard deviation
**CV: Coefficient of variation (%)

Table 2 Physicochemical composition of the different Bleu cheeses (*Bleu d'Auvergne*: BA; *Bleu des Causses*: BC) used for the partial least square regression models.

Physicochemical parameter	Cheese	Min	Max	Mean	SD*	CV**
DM (%)	BA	52.54	58.02	54.56	2.54	4.6
	BC	52.75	57.69	55.31	2.04	3.7
Fat (%)	BA	28.17	34.83	30.60	2.93	9.6
	BC	29.75	32.42	31.23	1.28	4.1
Ash (%)	BA	2.92	4.59	3.84	0.69	17.9
	BC	4.26	5.14	4.62	0.37	8.0
pH	BA	5.63	6.45	6.04	0.38	6.2
	BC	6.00	6.17	6.10	0.08	1.2
Protein (%)	BA	19.3	21.05	19.84	0.81	4.1
	BC	18.09	20.18	19.46	0.94	4.8
WSN (%)	BA	1.67	2.45	2.07	0.32	15.3
	BC	1.38	2.21	1.88	0.36	19.2

*SD: Standard deviation
**CV: Coefficient of variation (%)

Concerning the DM, significant difference was observed between FA-FM-BA cheeses and BC cheeses. BC cheeses presented the higher DM content probably due to its high ripening time usually performed (70 days). Concerning the Fat, significant difference was observed between the Fourme cheeses (FA-FM) and the Bleu cheeses (BA-BC). The Bleu cheeses presented higher Fat content. This is probably assigned to the different milk fat content. For example it is known that the Bleu cheeses are produced from raw milk and more particularly the BC cheeses, while the FA and FM cheeses are produced from skimmed milk. Considering

the Ash, significant difference was observed between the FA-BC and FM-BA cheeses, the last group presented higher values. Considering the pH, no significant difference was observed between FM, BA and BC cheeses, while FA cheeses presented lower significant value. This could be attributed to the oxidation level of cheeses. Based on the pH values the FA cheeses seem to be the less oxidized samples. The increase of the pH during ripening can be attributed to the depletion of lactic acid that induced a production of NH_3 [22,23].
Considering the protein, the FM cheese presented higher significant content than the BA, BC and FA. Concerning the WSN, three groups with significant differences were identified. The BA-BC cheeses presented the higher WSN content, followed by FA cheeses, and the FM cheeses presented the lowest WSN value.

To extract information from the data sets, PCA was applied to the mean centering physicochemical parameters of the different cheeses. The similarity map (**Fig. 1**) showed that three classes of cheese samples can be identified, the FA cheeses class, the BA - FM cheeses class and the BC cheeses class (**Fig. 1**). This similarity map suggested that the samples are not classified according to thermal treatment applied to the milk (e.g. thermized, pasteurized). Nonetheless, the difference between the cheese samples of the FA class is principally due to the ripening time based on PC1. The FA cheeses with high ripening time (45 days) have lower values on PC1compared to the FA cheeses with lower ripening time (30 days) (**Fig. 1**).

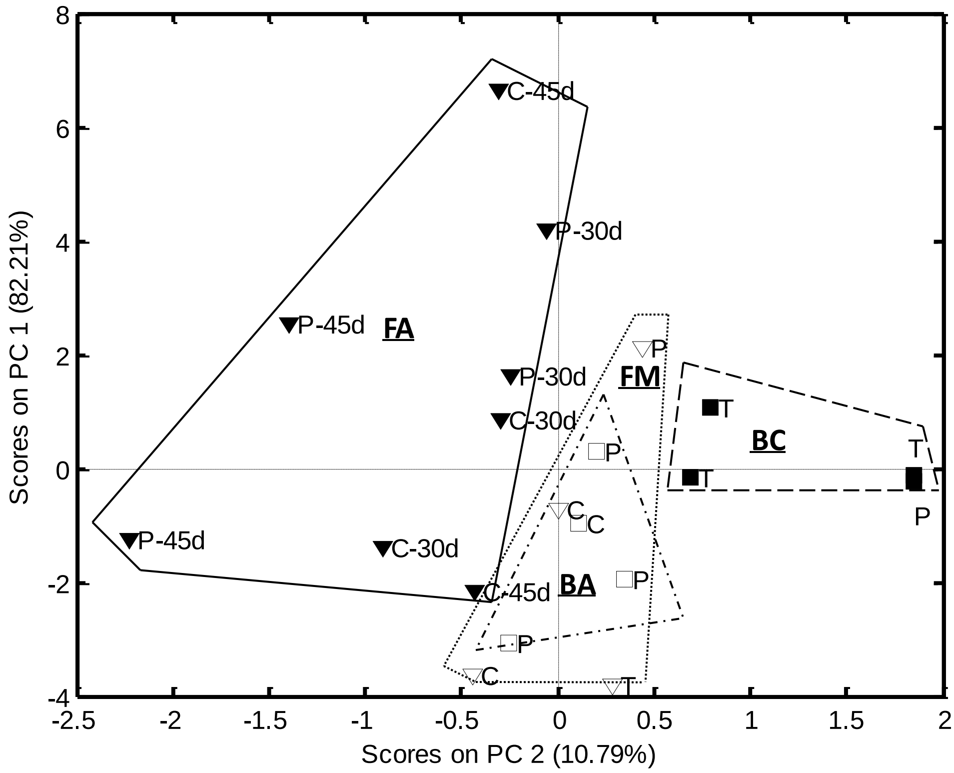


Fig. 1 Principal component analysis similarity map (scores and loadings) determined by principal components 1 (PC_1) and 2 (PC_2) for the different bleu veined cheeses *Fourme d'Ambert (FA: ▼), Fourme de Montbrison (FM: ▽), Bleu d'Auvergne (BA: ■)* and *Bleu des Causses (BC: □)*. (d: number of ripening days)

Synchronous fluorescence spectra

Fig. 2 presented an example of *SF spectra of four pasteurized* bleu veined cheeses. In order to highly the difference between the *four pasteurized* bleu veined cheeses the SF spectra was divided in two part the first one from 250-340 nm (**Fig. 2a**) and the second one from 340-500 nm (**Fig. 2b**). The band assigned to tryptophan excitation range is presented Fig. 2a. The tryptophan band exhibited a maximum located at 290 nm. The location of this band varied slightly from one cheese to another. Indeed, a slight red shift was observed for FA, FM and BA cheeses compared to BC cheeses. The shape of the spectra of tryptophan has been shown to be very sensitive to its environment [24].This shift can be explained by the exposing of more tryptophan residues to the aqueous phase of cheese [25]. This could be due the high level of proteolysis observed during ripening stage inducing an increase of the tryptophan residue exposed to solvent [26-28]. The WSN/TN for FA, FM, BA, and BC were 53.00%

(±0.85), 28.62% (±0.14), 68.57% (±0.62) and 68.54% (±2.84), respectively. These differences may also arise from the difference in cheeses pH that induced a difference in the tertiary and quaternary structures of casein micelles [26]. The pH of FA, FM, BA, and BC were 5.85% (±0.00), 5.57% (±0.05), 5.63% (±0.02) and 6.00% (±0.00), respectively. We can also note a difference in the width of this band from one cheese to another indicating a difference in the cheeses network structure partly resulting from different protein-protein interactions [26].

As the 4 categories of cheeses investigated in this study were manufactured by different processes leading to different textures, it was assumed that their structures, and as a consequence the environments of the fluorophores, were different [29].

The excitation fluorescence band assigned to vitamin A located in the fat globules of the cheeses showed a maximum around 322 nm. The fluorescence intensity of the spectra changed from one cheese to another suggesting a different physicochemical state of the triglyceride in cheese. The BC cheeses presented the highest fluorescence intensity, followed by BA, FM and FA. This can describe a difference in the crystallization level of lipids in cheese. Indeed, it has been previously reported [30,31,24], that the decrease in the vitamin A fluorescence intensities can be attributed to an increase of the lipids in a liquid state. This suggested that BC cheeses presented a high level of lipid in solid state and FA cheeses presented the lowest content of lipids in solid state. The difference could also be attributed to the protein-lipid and lipid-lipid interactions since previous investigation showed that the shape of vitamin A spectra depends on the physical state of triglycerides and the interactions [29,32,33] .

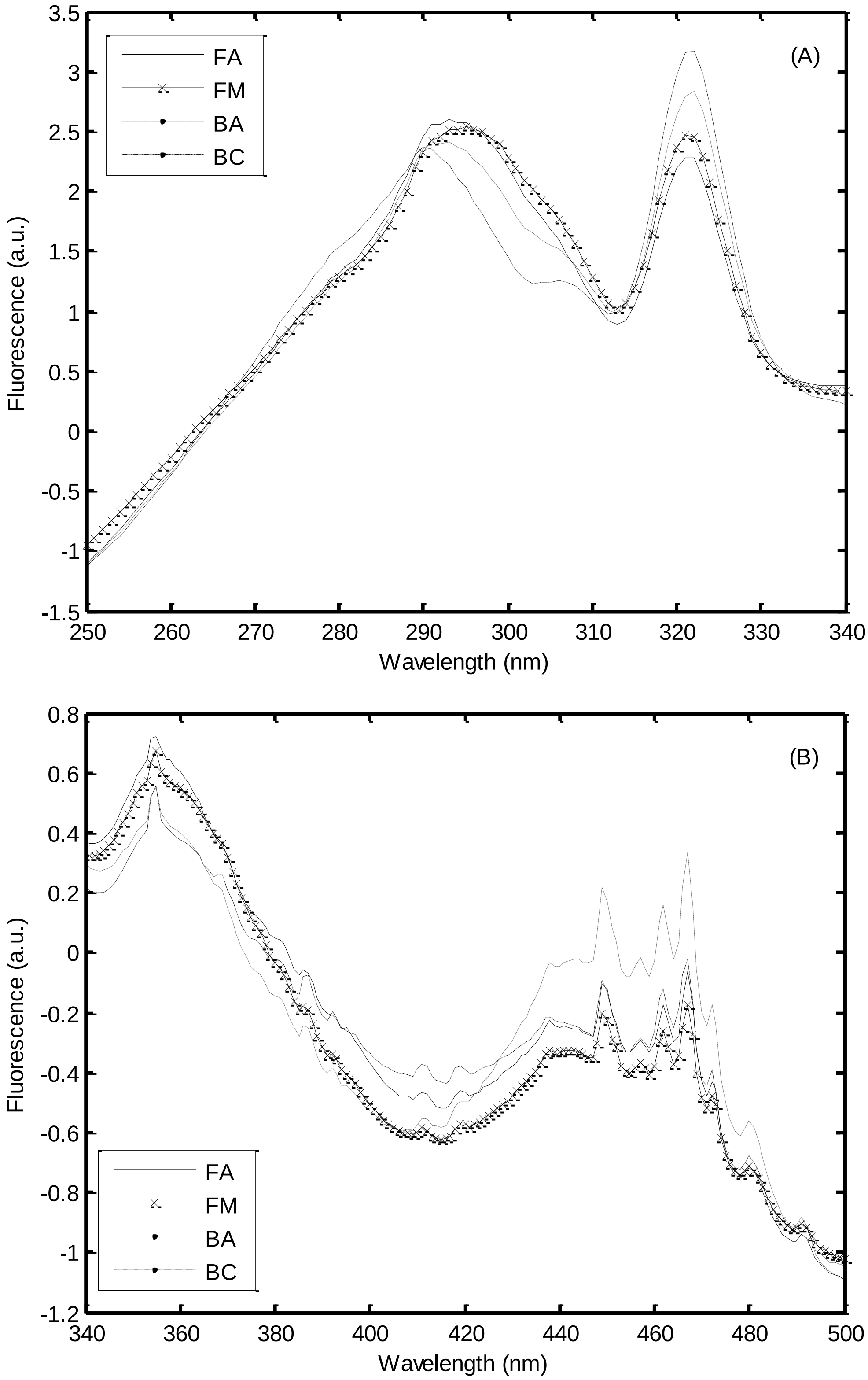


Fig. 2 Synchronous Fluorescence spectra of *Fourme d'Ambert* (FA), *Fourme de Montbrison* (FM), *Bleu des Causses* (BC), and *Bleu d'Auvergne* (BA) cheeses made from pasteurized milk and recorded between 250 – 500 nm. The spectra were divided in two parts to highly the differences between spectra: (a) from 250 – 340 nm, (b) from 340 – 500 nm.

The excitation fluorescence band centered around 355 nm and 460 nm were previously assigned to riboflavin [34,35], NADH/FADH in milk [34] and Maillard reaction products [36]. The Riboflavin is bound to proteins, predominantly albumin, but also immunoglobulins. Like other fluorophores, the fluorescence spectra of riboflavin showed difference of cheeses according to their manufacturing process; The difference could also be due to the level of oxidation in each cheese since the riboflavin spectra have been used as indicator of: (i) the oxidation in dairy products; and (ii) protein conformation and interaction changes in cheese matrix during ripening stage since it interacts with proteins [37,24]. It is well known that heat treatment applied to milk induced partial denaturation of milk proteins and formation of fluorescent Maillard reaction products. The four cheeses exhibited different fluorescence intensity of the bands at 355 nm and in the 440-465 nm range. Those differences could be assigned to the different matrix structure of cheeses (organization of the protein network) and different amounts of fluorescent Maillard products as pointed out by [36].

Univariate analysis is not really appropriate for the study of complex spectra. Multivariate analysis techniques (PCA, SIMCA, FDA, and PLSDA) are more appropriate in this case. For this purpose the 100 spectra collected from the four types of bleu cheeses were subjected to PCA after different preprocessing (normalization and standard normal variate) in order to compare the set of fluorescence spectra and to emphasize the similarities and differences underlined previously.

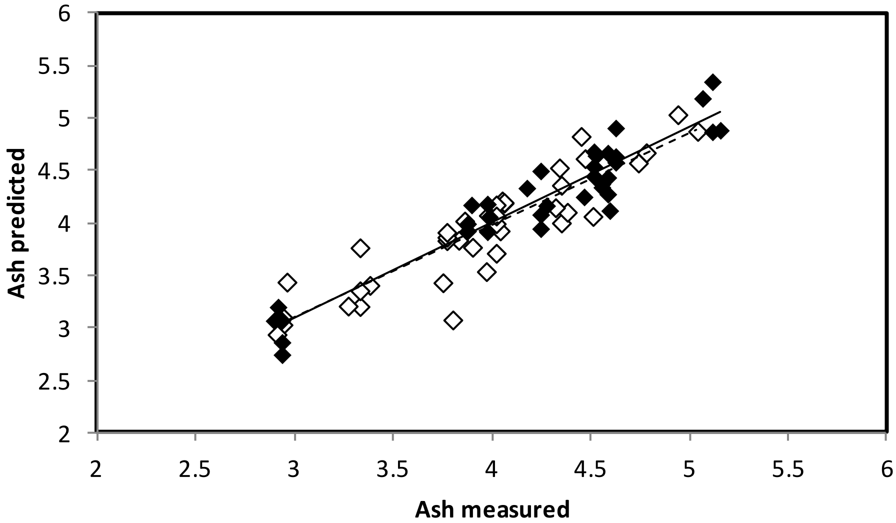
The PCA applied to the data sets showed that first two PCs took into account 68.5% of the total variation with PC_1 accounting for 38% of the total variance. The similarity map (data not shown) showed a slight discrimination of the four cheeses, since two groups were depicted named FA-FM and BA-BC, which was after confirmed by the PLSDA (data not shown).

The obtained results were not depicted by the PCA applied to the physico-chemical parameters; this difference is not surprising considering the fact that the manufacturing process of FA-FM differed from those named BA-BC. The FA and FM cheeses presented almost similar manufacturing process. The differences between them concerned principally the draining and salting techniques. The FA undergoes a reduced draining and salting with dry salt or brine on the surface; while the FM undergoes a pre-draining step, milling and salting in the mass of the curd, the drying stage is lying in the spruce gutters. Similarly, BA and BC cheeses presented mostly similar process since the difference is lies principally to their ripening times 28 days for the BA and 70 days for the BC.

Partial least squares regression

Three PLS-R models were considered: the first one including the *Bleu cheeses* (BA and BC), the second one including the *Fourme cheeses* (FA and FM) and the last one the 4 *Bleu veined cheeses* (FA, FM, BA and BC). The evaluation of the performance of the PLS models was investigated based on the R^2, RMSECV and the RPD for calibration and cross-validation sets (**Table 3**, **Fig. 3**).

(a)



(b)

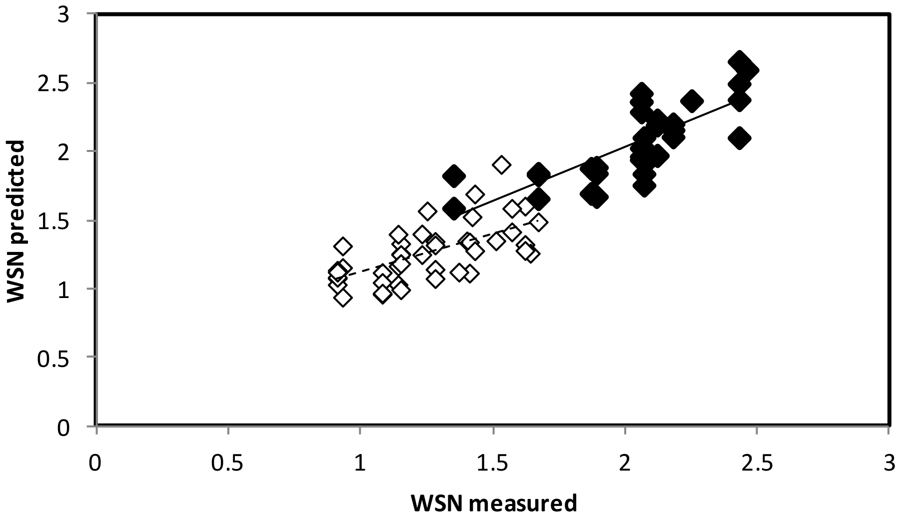
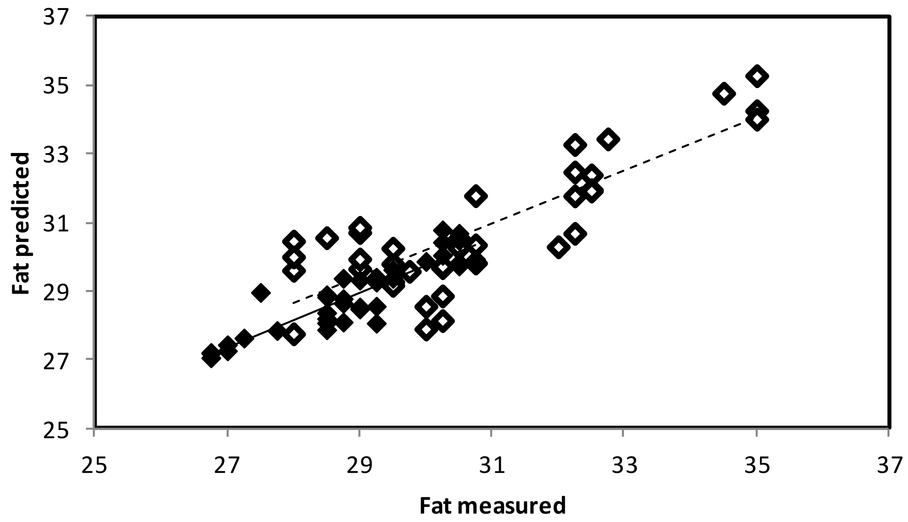


Fig. 3 Partial least squares regression models for the prediction of (a) Ash (%), (b) Fat (%dry mater), (c) Water Soluble Nitrogen (% dry mater), and (d) protein (% dry mater) for *Fourme cheeses* (◊) and *Bleu cheeses* classes (♦).

(c)



(d)

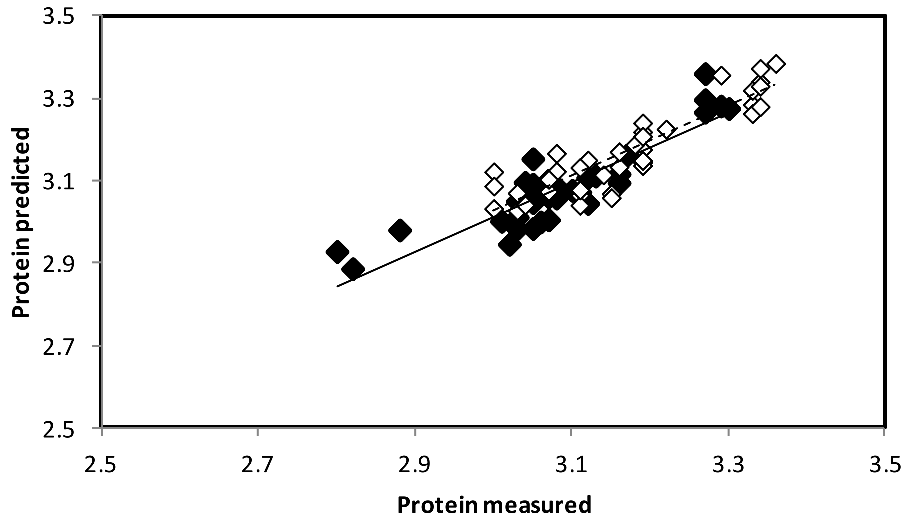


Fig. 3 (suite) Partial least squares regression models for the prediction of (a) Ash (%), (b) Fat (%dry mater), (c) Water Soluble Nitrogen (% dry mater), and (d) protein (% dry mater) for *Fourme cheeses* (◊) and *Bleu cheeses* classes (♦).

In a first step, PLS-R was applied to *Bleu cheeses* (BA and BC). For the calibration set, good correlations between the physicochemical values and the SF spectra were obtained for all the parameters $R^2 \geq 0.90$, excepting for fat where R^2 is of 0.83. The results concerning the cross validation models showed good correlations between ash and the SF spectra (R^2 = 0.90 and RPD = 3.17) (**Table 3**). Only approximate results were obtained for measurements of DM, SN, protein and pH although the high level of LV. While unsuccessful measurement was observed for WSN.

Regarding PLS-R obtained on *Fourme cheeses* (FA and FM), quite similar results to those found with *Bleu cheeses* (BA-BC) were observed (**Table 3, Fig. 3**). Approximate results were obtained for ash, fat and protein, while very poor result was observed for WSN. Acceptable for rough screening was found with pH.

When the two groups were pooled into one matrix and analyzed by PLS-R, the obtained results were almost less interesting compared to those obtained alone with *Fourme* or *Bleu* groups. Indeed, only approximate results were obtained with WSN, pH and fat, while those observed for ash, DM and protein could be considered as acceptable for only rough screening (**Table 3**). The obtained results are less better than those reported out by Karoui and others [25] on three different varieties of soft cheeses analyzed at the external and central zones since good results were obtained for pH, fat, DM, protein and WSN from tryptophan, vitamin A and riboflavin spectra, although the relatively low LV used in the PLS-R (less than 6) compared to the present study where a large numbers of LV was used. The results obtained in the present study could be due to several hypotheses. Firstable the huge heterogeneity of veined cheese samples due to the presence of blue and paste compared to soft cheeses; secondly, the difference in the results obtained by Karoui et al. and those obtained in the present investigation could arise from the small range of the physico-chemical parameters considered in the present investigation. Finally, SFS could not be appropriate for the prediction of physico-chemical parameters in blue cheeses; then it would be better to compare the obtained results with those obtained after excitation and emission wavelengths using classical fluorescence spectroscopy.

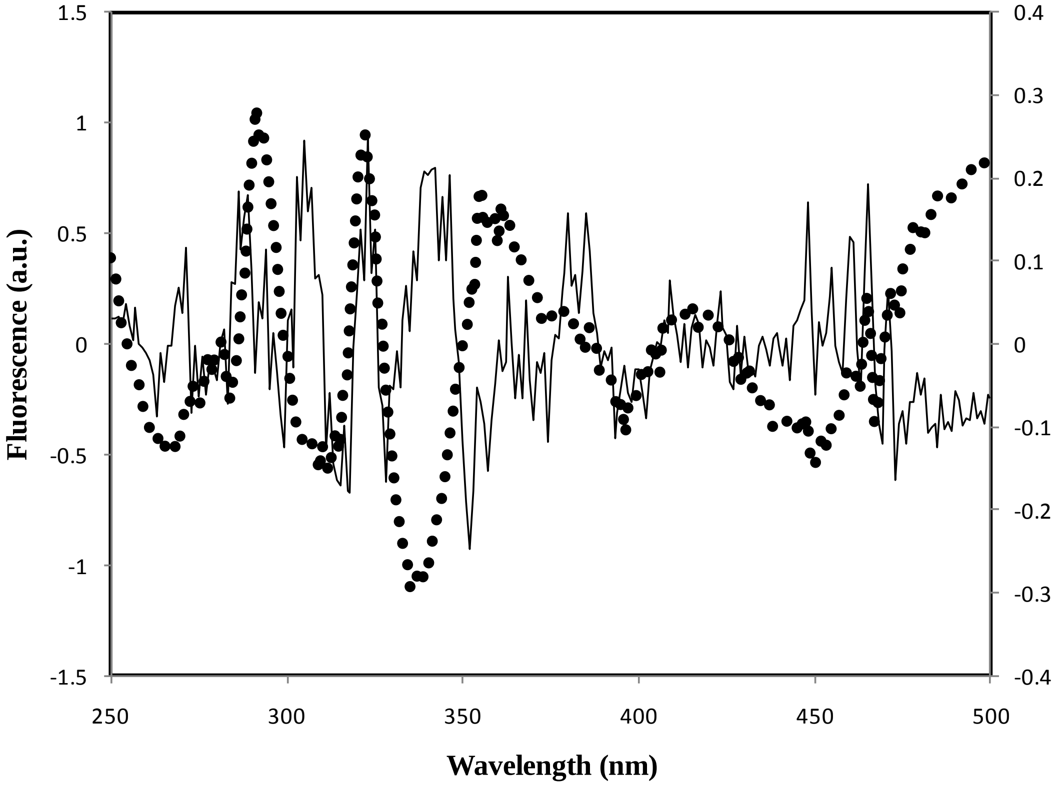
In order to identify which wavelength contributed most to the prediction of the physicochemical parameters the regression coefficient distribution were studied. Due to the poor model quality considering the global PLS-R model, only the regression coefficient of individual models for the *Bleu* and *Fourme cheese*s were studied. The regression coefficient spectra of Ash, DM, Fat, protein and pH for the *Fourme cheeses* (FA-FM) regression models and *Bleu cheeses* (BA-BC) regression models are presented in **Fig. 4**. The regression coefficients spectra of the different predictive models presented a noisy shape indicating a slight over-fitting of the models.

Table 3 Performance after leave-one-out cross-validation of the Partial least squares regression models of physicochemical composition for (Ash (%dry mater), Dry mater (DM%), Fat (%dry mater), Water Soluble Nitrogen (WSN% dry mater), protein (% dry mater), and pH) of bleu cheeses (RMSECV: Root mean square error of cross validation; R^2: Coefficient of determination of cross-validation; RPD: ratio of standard deviation to root mean square error of cross-validation; LV: loading variable). (FA-FM: model for the *Fourme cheeses*; BA-BC: model for the *Bleu cheeses*; BA-BC-FA-FM: model for all the *Bleu veined cheeses - Fourme* and *Bleu cheeses*-)

Physicochemical parameters	**Cheese**	**RMSECV**	**R^2**	**RPD**	**LV**
Ash	**BA-BC**	0.20	0.90	3.17	8
	FA-FM	0.241	0.81	2.29	10
	BA-BC-FA-FM	0.327	0.65	1.68	14
DM	**BA-BC**	1.00	0.80	2.22	9
	FA-FM	0.591	0.77	2.07	14
	BA-BC-FA-FM	1.067	0.64	1.67	12
FAT	**BA-BC**	1.15	0.67	1.75	7
	FA-FM	0.512	0.81	2.30	10
	BA-BC-FA-FM	0.976	0.75	2.01	14
WSN	**BA-BC**	1.15	0.57	1.52	12
	FA-FM	0.512	0.40	1.29	6
	BA-BC-FA-FM	0.210	0.80	2.21	10
Protein	**BA-BC**	0.05	0.80	2.24	7
	FA-FM	0.048	0.81	2.26	8
	BA-BC-FA-FM	0.070	0.62	1.62	8
pH	**BA-BC**	0.05	0.80	2.22	14
	FA-FM	0.212	0.62	1.62	13
	BA-BC-FA-FM	0.180	0.74	1.97	15

RMSECV: Root Mean Square Error of Cross Validation
R2: Coefficient of determination for cross-validation
RPD: ratio of standard deviation to root mean square error of cross-validation
LV: Loading Variable

(a)



(b)

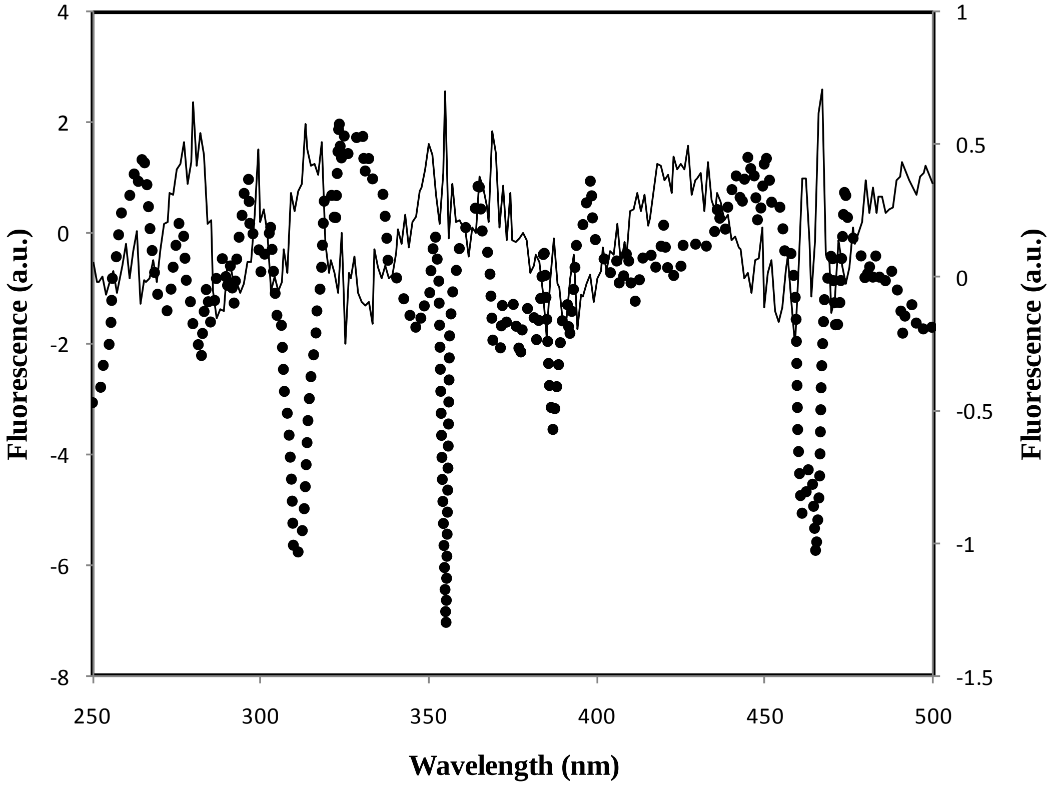


Fig. 4 Regression coefficient distribution over the 250-500 nm spectra of Partial least squares regression models of (a) Ash, (b) Fat, and (c) protein for *Fourme cheeses* (**--**) and *Bleu cheeses* classes (•••).

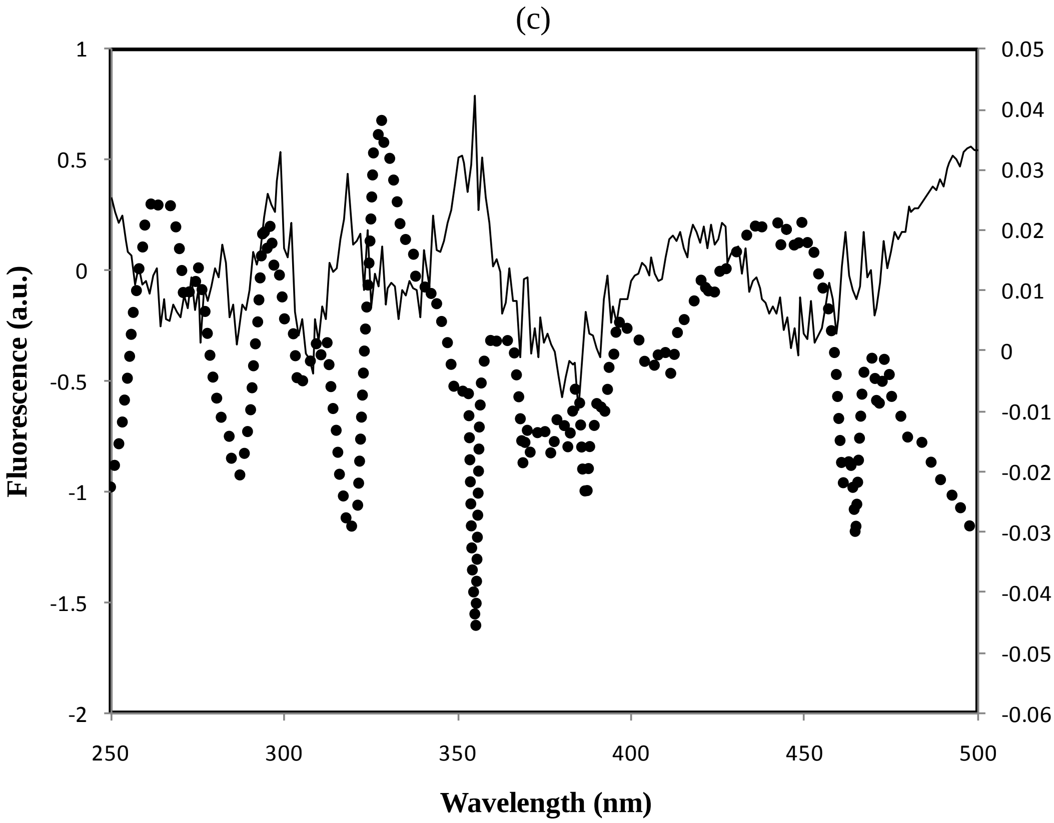


Fig. 4 (suite) Regression coefficient distribution over the 250-500 nm spectra of Partial least squares regression models of (a) Ash, (b) Fat, and (c) protein for *Fourme cheeses* (**--**) and *Bleu cheeses* classes (•••).

The regression coefficient of ash for the *Fourme cheeses* (FA-FM) regression models (**Fig. 4a**) presented different positive bands located at 305 (emission 385 nm), 313 (emission 393 nm), 323 (emission 403 nm), 338 (emission 418 nm), and one negative band at 352 nm (emission 432 nm). While the regression coefficient distribution of ash for the *Bleu cheeses* (BA-BC) model (**Fig. 4a**) presented two important positive bands located at 291 (emission 371 nm) and 321 (emission 401 nm) and one important negative band at 335 nm. The ones located to 323 and 321 are due to vitamin A, while that observed at 291 nm is due to tryptophan compound.

The regression coefficient distribution of the DM model for *Fourme cheeses* (FA-FM) (data not shown) identify three important positive bands at 305 (emission 385 nm), 313 (emission 393 nm), 355 nm (emission 465 nm) and one negative band at 286 nm (emission 316 nm). That obtained for Bleu cheeses (BA-BC) (data not shown) presented almost similar bands with an inversion of the sign at both 286 (emission 366 nm) and 355 nm (emission 435 nm) bands. The bands observed at 355 nm could be due to the emission of tryptophan compounds. The opposite band observed around 355 nm for the two groups of cheeses indicated that *Blue cheeses* and *Fourme cheeses* were in two different molecular environments (hydrophilic and hydrophobic) that could be due to the manufacturing process and the ripening time. A shift to higher wavelengths was observed for *Blue cheeses*.

The analysis of the regression coefficient distribution of fat for *Fourme cheeses* (FA-FM) model (**Fig. 4b**) presented different positive bands located at 280 (emission 360 nm), 313 (emission 393 nm), 355 (emission 393 nm), and 467 nm (emission 547 nm), while that for the *Bleu cheeses* (BA-BC) (Fig. 4c) presented 3 negative bands located at 310 (emission 390 nm), 355 (emission 435 nm), and 461 nm (emission 541 nm). The band observed at 310 and 313 nm could be due to vitamin A; again, an opposition between *Fourme group* and *Blue group* was observed around 310 nm indicating a difference in the crystallization level of triglyceride. The highest one was observed for *Blue cheeses*.

The regression coefficient distribution of WSN for the *Fourme cheeses* (FA-FM) (results not shown) presented different positive bands at 263 (emission 343 nm), 319 (emission 399 nm), 392 (emission 472 nm), 395 nm (emission 475 nm) and negative bands at 326 (emission 406 nm), 355 (emission 435 nm), 369 (emission 449 nm), and 461 nm (emission 541 nm), while that for the *Bleu cheeses* (BA-BC) (results not shown) presented positive bands at 307 (emission 387 nm), 355 (emission 435 nm), 411 (emission 491 nm), 462 nm (emission 542 nm) and three negative bands at 285 (emission 365 nm), 318 (emission 398 nm), and 385 nm (emission 465 nm).

The regression coefficient distribution of protein for the *Fourme cheeses* (FA-FM) (**Fig. 4c**) presented positive peaks at 295 (emission 375 nm), 318 (emission 398 nm), 355 nm (emission 435 nm) and two negative bands at 308 (emission 388 nm) and 450 nm (emission 530 nm). While, the regression coefficient distribution of protein for the *Bleu cheeses* (BA-BC) model (**Fig. 4c**) exhibited one positive peaks at 328 nm (emission 408 nm) and three negative bands at 355 (emission 435 nm), 387 (emission 467 nm) and 465 nm (emission 545 nm). The opposition in the bands located around 355 nm between the two groups could be due to the difference in the oxidation level. Indeed, it appeared from the spectral bands that blue cheeses are less oxidized than *Fourme cheeses*, in agreement with the results obtained in

Table 1. The 295 nm band can be assigned to the fluorescence of tryptophan amino acid, while the 355 nm band can be assigned to riboflavin.

The regression coefficient distribution of pH for *Fourme cheeses* (FA-FM) model (data not shown) presented four important negative peaks at 355 (emission 435 nm), 358 (emission 438 nm), 387 (emission 467 nm), and 462 nm (emission 543 nm). While the regression coefficient distribution for the *Bleu cheeses* (BA-BC) model (data not shown) presented 7 important bands. Four positive bands located at 298 (emission 378 nm), 312 (emission 392 nm), 344 (emission 424 nm), and 395 nm (emission 475 nm) and three negative bands located at 290 (emission 370 nm), 306 (emission 386 nm), and 360 nm (emission 440 nm).

Conclusion

This preliminary study demonstrated the potential of SFS to predict some physico-chemical parameter from heterogeneous and anisotropic matrix such as *Blue* and *Fourme cheeses* samples produced from raw, thermized and pasteurized milk samples. This study gives encouraging results, indeed SFS succeeded to predict ash and protein in *Blue* or *Fourme cheeses*. When the two groups were analysed jointly, the SFS failed to predict all the physico-chemical parameters. The regression coefficients allowed to identify the most interesting wavelengths allowing to differentiate between the two groups. A more complete calibration and validation phases (with a larger number of samples) will be necessary to obtain reliable SFS predictive models and to obtain a clear description of the chemical bands of the regression coefficient bands. More other, from the obtained results, it would be better to test the ability of SFS with only one variety of cheese (*Fourme d'Ambert*, *Fourme de Montbrison*, *Bleu d'Auvergne*, or *Bleu des Causes*) presenting the same manufacturing process from raw, thermized and pasteurized milk. This would probably help to emphasize the predictive results.

References

1. Downey G, Sheehan E, Delahunty C, O'Callaghan D, Guinee T, V.Howard (2005) Prediction of maturity and sensory attributes of Cheddar cheese using near-infrared spectroscopy. Int Dairy J 15:701–709

2. Karoui R, Mouazen AM, Dufour E, Schoonheydt R, De Baerdemaeker J (2006) Utilisation of front-face fluorescence spectroscopy for the determination of some selected chemical parameters in soft cheeses. Le Lait 86 (2):155-169

3. Karoui R, Mouazen AM, Dufour É, Pillonel L, Schaller E, Picque D, De Baerdemaeker J, Bosset JO (2006) A comparison and joint use of NIR and MIR spectroscopic methods for the determination of some parameters in European Emmental cheese. European Food Research and Technology 223 (1):44-50

4. Karoui R, Martin B, Dufour É (2005) Potentiality of front-face fluorescence spectroscopy to determine the geographic origin of milks from the Haute-Loire department (France). Lait 85 (3):223-236

5. Karoui R, Dufour E, Pillonel L, Picque D, Cattenoz T, Bosset JO (2004) Fluorescence and infrared spectroscopies: a tool for the determination of the geographic origin of Emmental cheeses manufactured during summer. Lait 84:359-374

6. Christensen J, Povlsen VT, Sorensen J (2003) Application of Fluorescence Spectroscopy and Chemometrics in the Evaluation of Processed Cheese During Storage. J Dairy Sci 86 (4):1101-1107

7. Christensen J, Becker E-CM, Frederiksen CS (2005) Fluorescence spectroscopy and PARAFAC in the analysis of yoghurt. Chemometrics and Intelligent Laboratory Systems 75:201-208

8. Ruoff Kea (2006) Authentication of the botanical and geographical origin of honey by front-face fluorescence spectroscopy. J Agric Food Chem 54:6858

9. Karoui R, Dufour E, Bosset JO, De Baerdemaeker J (2007) The use of front face fluorescence spectroscopy to classify the botanical origin of honey samples produced in Switzerland. Food Chemistry 101 (1):314-323

10. Karoui R, Cartaud G, Dufour E (2006) Front-face fluorescence spectroscopy as a rapid and nondestructive tool for differentiating various cereal products: A preliminary investigation Journal of Agricultural and Food Chemistry 54:2027–2034.

11. Karoui R, Schoonheydt R, Decuypere E, Nicolaï B, De Baerdemaeker J (2007) Front face fluorescence spectroscopy as a tool for the assessment of egg freshness during storage at a temperature of 12.2°C and 87% relative humidity Analytica Chimica Acta 582:83-91

12. Karoui R, NicolaÃ¯ B, De Baerdemaeker J (2008) Monitoring the Egg Freshness During Storage Under Modified Atmosphere by Fluorescence Spectroscopy. Food and Bioprocess Technology 1 (4):346-356

13. Karoui R, Kemps B, Bamelis F, De Ketelaere B, Merten K, Schoonheydt R, Decuypere E, De Baerdemaeker J (2005) Development of a rapid method based on front-face fluorescence spectroscopy for the monitoring of egg freshness: 2-evolution of egg yolk. Eur Food Res Technol

14. Karoui R, Thomas E, Dufour E (2006) Utilisation of a rapid technique based on front-face fluorescence spectroscopy for differentiating between fresh and frozen–thawed fish fillets Food Research International 39:349-355

15. Dufour E, Frencia JP, Kane E (2003) Development of rapid method based on front face fluorescence spectroscopy for the monitoring of fish freshness. Food Res Inter 36:415-423

16. Karoui R, Lefur B, Grondin C, Thomas E, Demeulemester C, Baerdemaeker JD, Guillard A-S (2007) Mid-infrared spectroscopy as a new tool for the evaluation of fish freshness. International Journal of Food Science & Technology 42 (1):57-64

17. Karoui R, Laguet A, Dufour E (2003) Fluorescence spectroscopy: A tool for the investigation of cheese melting - Correlation with rheological characteristics. Lait 83 (3):251-264

18. Purna SKG, Prow LA, Metzger LE (2005) Utilization of front-face fluorescence spectroscopy for analysis of process cheese functionality. Journal of dairy science 88 (2):470-477

19. Karoui R, Dufour E, Pillonel L, Picque D, Cattenoz T, Bosset JO (2004) Determining the geographic origin of Emmental cheeses produced during winter and summer using a technique based on the concatenation of MIR and fluorescence spectroscopic data. Eur Food Res Technol 219:184-189

20. Karoui R, Dufour E, Schoonheydt R, Baerdemaeker JD (2007) Characterisation of soft cheese by front face fluorescence spectroscopy coupled with chemometric tools: Effect of the manufacturing process and sampling zone. Food Chemistry 100: 632–642

21. Williams P (2003) Near-Infrared Technology-Getting the Best Out of Light. PDK Grain, Nanaimo, Canada.

22. Karoui R, Pillonel L, Schaller E, Bosset JO, De Baerdemaeker J (2007) Prediction of sensory attributes of European Emmental cheese using near-infrared spectroscopy: a feasibility study. Food Chemistry 101 (3):1121-1129

23. McSweeney PLH, Sousa MJ (2000) Biochemical pathways for the production of flavour compounds in cheese during ripening Le Lait 80:293-324

24. Karoui R, Dufour E, Baerdemaeker JD (2007) Monitoring the molecular changes by front face fluorescence spectroscopy throughout ripening of a semi-hard cheese. Food Chemistry 104: 409–420

25. Karoui R, Mouazen AM, Dufour E, Schoonheydt R, Baerdemaeker JD (2006) Utilisation of front-face fluorescence spectroscopy for the determination of some selected chemical parameters in soft cheeses. Lait 86 (2):155-169

26. Herbert S, Mouhous Riou N, Devaux MF, Riaublanc A, Bouchet B, Gallant DJ, Dufour É (2000) Monitoring the identity and the structure of soft cheeses by fluorescence spectroscopy. Le Lait 80 (6):621-634

27. Mazerolles G, Devaux MF, Dufour E, Qannari EM, Courcoux (2002) Chemometric methods for the coupling of spectroscopic techniques and for the extraction of the relevant information contained in the spectral data tables. Chemometrics and Intelligent Laboratory Systems 63 (1):57-68

28. Mazerolles G, Devaux MF, Duboz G, Duployer MH, Mouhous Riou N, Dufour E (2001) Infrared and fluorescence spectroscopy for monitoring protein structure and interaction changes during cheese ripening. Lait 81:509-527

29. Dufour E, Devaux MF, Fortier P, Herbert S (2001) Delineation of the structure of soft cheeses at the molecular level by fluorescence spectroscopy—relationship with texture. International Dairy Journal 11 (4-7):465-473

30. Dufour E, Lopez C, Riaublanc A, Mouhous Riou N (1998) La spectroscopie de fluorescence frontale : une approche non invasive de la structure et des interactions entre les constituants des aliments. Agoral 10:209-215

31. Boubellouta T, Dufour E (2010) Cheese-Matrix Characteristics During Heating and Cheese Melting Temperature Prediction by Synchronous Fluorescence and Mid-infrared Spectroscopies. Food and Bioprocess Technology:1-12

32. Karoui R, Mazerolles G, Dufour É (2003) Spectroscopic techniques coupled with chemometric tools for structure and texture determinations in dairy products. International Dairy Journal 13 (8):607-620

33. Karoui R, De Baerdemaeker J, Dufour E (2008) Utilisation of front face fluorescence spectroscopy as a tool for the prediction of some chemical parameters and the melting point of semi-hard and hard cheeses: A preliminary study Eur Food Res Technol 226:119-1126

34. Kulmyrzaev AA, Levieux D, Dufour E (2005) Front-Face Fluorescence Spectroscopy Allows the Characterization of Mild Heat Treatments Applied to Milk. Relations with the Denaturation of Milk Proteins. Journal of Agricultural and Food Chemistry 53 (3):502-507

35. Karoui R, Dufour É (2003) Dynamic testing rheology and fluorescence spectroscopy investigations of surface to centre differences in ripened soft cheeses. International Dairy Journal 13 (12):973-985

36. Kulmyrzaev A, Dufour E (2002) Determination of lactulose and furosine in milk using front-face fluorescence spectroscopy. Lait 82 (6):725-735

37. Karoui R, Dufour E, Baerdemaeker JD (2007) Front face fluorescence spectroscopy coupled with chemometric tools for monitoring the oxidation of semi-hard cheeses throughout ripening. Food Chemistry 101 1305–1314

III. Potentiel de la spectroscopie de fluorescence synchrone pour l'étude des changements structuraux de 4 types de fromages à pâte persillée lors de traitements thermiques

Ce chapitre est structuré en deux parties (III.3 et III.4) :

- la première partie décrit le potentiel de la spectroscopie de fluorescence ainsi que de la rhéologie (test de compression dynamique) pour décrire les modifications de la structure (micro et macrostructures) de quatre fromages à pâte persillée au cours du chauffage ;
- la deuxième partie décrit le potentiel de la spectroscopie de fluorescence ainsi que de la rhéologie (test de compression dynamique) pour décrire les modifications de la structure de quatre fromages à pâtes persillée au cours du refroidissement après un premier cycle de chauffage.

Ce chapitre fera l'objet d'un article scientifique qui sera intitulé «Potential of synchronous fluorescence spectroscopy to determine the physicochemical changes of four French blue cheeses during heating and cooling: correlation with rheological characteristics».
Les paragraphes III.3. et III.4. ont été rédigés en anglais et structurés sous forme de publication afin de faciliter la publication de l'article cité ci-dessus (articles N°4-1 et 4-2).

III.1. Objectifs et méthodologie

III.1.1. Objectifs

La consommation et les modes de consommations alimentaires évoluent au fil des années. Le consommateur s'oriente de plus en plus vers la consommation de plats élaborés et prêts à l'emploi. Dans le secteur des produits laitiers, le fromage est de plus en plus utilisé par le secteur industriel dans la composition et la formulation d'un vaste éventail de produits alimentaires (pizzas, sandwichs, gratins, plats cuisinés, gâteaux au fromage, desserts lactés, etc.). Ces fromages sont également utilisés en tant qu'ingrédients (fromages râpés, poudres de fromage, etc.). Les préparations industrielles ou non industrielles (chez le consommateur) de ces nombreux produits alimentaires nécessitent le plus souvent un traitement thermique. Le comportement des fromages sous l'action de la température dépend de leur texture qui dépend à la fois de la composition physicochimique des laits de départs mais aussi des procédés de fabrication (Dufour et al 2001 ; Herbet et al 1999 ; Herbert et al 2000 ; Karoui et al 2003). Il est donc important de caractériser le comportement des fromages lors de traitements thermiques, notamment pendant le chauffage et le refroidissement. En effet, les changements de température provoquent des altérations dans les propriétés physiques et chimiques des constituants du fromage qui influencent les propriétés globales du produit final (goût, apparence, texture, etc.). Une meilleure caractérisation du comportement des fromages sous l'action de la température représente l'une des clés de l'utilisation de ces produits comme ingrédient dans les plats cuisinés et du développement de fromages avec des propriétés fonctionnelles à chaud bien identifiées.

Il y a eu de nombreuses études sur l'évaluation des propriétés fonctionnelles à chaud des fromages de différentes variétés. Cependant, il y a peu d'informations disponibles dans la littérature sur les propriétés fonctionnelles à chaud et sur les changements structuraux des fromages à pâte persillée.

- Le premier objectif de ce travail était d'évaluer le potentiel de la spectroscopie de fluorescence synchrone ainsi que du test de compression dynamique pour caractériser les modifications structurales au cours du chauffage de fromages à pâte persillée (Fourme d'Ambert, Fourme de Montbrison, Bleu d'Auvergne et Bleu des Causses).

- Le deuxième objectif de ce travail était d'évaluer le potentiel de la spectroscopie de fluorescence synchrone ainsi que du test de compression dynamique pour déterminer les modifications structurales de fromages à pâte persillée (Fourme d' Ambert, Fourme de Montbrison, Bleu d' Auvergne et Bleu des Causses) lors d'un cycle de refroidissement faisant suite à un premier chauffage.

- Le troisième objectif était d'étudier les structures aux niveaux moléculaire et macroscopique de ces fromages et de mettre en évidence les corrélations entre ces 2 niveaux d'observation. Dans cette optique, la régression par les moindres carrés partiels (PLS-R) ainsi que l'analyse canonique des corrélations (ACC) ont été utilisées pour rechercher les corrélations entre ces deux domaines de mesure.

III.1.2. Méthodes

III.1.2.1. Les fromages à pâte persillée

20 fromages (tableau n°1) à pâte persillée trouvés dans le commerce et issus de dix entreprises différentes (8 fourmes d'Ambert, 4 fourmes de Montbrison, 4 bleus des Causses et 4 bleus d'Auvergne) ont été analysés dans cette étude. Ces fromages ont l'avantage d'être issu d'une large diversité de procédés de fabrications tant au niveau du traitement du lait (cru, thermisé, pasteurisé) que de celui du temps d'affinage (30 à 45 jours).

III.1.2.2. Analyses physicochimiques

Les analyses qui ont été réalisées sont la matière sèche (NF EN ISO 5534), la matière grasse (NF V04-287), l'azote total et soluble (NF EN ISO 8968-1), et les minéraux totaux (cendres). Le pH a été mesuré à l'aide d'un pH mètre (Schott CG840, Paris, France) après avoir dispersé 10 g de fromage râpé dans 50 ml d'eau distillée à 20°C.

III.1.2.3. Analyse par SFS et analyse rhéologique

Les analyses SFS et rhéologiques (test de compression dynamique) ont été réalisés en parallèles sur l'ensemble des 20 fromages à pâte persillée. Ces mesures ont été entreprises au cours du chauffage (20 à 80°C ; 3°C. min^{-1}) et du refroidissement (20 à 80°C ; 3°C. min^{-1}).

III.1.2.4. Analyses chimiométriques

Même si des différences apparaissent entre les spectres, l'évaluation de l'ensemble des données ne peut se faire qu'aux moyens des méthodes statistiques multidimensionnelles.
Dans un premier temps l'ACP ainsi que l'analyse PLSDA ont été appliquées séparément puis conjointement aux tableaux de données de spectroscopie et de rhéologie (test de compression dynamique) de chaque catégorie de fromage (FA, FM, BA, et BC) obtenu lors de la cinétique de chauffage (entre 20 et 80°C) et de refroidissement (entre 80 et 20°C) afin de définir les spécificités et les liens existants entre ces différents fromages.
L'analyse canonique des corrélations (CCA) ainsi que la régression par les moindres carrés partiels (PLS-R) ont été appliquées au tableau de données de spectroscopie de fluorescence et au tableau comprenant les données rhéologiques (G', G", tan(δ)) pour les températures 20, 35, 50, 65, 80 °C afin de définir les corrélations existantes entre ces deux jeux de données.

III.2. Résultats et Discussion

Dans cette étude préliminaire 20 fromages à pâte persillée appartenant à quatre catégories (FA, FM, BA et BC) ont été étudiés par les méthodes SFS et rhéologique (compression dynamique) pour tenter de caractériser leur structure et de leur texture lors du chauffage (20 à 80 ° C) et du refroidissement (80 à 20°C). Les résultats obtenus au cours de cette étude, sur un nombre certes limité de fromages, sont encourageants et laissent entrevoir la possibilité de prédire les caractéristiques rhéologiques à chaud et après refroidissement des fromages à pâte persillée à partir des données de spectroscopie de fluorescence. Comprendre l'origine de la texture des aliments nécessite une intégration de différents éléments, les plus importants pourraient être la composition chimique, la structure physique, et les caractéristiques sensorielles. Ainsi, il pourrait être intéressant d'étudier le lien ou les corrélations existantes entre ces différents éléments afin d'essayer de développer des produits ayant des fonctionnalités répondant aux besoins des industriels et aux attentes des consommateurs. Les méthodologies employées pour caractériser ces quatre catégories de fromages constituent un nouveau challenge en particulier pour l'innovation et la formulation de nouveaux produits à base de fromage à pâte persillée.

III. 3

ARTICLE 4.1: THE EFFECT OF HEATING ON THE STRUCTURE OF FOUR TYPES OF BLUE CHEESES BY FLUORESCENCE SPECTROSCOPY AND RHEOLOGY METHODS: PRELIMINARY STUDY

Introduction

Dairy products, like cheese, are mass consumption product other the world. Those products are subjected to different manufacturing process allowing developing their specific organoleptic quality (e.g. color, flavor, texture). Nonetheless, cheese texture is one of the most important factors in cheese quality. Indeed, food texture is one of the key properties consumers evaluate when determining food quality and acceptability. Food texture is defined as “all the rheological and structural (geometric and surface) attributes of the product perceptible by means of mechanical, tactile, and where appropriate, visual and auditory receptors” [31]. Thus, the cheese texture is a reflection of cheese structure at the microscopic and molecular levels. Structurally, cheese is a complex matrix of milk proteins principally caseins, fats, minerals, and other components including water. Cheese variety and composition influence component distribution which in turn largely determines the structural characteristics. It has been suggested that higher moisture content allows for greater movement of the casein matrix in hard cheeses and reduces resistance to, and increases recovery from, deformation in hard cheeses, i.e., recovery of the test sample to its original shape after deformation [19]. Moisture content has been reported to influence the fracture mechanism during biting and mastication [43]. Moreover, changing composition, such as decreasing fat content, is often associated with undesirable changes in texture [33]. Heat treatment is more and more performed on cheese due to the development of cheese ingredients [34]. The texture of cheese and more precisely the viscoelastic properties of cheese are strongly dependent on temperature. Temperature changes cause alterations in the physical and chemical properties of food components which influence the overall properties of the final product (e.g., taste, appearance, and texture). Several methods have been used to evaluate viscoelastic behavior of cheese [1] most of them are empirical and have a low repeatability. The development of the dynamic rheological tests allowed measuring the viscoelastic properties of cheese subjected to sinusoidal varying stresses or strains while recording the material response within the linear viscoelastic region. Generally, rheological measurements in the linear viscoelastic region are designed to probe the structure of a soft solid without damaging the structure. This method allows to obtain information on the sample macrostructure and to give information on the modification of the product texture.

Only few techniques enable the monitoring, at a molecular level, of the structural evolution of food samples during heat treatment. During the last two decades, there was a huge development of research based on spectroscopic methods for investigating intact food products and exploring structure–quality relationships. The most important spectroscopic method used in the dairy industry is the infrared spectroscopy in the mid (MIR) and near infrared (NIR) region of the electromagnetic radiation. The reasons for its importance are its speed, its reliability, its price etc. The fluorescence spectroscopy gain more and more interest in the analysis of dairy products because it is also a sensitive, rapid and noninvasive analytical technique that can provide information on the presence of fluorescent molecules and their environment in all sorts of biological samples. The development and improvement of chemometric methods [6,7] combined with the technical and optical development of spectrofluorometers have in recent years increased the possibilities for the use of fluorescence spectroscopy. Thus, online monitoring sensors that enable measurements of complete excitation emission spectra (fluorescence landscapes) are now commercially available. The interests of fluorescence and infrared spectroscopic methods for the nondestructive investigation of dairy products have been reviewed by several authors [10,20,22]. Previously heat treatment and structural changes during coagulation have successfully been investigated in milk using fluorescence spectroscopy [3,14,18]. Changes in fat and protein composition and structure have been characterized by the means of measuring the tryptophan and vitamin A fluorescence of cheeses during ripening [13,37] and for identification of different cheeses at a molecular level [11,17].

A large number of cheeses (Roquefort, Stilton, Gorgonzola, Fourme d'Ambert, Bleu d'Auvergne, Cabrales, etc.) are grouped under the category of blue cheeses. All blue cheeses share the same flavor-producing agent, the mould *Penicillium roquefortii*. Despite similarities, the blue cheeses exhibit differences, i.e. origin of the milk (cow's, ewe's), shape of cheeses, strains of the *Penicillium roquefortii*, and color of the blue veins [29].

Fourme d'Ambert (FA), Fourme de Montbrison (FM), Bleu d'Auvergne (BA) and Bleu des Causses (BC) are Protected Designation of Origin (PDO) cheeses both produced from cow's milk to a limited extent in Auvergne (France). PDO is a guarantee for consumers of the uniqueness of a food product, particularly cheeses, and an effective marketing tool for the producers [29]. There have been numerous studies on the effects of heating on viscoelastic characteristics of cheese varieties [15,16,28,33]. However, there is limited information available on changes in the viscoelastic properties during heating of blue cheeses. In France, a large proportion of blue cheeses can be used as an ingredient. Blue cheeses are used extensively in cooking, as an ingredient or as a condiment or as portions of grated, or to cover the meat and vegetables.

The objectives of the present study are to investigate the ability of the synchronous fluorescence spectroscopy method to determine physicochemical changes in 4 categories of French-blue cheeses during heating and to investigate the relationship between changes in spectral characteristics and rheological characteristics of cheese during heating. In a first part the analysis was focused on cheeses produced from milks that have undergone the same heating process (pasteurization). In a second part the analysis was generalized to the 20 French bleu veined cheeses.

Material and methods

French blue cheeses

This study was performed on 20 French blue veined cheeses produced from raw, thermized or pasteurized milk and belonging to four denominations: 8 *Fourme d'Ambert* (FA), 4 *Fourme de Montbrison* (FM), 4 *Bleu des Causses* (BC) and 4 *Bleu d'Auvergne* (BA) obtained from different companies and supermarkets located in the Auvergne region. The FA cheeses investigated presented different ripening times (30 and 45 days), while the ripening times of the other bleu cheeses were unknown. Upon arrival to our laboratory, each bleu veined cheese was frozen rapidly to -20°C and kept until analysis. To go deeper in the results analysis and interpretation one pasteurized cheese sample from one brand category were investigated and at the end all the cheeses were analyzed to generalize the observations and conclusions.

Physicochemical analysis

The physicochemical analyses performed on cheese samples were dry matter (DM), fat, protein and water-soluble nitrogen (WSN) contents using standards NF EN ISO 5534, NF V04-287, and NF EN ISO 8668-1, respectively (AFNOR, 2002). The pH was measured by a pH meter (Schott, CG840, Paris, France) after grating 10 g of cheese and dispersing it in 50 ml of ionized water. Salt content in cheeses was determined according to French standard (AFNOR: NF ISO 5843) using an automatic titrator (TitroLine easy, Model III, Schott, France) which is based on Volhard titrimetric test according to Marchall method [35]. Ash content was determined using the method of incineration of a sample (5 g) in a muffle furnace at 550°C for 6 h. All analyses were performed on cheeses produced from pasteurized milks and were done in triplicate. The results were reported as mean ± standard deviation.

Rheological Measurements

For rheological analysis, cheeses were sliced into thin disks (2 mm thick and 20 mm diameter) with a cheese slicer. The dynamic oscillatory analyses were performed with a rheometer (CP 20, TA Instrument, Guyancourt, France) equipped with a plate geometry of 20 mm diameter and a Peltier plate that provided very accurate and rapid temperature control. To determine viscoelastic properties with dynamic tests, oscillation analyses were performed in the linear viscoelastic region by applying a constant force of 0.1 N and a constant frequency of 1 Hz (force sweep tests indicated that this was within the linear viscoelastic region, results not shown). All analyses were carried out during heating from 20 to 80°C a rate of $3°C.min^{-1}$. The recorded data included the elastic component G' (storage modulus), the viscous component G" (loss modulus), and Tan δ (= G"/G'). All analyses were made in triplicate.

Synchronous fluorescence spectroscopy

Cheese SF (Synchronous fluorescence) spectra were recorded using a FluoroMax-2 spectrofluorimeter (Spex-Jobin Yvon, Longjumeau, France). The broad nature of conventional fluorescence spectrum and spectral overlap can be overcome and enhanced selectivity by using this technique. In SFS, the excitation wavelength (λ_{ex}) and emission wavelength (λ_{em}) are scanned simultaneously (synchronously), usually maintaining a constant wavelength interval, $\Delta\lambda$, between λ_{ex} and λ_{em}. In this study a $\Delta\lambda$ = 80 nm was identified as being the most suited value after analyzing spectral shape obtained at different $\Delta\lambda$ = 20, 40, 80, 100, 120 and 140 nm (data not shown). Before acquisition of spectra, cheeses were sliced into thin samples (2 cm x 1 cm x 0.3 cm) and placed in a quartz cell which was then mounted on a front face accessory, such that the incidence angle of the excitation radiation was set at 56°. SF spectra were recorded between 250 and 500 nm at different temperatures on the same samples during heating from 20 to -80°C at a rate of 3°C.min^{-1}. The spectrofluorimeter was equipped with a thermostatically controlled cell-holder and the temperature was controlled by a Haake temperature controller (Haake, Champlan, France). The SF spectra were recorded five times per sample.

Principal component analysis

Principal component analysis (PCA) is a modeling method that gives an interpretable overview of the main information in a multidimensional data table [2]. It produces a new smaller set of variables called principal components (PCs). By plotting the PCs, it is possible to distinguish interrelationships between different variables, and detect and interpret sample patterns, groupings, similarities or differences. The PCA method was applied to SF spectra after normalization (Standard Normal Variate).

Partial least squares regression

PLS-R is a recent technique that generalizes and combines features from PCA and multiple regressions. Its goal is to predict or analyze a set of dependent variables from a set of independent variables or predictors. This prediction is achieved by extracting from the predictors a set of factors called latent variables that realize a compromise between the variance explanation and the prediction of the response. In the present study PLS-R was used in order to predict rheological parameters from SF spectra recorded on four cheese brands. As the number of observations was small the regression models were validated by leave-one-out cross-validation.

Results and discussion

Physicochemical

The physicochemical characteristics of the four blues cheeses obtained from pasteurized milks are presented in **Table 1**. In order to identify the difference between physicochemical characteristics of the blue veined cheeses an ANOVA test ($P < 0.05$) was applied to the different data.

Considering DM content, no significant difference was observed between FM and BA, while significant difference was observed between FM-BA, FA and BC. These differences are probably due to the ripening conditions and more particularly to the drying stage that induced a difference in the evaporation level of water.

Considering WSN/TN %, FA, BA and BC presented the highest values with no significant difference between them while the FM presented the lowest value. These differences can be due to the ripening condition (e.g. temperature, duration) and proteolytic activities (e.g. plasmin, chymosin). It is well known that the WSN/TN % increases during the ripening time due to proteolysis [42]. For example, it is well known that one of the specificity of the BC cheese deals with its long ripening time usually performed (*i.e.* 70 days) compared to the ripening times usually performed for the other bleu cheeses (28 to 32 days).

Considering the salt content significant difference was observed between the four cheeses. The lowest salt content is obtained for FM, followed by BA, FA and finally BC. The significant differences in the salt content can be explained by the salting process that is conducted manually from one cheese to another and is subjected to variations. The highest difference between the salting process of the four bleu cheeses can be explained by the fact that the salting of the FA, BC, and BA is always performed by applying salt at the surface of the cheese, while the salting of the FM is always performed with salt in a solid state added in the curd and then mixed to be homogenized. It is well known that salt content influences the rate of casein hydrolysis during cheese ripening, and the extension of this degradation process has an important role in the development of the flavour and texture.

Considering the protein content, significant difference was observed between the four cheeses. The lowest protein content is obtained for BC, followed by FA, BA and finally FM.

Table 1 Physicochemical characteristics of French Blue cheeses obtained from pasteurized milks (FA: *Fourme d'Ambert*, FM: *Fourme de Montbrison*, BA: *Bleu d'Auvergne*; BC: *Bleu des Causses*)*.

physico-chemical Parameters	Blue cheese varieties			
	FA	FM	BA	BC
pH	5.85 (±0.00)[b]	5.57 (±0.06)[d]	5.63 (±0.03)[c]	6.00 (±0.00)[a]
Fat (%)	27.42 (±0.14)[c]	30.25 (±0.25)[a]	30.17 (±0.14)[a]	29.75 (±0.25)[b]
DM (%)	52.08 (±0.22)[c]	55.05 (±0.05)a	54.98 (±0.07)[a]	52.64 (±0.19)[b]
Fat in DM (%)	52.64 (±0.44)[c]	54.95 (±0.49)[b]	54.87 (±0.23)[b]	56.51 (±0.48)[a]
Protein (%)	19.21 (±0.10)[c]	20.33(±0.02)[a]	19.62 (±0.14)[b]	18.09 (±0.28)[d]
WSN/TN (%)	60.28 (±12.63)[a]	32.43(±6.60)[b]	68.57 (±0.62)[a]	68.54 (±2.82)[a]
Ash (%)	4.34 (±0.01)[b]	3.33 (±0.05)[d]	3.96 (±0.04)[c]	5.12 (±0.05)[a]
Salt (%)	1.82 (±0.07)[b]	1.01 (±0.02)[d]	1.56 (±0.00)[c]	2.17 (±0.01)[a]

*Values in brackets are standard deviation; DP: dropping point; SP: softening point; DM: dry matter

Considering the pH value, the four cheeses presented significant different pH values. The higher pH was observed for the BC cheese, followed by the FA cheese, then by the BA and finally the FM presented the lowest pH value. This could be attributed to the oxidation level of cheeses. The increase of the pH during ripening can be attributed to the depletion of lactic acid that induced a production of NH_3 [42]. Based on the pH values the FM cheeses seem to be the less oxidized samples. The variation of the pH was previously assigned to the firmness of the cheeses [40]. It was assumed that cheeses with low pH had the highest firmness. This was not confirmed in our study probably due to the heterogeneity of the structure due to the *Penicillium*. Indeed the study of Vassal and coworkers [40] was performed on Camembert cheeses without *Penicillim*.

Regarding the fat in dry matter content two groups can be identified with significant differences the FM-BA group and the FA-BC. The difference in fat content is probably assigned to the difference in milk fat content and to the difference in cheese manufacturing process.

Rheology

The dynamic viscoelastic parameters (G' and G'') of the four blue cheeses measured as a function of temperature during heating are shown in figure 1. For the 4 cheeses when the temperature increased, G' and G" decreased (Fig. 1) while Tan δ increased (data not shown). In the temperature region from ~20 to 35°C, the dynamic viscoelastic values decreased in a high extent, whereas in the temperature region from ~35 to 80°C, they decreased in a low extent. Those observations are in agreement with [4,24,39]. This can be attributed to the effect of temperature on protein-protein and protein-lipids interactions [4,24].

The drastic change of G' and G'' observed between ~20 and35°C is probably induced by fat liquefaction [32,33]. Lucey et al [33] noted that the increase in Tan δ occurs after the melting of fat in cheese (~ 35°C), and therefore must be caused by changes in the bonds and interactions involving the protein matrix. The limited changes in G' and G'' from ~ 35 to 80°C could be due to a mechanical resistance of the cheese network in relation to the thermal stability of the proteins. Studies conducted by [38] have shown that at high temperature the rigid structure of the casein micelles becomes increasingly mobile, which might be contributing to this fluid-like behavior. It appears that at high temperature, above ~ 65°C, there was some bond formation, which slightly increased the stiffness of the weak matrix. Hydrophobic interactions are known to increase until ~ 65°C, after which they start to diminish [8].

From figure 1, it appears that during the increase in temperature, FA cheese presented a less marked liquid like behavior since it is the firmest and the most rubbery cheese. Indeed, whatever the temperature, the FA cheese exhibited the highest values of G' and G", then the FM, then the BA and finally the BC cheese showed the lowest ones. These differences may be explained by the characteristics of the protein network and the gross composition of the cheeses (Table 1). Indeed, it was reported that the factors such pH, the salt concentration, moisture and protein breakdown markedly affect the viscoelastic properties of cheeses [41].

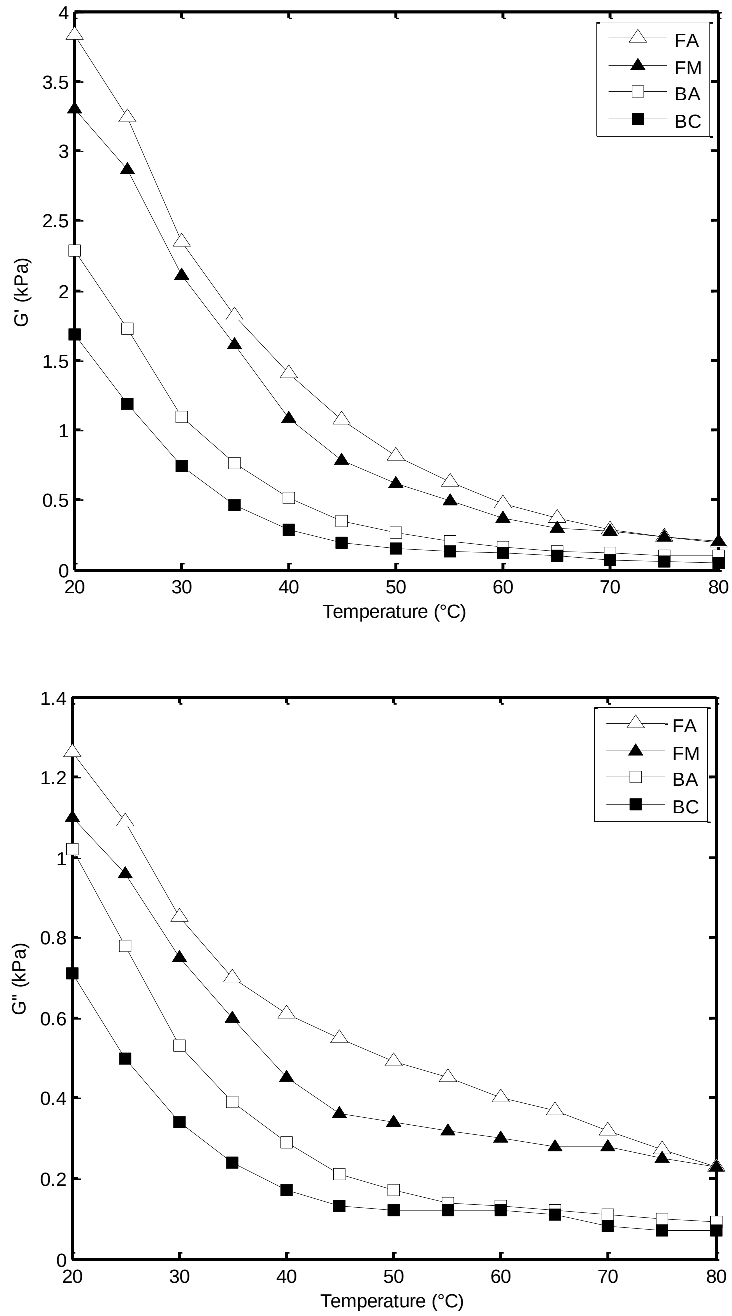


Figure 1 Viscoelastic parameters (G' and G") measured during heating (from 20 to 80°C) for the mean of Blue veined cheeses obtained from pasteurized milk (FA- *Fourme d'Ambert*, FM- *Fourme de Montbrison*, BA- *Bleu d'Auvergne*; BC- *Bleu des Causses*).

Two important parameters can be obtained by analyzing the cheese rheology data assigned to the cheese matrix and fat melting points. The value of Tan δ = 1 indicates the temperature where the viscous properties prevail, (G'' starts to dominate the G') i.e., the cheese starts to melt [33]. For the four cheeses almost the same cheese melting temperature was observed, 60.9°C (± 1.48°C), 60°C (± 0.84°C), 60.6°C (± 2.17°C) and 60.66 (± 2.75°C) for the FA, FM, BA and BC, respectively. These results are in the range of the melting points (55-60°C) reported for other varieties of cheeses (e.g. Emmental, Comté, …) [4]. The fat melting temperature in cheese is obtained from plotting log (η*) versus temperature during heating experiments [4]. The fat melting temperature obtained for FA, FM, BA and BC, are 28.3°C (± 3.38°C), 31.7°C (± 0.31°C), 31.7°C (± 1.28°C), and 32.6°C (± 0.45°C), respectively.

Direct Analysis of Fluorescence Spectra

Figure 2 a-d presents bleu cheeses SF spectra recorded at 20, 35, 50, 65, and 80°C. Almost the same fluorescence bands and spectral modifications were observed from one cheese to another. A Sharp and intense bands located at 295 nm (emission at 375 nm) and 322 nm (emission at 402 nm) were assigned to tryptophan residues of proteins and vitamin A, respectively [5,14,17,18,27]. A broader band centred at 355 nm (emission at 435 nm) and some small peaks around 449, 462, 467, 480 and 491 nm can be attributed to riboflavin and coenzymes (*e.g.* NADH, FADH) - riboflavin - Maillard compounds [5,26,30]. An increase of temperature from 20 to 80 °C drastically changed the synchronous spectrum of investigated cheeses. As previously reported by Boubellouta and Dufour [4] on Comté and Raclette cheeses, depending on the temperature values, bands tend to appear and disappear on the spectra. The highest intensity observed for the band at 322 nm (emission at 402 nm) (vitamin A) decreases drastically at 80°C. This modification could describe a degradation of the vitamin A and a modification of the physical state of the triglycerides. Indeed, different studies [14] demonstrated that the fluorescent properties of fluorophores are very sensitive to the changes in the solvent viscosity and physical state of triglycerides. For example, Dufour and other [12] demonstrated that the shape of the vitamin A excitation spectra in reconstituted milk fat globules is temperature dependent and therefore depends to the liquid or crystalline state of triglycerides. It was also demonstrated that a very good correlation exist ($R^2 = 0.997$) between the fluorescence data and the lipid level in liquid state determined by differential scanning calorimetry. The difference could also be attributed to the difference between protein-lipid and lipid-lipid interactions since previous investigation showed that the shape of vitamin A spectra depends on the physical state of triglycerides and the interactions [11,24].

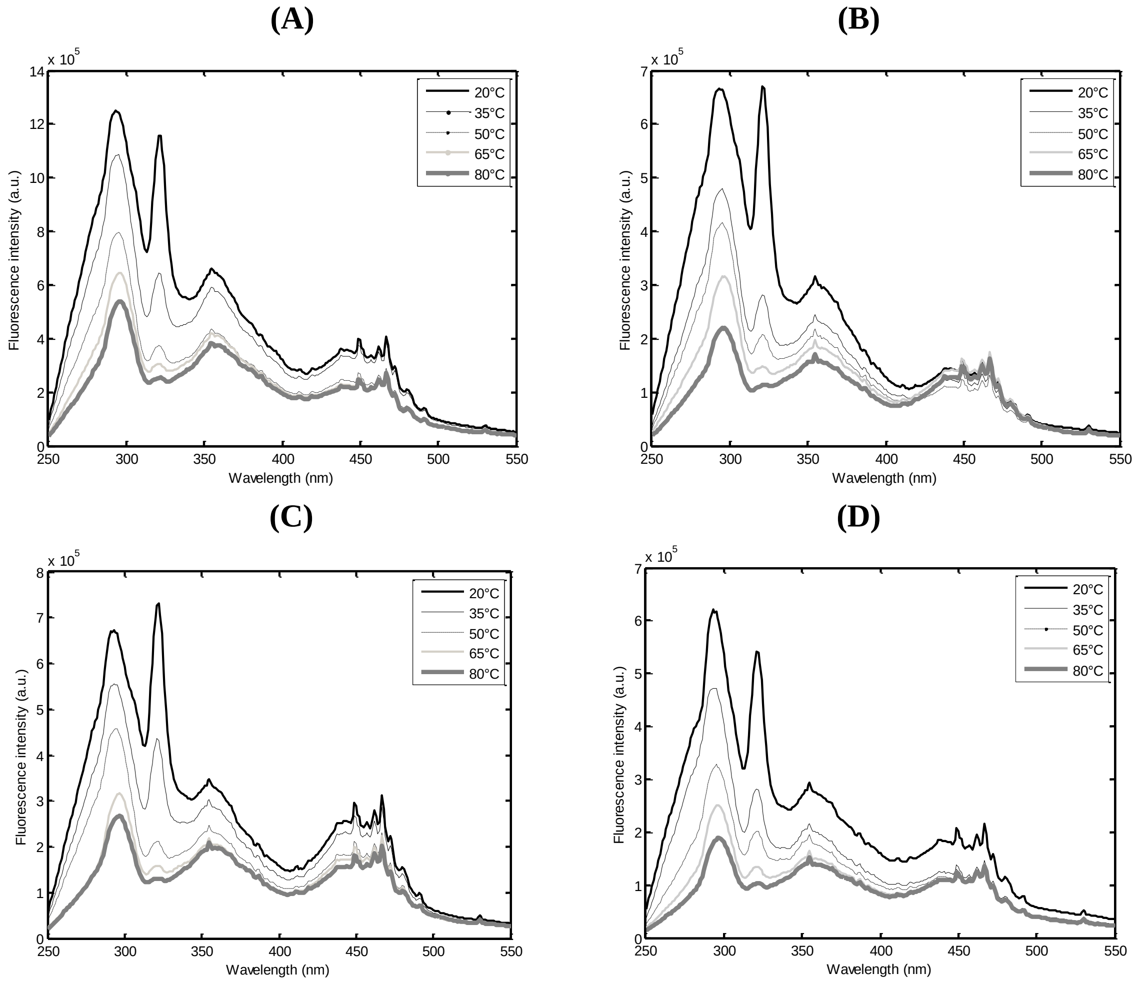


Figure 2. Synchronous fluorescence spectra (Δλ = 80 nm) recorded during heating from 20 to 80°C of bleu veined cheeses obtained from pasteurized milk. (A- Fourme d'Ambert cheese; B- Fourme de Montbrison cheese; C- Bleu des Causses cheese; D- Bleu d'Auvergne cheese).

The fluorescence emission of riboflavin is highly sensitive to its local environment, and it can be used as an indicator group for the oxidation in dairy products. It can also be used as an indicator group for protein conformation and interaction changes in cheese matrices during ripening since it interacts with proteins [25]. Thus, the decrease of the band centered at 450 nm during heating can suggest a modification of the protein matrix structure during heating and also the degradation of riboflavin [5].

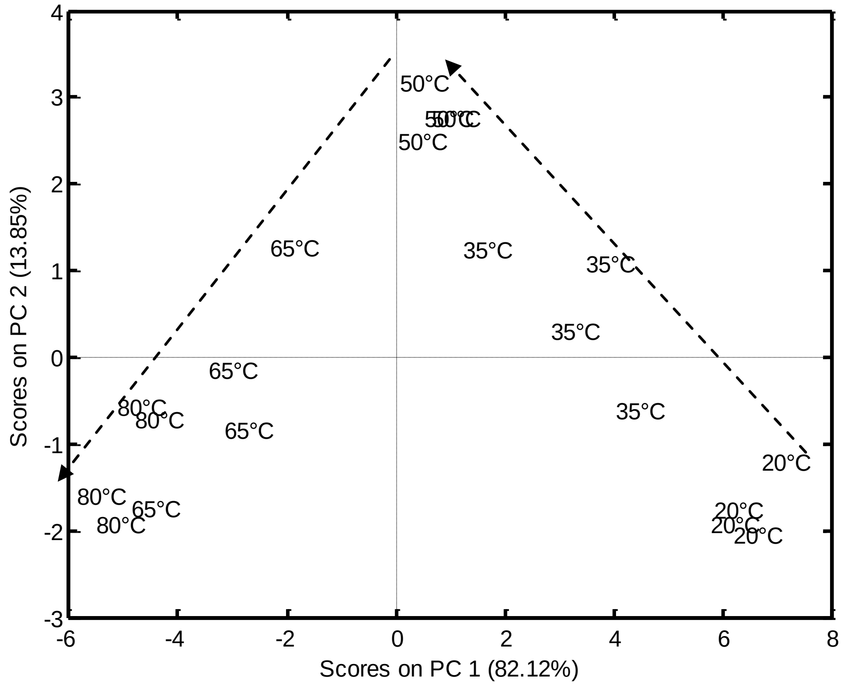
The other main changes were observed for band assigned to tryptophan residue of proteins. The intensity of tryptophan band drastically decreased during heating. In addition, as previously reported a slight shift of the band was also observed when the temperature increased: the maxima at 295 and 297 nm were observed for 20 and 80 °C, respectively. This shift can be explained by the exposing of more tryptophan residues to the aqueous phase of cheese [21]. Structural changes leading to the hydrophilic environment of tryptophan residues in protein can be related to the decrease of fluorescence intensity during cheese heating.

Principal Component Analysis of Fluorescence Spectra

PCA were applied individually on SF spectra of bleu cheeses to check the ability of synchronous spectra to retrieve information about the changes on the molecular structure during the melting of the cheese matrix. The first two principal components describe more than 95% of the total variance with a large predominance of the principal component 1 (82.12%). Considering the map defined by principal components 1 and 2, it appeared that spectra recorded at temperatures below 50°C exhibited positive scores according to the first principal component, whereas those recorded above 50°C exhibited negative scores (Fig. 3a). The loading associated to the first principal component presented a high positive peak located at 322 nm (vitamin A) and very small negative bands (close to zero) located at 290 (tryptophan), 360 (riboflavin) and 460 nm (riboflavin). This loading spectrum can be related to the modification of physical state of triglycerides during heating. This observation is in agreement with the studies of [4,5].

Considering the loading spectrum assigned to the principal component 2, the associated spectral pattern showed an opposition between a high negative band at 322 nm (vitamin A) and a high positive bands located at 290 nm (tryptophan) (Fig. 3b). This opposition between the bands at 299 and at 322 nm may be related to the changes in protein–protein and protein–lipid interactions during heating and to the different network structures resulting from cheese matrix melting [4,18,37]. In addition according to principal component 2, two anti-parallel phases in the changes of bleu cheese-matrix structure with temperature can be noted on the similarity map: the first one range between 20 and 50°C and the second one range between 50 and 80 °C. The top of this bell-shaped curve was observed for 50°C which is close to the melting temperature (60.6 °C) determined from rheology measurements. Similar results were obtained following the analysis of the SF spectra of the other bleu cheeses (results not shown). Those observations are consistent with the study performed on other cheese varieties [4].

(A)



(B)

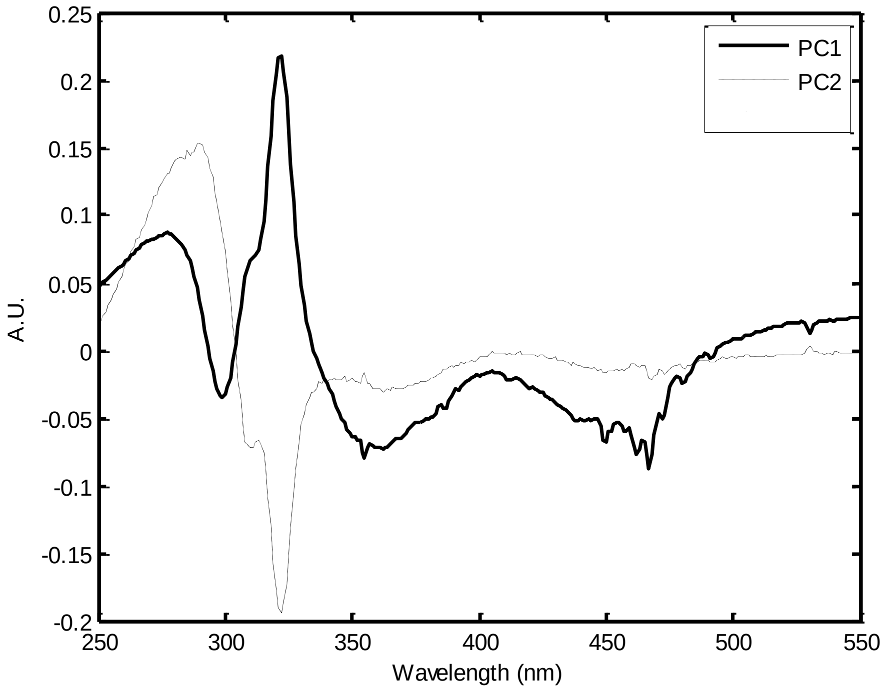


Figure 3. PC1 and PC2 Similarity maps (A) and associated spectral loadings (B) obtained after PCA of SF spectra recorded during heating (from 20 to 80°C) for the *Bleu d'Auvergne* cheeses obtained from pasteurized milk.

Principal Component Analysis of the Four Cheeses

Then, PCA was applied to the four Bleu cheeses obtained from pasteurized milk (Fourme d'Ambert, Fourme de Montbrison, Bleu d'Auvergne and Bleu des Causses) to check the ability of SF spectra to retrieve information about the changes and differences on the molecular structure during the melting of the different bleu cheeses matrix. The similarity map defined by the principal components 1 and 2 took into account 70.29% and 16.69% of the total variance, respectively. The similarity map described a good discrimination of the data sets on the principal component 1 according to temperature. The samples from 20 to 50°C presented positive scores while negative scores were noted from 65 to 80°C. The similarity map showed a relatively good discrimination of Bleu cheeses (BA and BC) from Fourme cheeses (FA and FM) between 20 and 50°C according to principal component 2 (Fig. 3). While a poorer discrimination of the four cheeses from 65 to 80°C was observed especially for BA, FA and FM according to the principal component 2 (Fig. 4a). The examination of the loading spectrum associated to the first principal component 1 presented a shape similar to the loading spectrum associated to principal component 1 presented in Fig. 4b. This loading spectrum presented a high positive peak located at 322 nm (vitamin A) and negative bands located at 290 (tryptophan), 360 (riboflavin) and 460 nm (riboflavin). This loading spectrum was assigned to the physical state modifications of triglycerides during heating. This observation could imply that the same physicochemical modifications of triglycerides occur during heating of bleu cheeses. The loading spectrum associated to the principal component 2 presented an opposition between one negative band located at 299 nm (tryptophan) and one positive band located at 322 nm (vitamin A). This loading spectrum was previously assigned to changes in protein– protein and protein–lipid interactions during heating and to the different network structures resulting from cheese matrix melting. This observation indicates that BC cheese presented a specific behavior during heating of cheese due to its specific microstructure especially from 50 to 80°C (**Fig. 4a**).

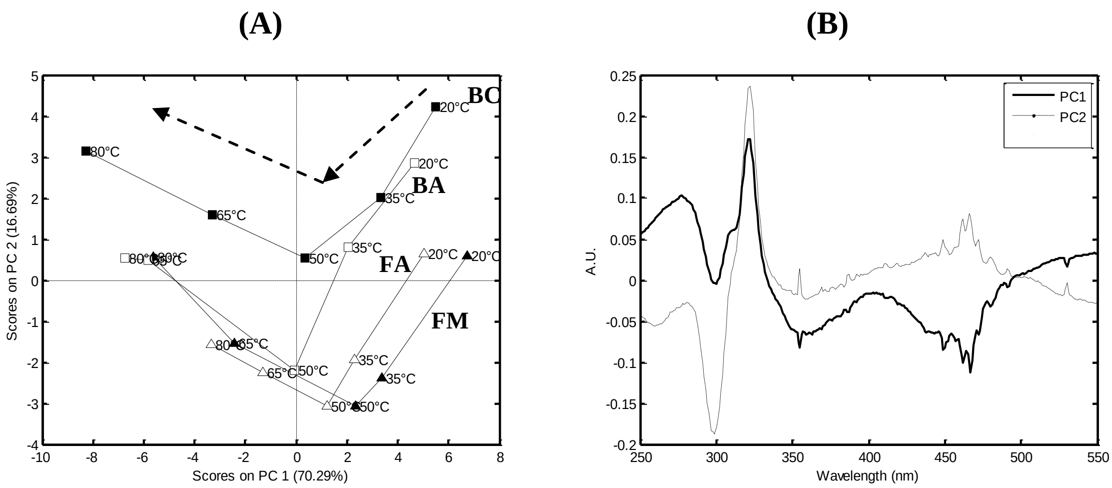


Figure 4. PC1 and PC2 Similarity maps (A) and associated spectral loadings (B) obtained after PCA of SF spectra recorded during heating (from 20 to 80°C) for the different blue cheeses obtained from pasteurized milk (FA: *Fourme d'Ambert*, FM: *Fourme de Montbrison*, BA: *Bleu d'Auvergne*; BC: *Bleu des Causses*)

Generalization of the analysis

Principal Component Analysis of rheological data

In order to generalize the observations previously noted for the bleu veined cheeses viscoelastic behavior. The viscoelastic parameters (G', G", and tan δ) recorded for the four bleu cheeses (5 repetitions of each type of cheese) during the heating cycle (20 to 80°C) were pooled in one matrix (x line and x column) and this table was analyzed by PCA. The principal component 1 and 2 took into account 75.37% and 20.82% of the total variance, respectively.

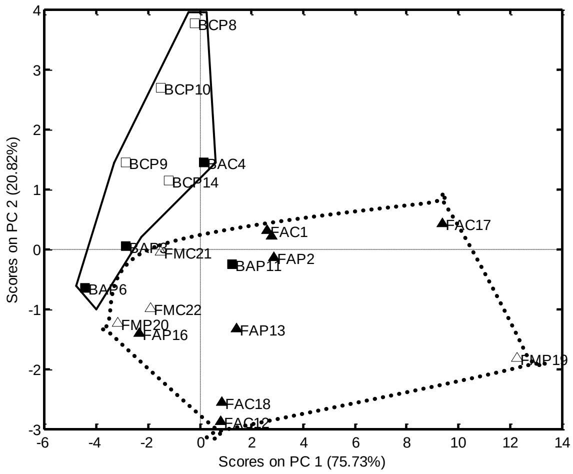


Figure 5. PC1 and PC2 Similarity maps obtained after PCA of rheological data (G', G" and tan δ) recorded during heating (from 20 to 80°C) for the different bleu cheeses (FA: *Fourme d'Ambert*, FM: *Fourme de Montbrison*, BA: *Bleu d'Auvergne*; BC: *Bleu des Causses*).

Considering the similarity map defined by principal component 1 and 2 (figure 5), it can be noted that the Fourme cheeses (FA and FM) can be discriminated from Bleu cheeses (BA and BC). A discrimination of FA cheeses from the other cheeses (FA- BA and BC) was essentially observed according to the principal component 1. Fourme d'Ambert cheeses, exhibiting the highest values of G' and G" (Figure 5), were on the positive side. The principal component 2 mainly discriminated BC cheeses from the other cheeses.

Principal Component Analysis of SF data

In order to generalize the observation previously reported about the changes on the molecular structure during the melting of the cheese matrix, the PCA was applied on the total samples of each brand. PCA was applied to the SF spectra recorded on the 8 FA (100 SF spectra), to the 4 FM (100 SF spectra), to the 4 BC (100 SF spectra) and finally to the 4 BA (100 SF spectra) cheeses. Thus four different PCA results were obtained (**Fig. 6 to 9**). The first two principal components describe more than 85% of the total variance with a large predominance of the principal component 1 (≥58.76%) whatever the cheese brand. Considering the map defined by principal components 1 and 2, it appeared that principal component 1 tends to discriminates the spectra according to temperature. It appears that the spectra recorded at temperatures below 50°C exhibited positive scores according to the first principal component, whereas those recorded above 50°C exhibited negative scores (**Fig. 6 to 9**). The loading associated to the first principal component presented a high positive peak located at 322 nm (vitamin A) and very small negative bands (close to zero) located at 299 (tryptophan), 360 (riboflavin) and 460 nm (riboflavin). This loading spectrum can be related to the modification of physical state of triglycerides during heating. Those observations are in agreement with our previous conclusions.

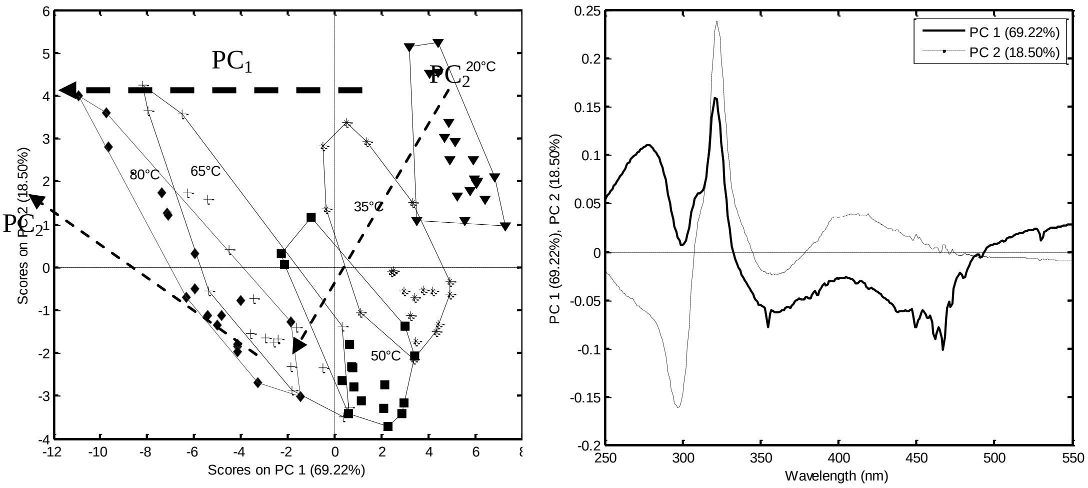


Figure 6. PC1 and PC2 Similarity maps and associated spectral loadings obtained after PCA of SF spectra recorded during heating (from 20 to 80°C) for the *Fourme d'Ambert cheese*

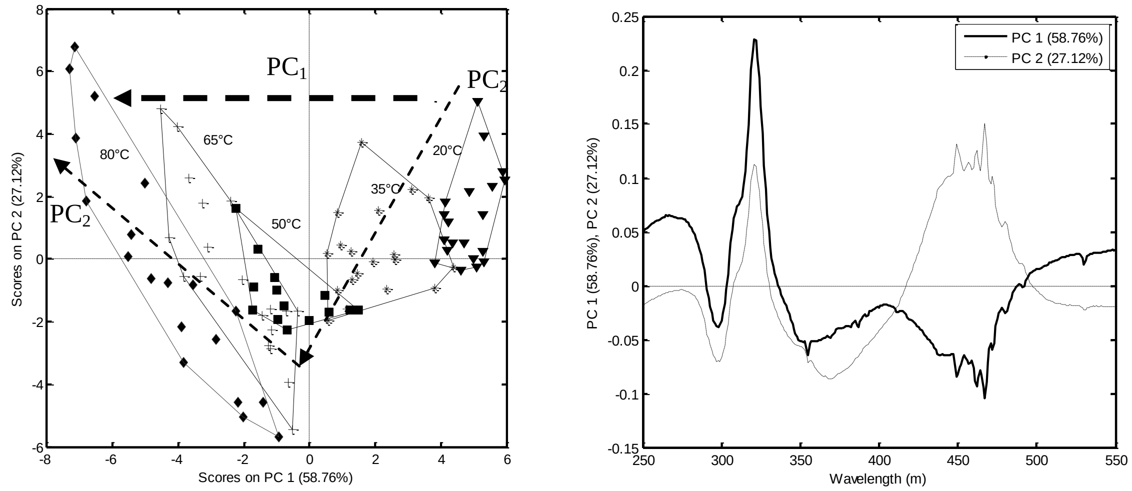


Figure 7. PC1 and PC2 Similarity maps and associated spectral loadings obtained after PCA of SF spectra recorded during heating (from 20 to 80°C) for the *Fourme de Montbrison cheese*

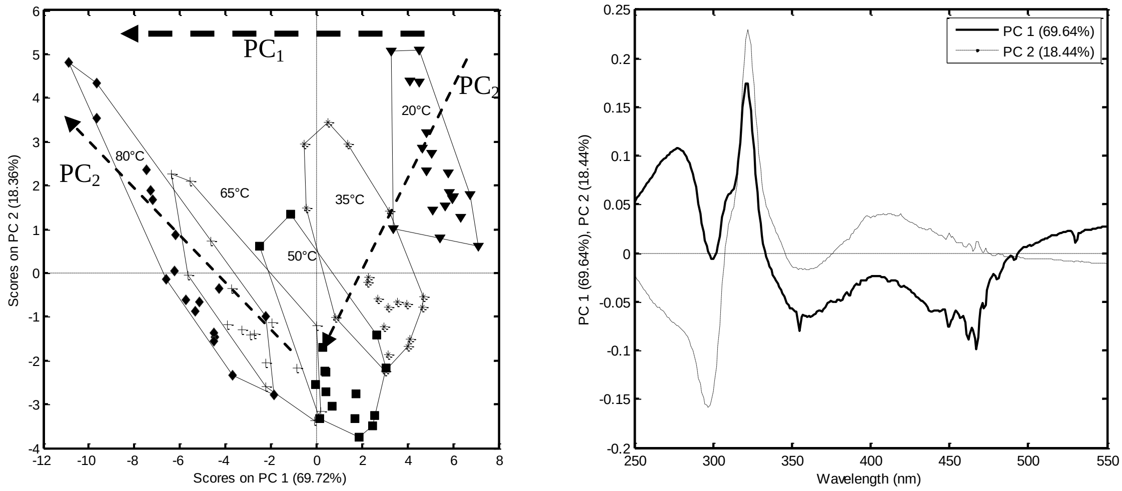


Figure 8. PC1 and PC2 Similarity maps and associated spectral loadings obtained after PCA of SF spectra recorded during heating (from 20 to 80°C) for the *Bleu d'Auvergne cheese*

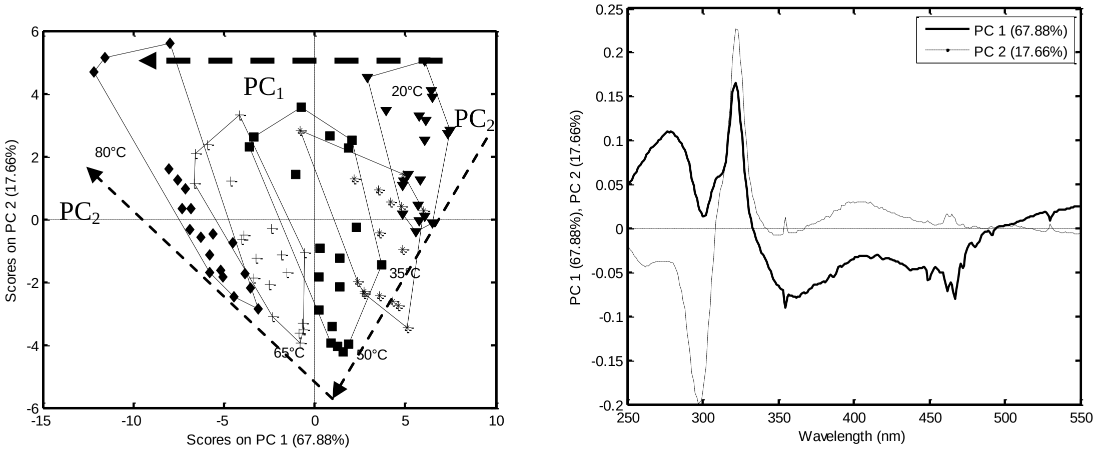


Figure 9. PC1 and PC2 Similarity maps and spectral loadings obtained after PCA of SF spectra recorded during heating (from 20 to 80°C) for the *Bleu des Causses cheeses*

Considering the loading spectrum assigned to the principal component 2, the associated spectral pattern showed an opposition between a high negative band at 322 nm (vitamin A) and a high positive bands located at 290 nm (tryptophan) (**Fig. 6-9)**. This opposition between the bands at 299 and at 322 nm was related to the changes in protein–protein and protein–lipid interactions during heating and to the different network structures resulting from cheese matrix melting. In addition according to principal component 2, two anti-parallel phases in the changes of bleu cheese-matrix structure with temperature can be noted on the similarity map: the first one range between 20 and 50°C and the second one range between 50 and 80 °C. Those observations are consistent with our previous conclusions.

Then, the SF spectra recorded during heating of the different bleu cheeses were gathered in one matrix and analyzed by PCA. The PCA results showed that the first two principal components took into account 79.30% of the total variance with a large predominance of principal component 1 (60.12%), discriminating the samples as a function of temperature (data not shown). The SF spectra recorded at low temperature (<50°C) had positive scores according to the principal component 1, whereas negative scores were observed for the ones recorded at high temperatures. No clear discrimination was observed between each bleu cheese brand, nonetheless the BA and BC scores on the similarity map composed by principal components 1 and 2 were closer and the same was noted for the FA and FM scores.

Partial Least Square Regression (PLS-R)

In order to identify the real link between the rheological data and the SF spectra PLS-R models were performed for each cheese brand separately (Fourme and Bleu cheeses) and for all the bleu veined cheeses (Fourme cheeses + Bleu cheeses). Indeed, in several cases (e.g. prediction of maturity and sensory attributes of cheeses), the analysis of the regression spectra can provide the fluorescence bands associated to rheological parameters of cheese [9]. Accordingly, the models developed for the prediction of rheological parameters are expected to provide realistic results [36].

The PLS-R results for the prediction of the tan δ (=G'/G") was investigated to identify the linl between rheological data and SF spectra. The performance of the PLS-R models can be evaluated by analyzing different factors (e.g. the root mean square error of cross validation — RMSECV, the determination coefficient of cross-validation - R^2 and the ratio of standard deviation to root mean square error of cross-validation — RPD).

According to R^2 value, the model is considered good for prediction if R^2 is > 0.81 and adequate only for approximate quantitative predictions if R^2 is between 0.66 and 0.81 [23]. The RPD factor was calculated by dividing the standard deviation of the data set by the RMSECV results. Karoui and Dufour [23] reported that a ratio greater than 2 indicates a good calibration whereas a ratio <1.5 indicates incorrect predictions.

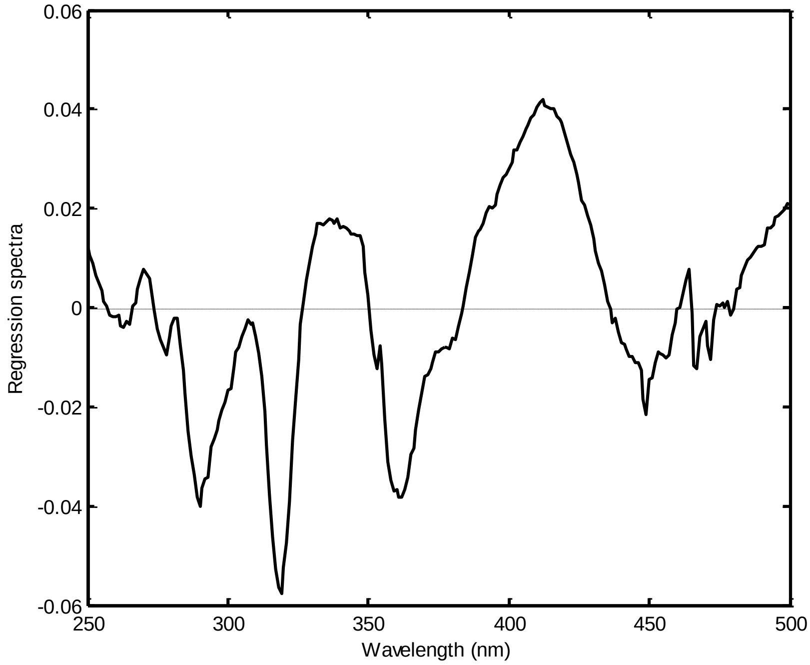
The results concerning the cross validation models showed good correlations between tan δ values and the SF spectra for the Bleu cheese class (R^2 = 0.82 and RPD = 2.36), the Fourme cheese class (R^2 = 0.81 and RPD = 2.29) and poor correlation values for the global model including all the bleu veined cheeses (R^2 = 0.77 and RPD = 2.08) (Table 2).

In order to identify which wavelength contributed most to the prediction of the rheological parameters the regression coefficient spectra for the FA-FM and BA-BC of the PLS-R models were studied (Figure 10). The regression spectra for the four categories of cheeses (BA-BC-FA-FM) were not analyzed due to its noisy profile (results not shown) resulting from the high number of latent variables used in the model (LV = 14). The analysis of the regression coefficient distribution of tan δ for *Fourme cheeses* (FA-FM) model (Fig. 10a) presented different negative bands located at 288 nm (emission 368 nm), 301 nm (emission 381 nm) , 321 nm (emission 401 nm) and 361 nm (emission 441 nm), and a positive band at 411 nm (emission 491 nm), while that for the *Bleu cheeses* (BA-BC) (Fig. 10b) presented 3 negative bands located at 277 nm (emission 357 nm), 321 (emission 401 nm), and 429 (emission xxx nm), and one positive band at 301 nm (emission 381 nm). The band observed at 277-288 nm could be due to tryptophan residue. The location of this band varied slightly from one cheese class to another. This suggests that the environment of the tryptophan residue is different between the Fourme cheeses (more hydrophilic) and the Bleu cheeses (less hydrophilic). Indeed, the shape of the spectra of tryptophan has been shown to be very sensitive to its environment. The bands located at 321 – 319 nm can be due to vitamin A. The bands located at 356-362 nm can be assigned to riboflavin and the large bands located at 411 - 429 nm can be assigned to riboflavin and Maillard reaction products.

Table 2: Performance after leave-one-out cross-validation of the Partial least squares regression models of rheological parameters (tan δ = G'/G") of bleu cheeses (RMSECV: Root mean square error of cross validation; R^2: Coefficient of determination of cross-validation; LV: loading variable; RPD:). (FA-FM: model for the *Fourme cheeses*; BA-BC: model for the *Bleu cheeses*; BA-BC-FA-FM: model for all the *Bleu veined cheeses* - *Fourme* and *Bleu cheeses*-)

	LV	RMSECV	R^2	RPD
FA-FM	5	0.11	0.82	2.36
BA-BC	3	0.10	0.81	2.29
BA-BC-FA-FM	14	0.12	0.77	2.08

(A)



(B)

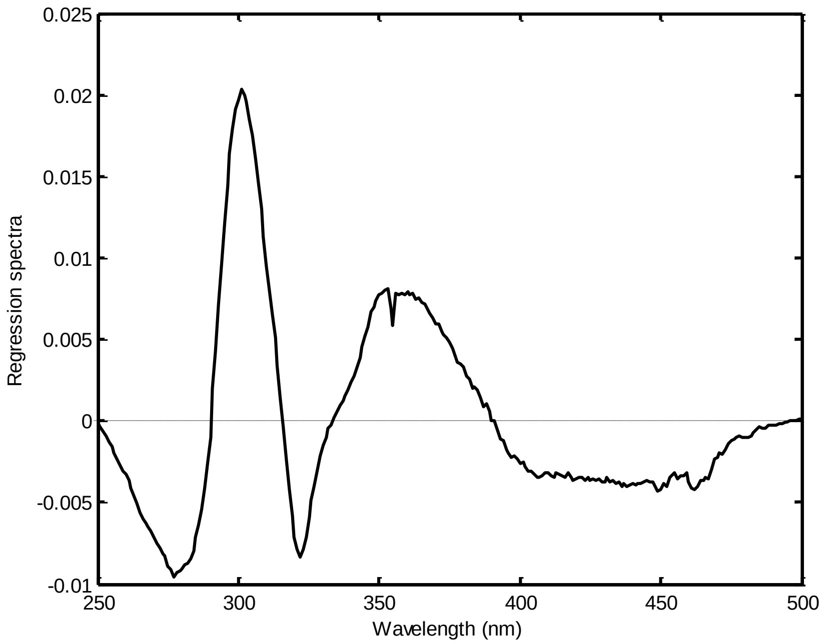


Figure 10. Regression coefficient distribution over 250-500 nm range of Partial least squares regression models of tan δ for *Fourme cheeses* (**A**) and *Bleu cheeses* classes (B).

Conclusion

In this preliminary study bleu veined cheeses belonging to four brands (FA, FM, BA and BC) were studied by SFS and dynamic testing rheology measurements in attempt to monitor changes in structure and texture occurring during heating from 20 to 80°C. It was demonstrated that viscoelastic measurements of bleu veined cheeses is a valuable tool to monitor and characterize the melting point of fat in cheese and the melting of cheese matrix. This study demonstrated also that SFS is a valuable tool to outline monitor and characterize modification of the microstructure of bleu veined cheeses, based (*i*) on the analysis of the SF spectra by chemometrics methods and (*ii*) on its correlation with the viscoelastic measurement characteristics (Tan δ) measured by PLS-R. Thus the rheological attributes of cheeses may be derived from SF spectra.

Understanding food structure and texture requires an integration of different element, ones of the most important could be the chemical, physical, and sensorial elements. Thus it could be interesting to study the link or correlation between the sensory, the fluorescence spectroscopy and the instrumental textural data that will permit to develop techniques for determining effects of process and/or composition on texture and to develop new food products that meet consumer expectations.

References

[1] Ak M.M., Gunasekaran S., Linear Viscoelastic Methods, in: S. Gunasekaran (Ed.), Nondestructive food evaluation Marcel Dekker, Inc., New York, 2001, p. 287.

[2] Bertrand D., Les méthodes d'analyse rapides dans les industries agroalimentaires, in: D. Bertrand, E. Dufour (Eds.), La spectroscopie infrarouge et ses applications analytiques, Lavoisier, Paris, France, 2006, pp. 4-28.

[3] Birlouez-Aragon I., Nicolas M., Metais A., Marchond N., Grenier J., Calvo D.A., Rapid Fluorimetric Method to Estimate the Heat Treatment of Liquid Milk, International Dairy Journal 8 (1998) 771–777.

[4] Boubellouta T., Dufour E., Cheese-Matrix Characteristics During Heating and Cheese Melting Temperature Prediction by Synchronous Fluorescence and Mid-infrared Spectroscopies, Food and Bioprocess Technology (2010) 1-12.

[5] Boubellouta T., Dufour E., Effects of Mild Heating and Acidification on the Molecular Structure of Milk Components as Investigated by Synchronous Front-Face Fluorescence Spectroscopy Coupled with Parallel Factor Analysis, Applied Spectroscopy 62 (5) (2008) 490-496.

[6] Bro R., PARAFAC. Tutorial and applications, Chemometrics and Intelligent Laboratory Systems 38 (2) (1997) 149-171.

[7] Bro R., Van den Berg F., Thybo A., Andersen C.M., Jorgensen B.M., Andersen H., Multivariate Data Analysis as a Tool in Advanced Quality Monitoring in the Food Production Chain. , Trends in Food Science and Technology 13 (2002) 235-244.

[8] Bryant C.M., McClements D.J., Molecular basis of protein functionality with special consideration of cold-set gels derived from heat-denatured whey, Trends Food Sci. Technol. 9 (1998) 143-151.

[9] Downey G., Sheehan E., Delahunty C., O'Callaghan D., Guinee T., V.Howard, Prediction of maturity and sensory attributes of Cheddar cheese using near-infrared spectroscopy, Int. Dairy J. 15 (2005) 701–709.

[10] Dufour É., Recent advances in the analysis of dairy product quality using methods based on the interactions of light with matter, International Journal of Dairy Technology 64 (2) (2010) 153-165.

[11] Dufour E., Devaux M.F., Fortier P., Herbert S., Delineation of the structure of soft cheeses at the molecular level by fluorescence spectroscopy-relationship with texture, International Dairy Journal 11 (4-7) (2001) 465-473.

[12] Dufour E., Lopez C., Riaublanc A., Mouhous Riou N., La spectroscopie de fluorescence frontale : une approche non invasive de la structure et des interactions entre les constituants des aliments, Agoral 10 (1998) 209-215.

[13] Dufour E., Mazerolles G., Devaux M.F., Duboz G., Duployer M.H., Mouhous Riou N., Phase transition of triglycerides during semi-hard cheese ripening, International Dairy Journal 10 (1-2) (2000) 81-93.

[14] Dufour E., Riaublanc A., Potentiality of spectroscopic methods for the characterisation of dairy products. I. Front-face fluorescence study of raw, heated and homogenised milks, Le Lait 77 (6) (1997) 657-670.

[15] Foegeding E.A., Brown J., Drake M.A., Daubert C.R., Sensory and mechanical aspects of cheese texture, Int. Dairy J 13 (2003) 585-591.

[16] Gunasekaran S., Ak M.M., Measuring cheese melt and flow properties, in Cheese Rheology and Texture in: B.R. CRC Press, FL, (Ed.), United States of America, 2003, pp. 331–375.

[17] Herbert S., Mouhous Riou N., Devaux M.F., Riaublanc A., Bouchet B., Gallant D.J., Dufour É., Monitoring the identity and the structure of soft cheeses by fluorescence spectroscopy, Le Lait 80 (6) (2000) 621-634.

[18] Herbert S., Riaublanc A., Bouchet B., Gallant D.J., Dufour E., Fluorescence Spectroscopy Investigation of Acid-or Rennet-Induced Coagulation of Milk, J. Dairy Sci. 82 (10) (1999) 2056-2062.

[19] Jack F.R., Paterson A., Piggott J.R., Relationships between rheology and composition of Cheddar cheeses and texture as perceived by consumers, International Journal of Food Science and Technology 28 (3) (1993) 293-302.

[20] karoui R., Blecker C., Fluorescence Spectroscopy Measurement for Quality Assessment of Food Systems—a Review, Food and Bioprocess Technology DOI 10.1007/s11947-010-0370-0 (2010).

[21] Karoui R., Cartaud G., Dufour E., Front-face fluorescence spectroscopy as a rapid and nondestructive tool for differentiating various cereal products: A preliminary investigation Journal of Agricultural and Food Chemistry 54 (2006) 2027–2034.

[22] Karoui R., De Baerdemaeker J., A review of the analytical methods coupled with chemometric tools for the determination of the quality and identity of dairy products, Food Chemistry 102 (3) (2007) 621-640.

[23] Karoui R., Dufour É., Development of a portable spectrofluorometer for measuring the quality of cheese, Dairy Science and Technology 88 (4-5) (2008) 477-494.

[24] Karoui R., Dufour É., Dynamic testing rheology and fluorescence spectroscopy investigations of surface to centre differences in ripened soft cheeses, International Dairy Journal 13 (12) (2003) 973-985.

[25] Karoui R., Dufour É., Prediction of the rheology parameters of ripened semi-hard cheeses using fluorescence spectra in the UV and visible ranges recorded at a young stage, International Dairy Journal 16 (12) (2006) 1490-1497.

[26] Karoui R., Dufour E., Baerdemaeker J.D., Front face fluorescence spectroscopy coupled with chemometric tools for monitoring the oxidation of semi-hard cheeses throughout ripening, Food Chemistry 101 (2007) 1305–1314.

[27] Karoui R., Mazerolles G., Dufour É., Spectroscopic techniques coupled with chemometric tools for structure and texture determinations in dairy products, International Dairy Journal 13 (8) (2003) 607-620.

[28] Kindstedt P.S., Caric M., Milanovic S., Pasta-Filata cheeses. In: Cheese Chemistry, Physics and Microbiology, Vol. 2, Major Cheese Groups, 3rd edn, pp. 251–277, Elsevier Academic Press, Amsterdam., 2004.

[29] Kulmyrzaev A., Bertrand D., Dufour E., Characterization of different blue cheeses using a custom-design multispectral imager, Dairy Sci. Technol. 88 (4-5) (2008) 537-548.

[30] Kulmyrzaev A., Dufour E., Determination of lactulose and furosine in milk using front-face fluorescence spectroscopy, Lait 82 (6) (2002) 725-735.

[31] Lawless H.T., Heymann. H., Sensory Evaluation of Food, Aspen Publishers, Gaithersburg, MD., 1999.

[32] Lefevere I., Dewettinck K., Huyghebaert A., Cheese fat as driving force in cheese flow upon melting, Milchwissenschaft 55 (2000) 563-565.

[33] Lucey J.A., Johnson M.E., Horne D.S., Perspectives on the basis of the rheology and texture properties of cheese, Journal of dairy science 86 (9) (2003) 2725-2743.

[34] Mann E., Cheese product innovations, Dairy Industries International 65 (10) (2000) 17-18.

[35] Marshall R., Chemical and physical methods. In: Standard methods for the examination of dairy products. 16th ed.Washington, D.C.: American Public Health Assn. p 4333–529., (1993).

[36] Martín-del-Campo S.T., Picque D., Cosío-Ramírez R., Corrieu G., Middle infrared spectroscopy characterization of ripening stages of Camembert-type cheeses, International Dairy Journal 17 (7) (2007) 835-845.

[37] Mazerolles G., Devaux M.F., Duboz G., Duployer M.H., Mouhous Riou N., Dufour E., Infrared and fluorescence spectroscopy for monitoring protein structure and interaction changes during cheese ripening, Lait 81 (2001) 509-527.

[38] Rollema H.S., Brinkhuis J.A., A H-NMR study of bovine casein micelles; influence of pH, temperature and calcium ions on micellar structure., Journal of Dairy Research 56 (1989) 417-425.

[39] Rosenberg M., Wang Z., Chuang S.L., Shoemaker C.F., Viscoelastic property changes in cheddar cheese during ripening, Journal of Food Science 60 (3) (1995) 640-644.

[40] Vassal L., Monnet V., Le Bars D., Roux C., Gripon J.C., Relation entre le pH, la composition chimique et la texture des fromages de type Camembert, Le Lait 67 (2) (1987) 173-185.

[41] Visser J., Factors affecting the rheological and fracture properties of hard and semi-hard cheese, Bulletin of the International Dairy Federation 268 (1991) 49-61.

[42] Waagner N.E., North European varieties of cheese. In P. F. Fox (Ed.).Cheese, chemistry, physics and microbiology (Vol. 2). London: Chapman and Hall. pp. 253., 1993.

[43] Walstra P., Van Vliet T., The physical chemistry of curd making. , Neth. Milk Dairy J. 40 (1986) 241-259.

III. 4

ARTICLE 4.2: STUDY OF THE PHYSICOCHEMICAL CHANGES OF BLUE CHEESE DURING HEATING AND COOLING BY SPECTRAL AND RHEOLOGICAL METHODS

INTRODUCTION

Cheese is used extensively by the industrial sector for the commercial manufacture of a vast array of assembled food (e.g., pizza pie, sandwiches) or formulated foods (e.g., gratins, prepared meals, cheese cake, dairy desserts) and cheese ingredients (shredded cheeses, cheese blends, dried grated cheeses, and cheese powders). There is considerable interest in investigating the changes in the properties of cheese as a function of temperature since the amount of cheese used as an ingredient in prepared foods has been rising [1]. Since prepared foods often undergo thermal processing before consumption, it is important to characterize cheese behavior during heating and cooling. The viscoelastic characterization of cheeses is important for the identification of cheese quality and identity. The viscoelastic properties of cheese are strongly dependent on temperature. Temperature changes cause alterations in the physical and chemical properties of food components which influence the overall properties of the final product (e.g., taste, appearance, and texture). It is generally assumed that at room temperature and for a given manufacturing process, milk proteins contribute to firmness and milk fats provide smoothness to cheese: the higher the fat content, the softer the cheese [2]. Several methods have been used to evaluated viscoelastic behavior of cheese [3] most of them are empirical and have a low repeatability. The development of the dynamic rheological tests allowed measuring the viscoelastic properties of cheese subjected to sinusoidal varying stresses or strains while recording the material response within the linear viscoelastic region. Generally, rheological measurements in the linear viscoelastic region are designed to probe the structure of a soft solid without damaging the structure. Those methods are used to determine the elastic or storage modulus (G'), viscous or loss modulus (G"), and Tan δ (=G''/G') [4]. The dynamic viscoelastic values are indicators of physical properties of the structure and number and strength of bonds present in the cheese system [5]. In food texture analysis, the physical properties of food structures and how they break down are of primary interest.

Only few techniques enable the monitoring, at a molecular level, of the structural evolution of food samples. Fluorescence spectroscopy, which is a sensitive, rapid and non-invasive analytical technique that provides information on the presence of fluorescent molecules and their environment in biological samples, may be a good candidate for this purpose. This technique has been used to assess the impact of storage on processed cheese [6], yogurt [7] and fish [8,9] quality, freshness [10]. Fluorescence may be applied to check food authenticity, for example to determine the geographic origin of cheese [9], and honey [11] or for the authentication of cereal products [12] and wines [13]. This fast method is able to record spectra directly on the food sample. The spectrum is a fingerprint that retains information about its physico-chemical characteristics. Changes in food viscoelastic properties primarily come from chemical modifications that can be assessed through spectroscopic investigations. Synchronous fluorescence spectroscopy (SFS) has been used to investigate the changes in milk component structures and interactions [14].

Milk contains proteins with aromatic amino-acids. Herbet et al [15] noted that fluorescence properties of aromatic amino-acids [16,17] can be used to study protein structure and protein interactions with hydrophobic molecules or ions. The aqueous phase of bovine milk contains six major proteins: β-lactoglobulin, α-lactalbumin, αs1 and αs2 caseins, β-casein and κ-casein. The amino-acid compositions of all these proteins include at least one tryptophan residue [15]. Depending on their structures, each protein exhibits, following excitation in the region 280-295 nm, a characteristic fluorescence emission spectrum defined by its maximum emission wavelength and the tryptophan quantum yield [18]. It is admitted that the shape of the tryptophan spectra characterize modifications of the environment of tryptophan residues in protein [15]. Milk also retains vitamin A located in the core and in the membrane of the fat globule. Due to its conjugated double bonds, vitamin A is a good fluorescent probe with excitation and emission wavelengths at about 330 and 450 nm, respectively [19]. The shape of the vitamin A excitation spectrum is correlated with the physical state of the triglycerides in the fat globule [20]. The lipids of milk fat globules contain hundreds of triacylglycerol species [2], for which melting occurs over a large temperature range, i.e., between -30 and +40°C [15]. It has been demonstrated that SFS is a valuable tool to also investigate changes in protein/fat interactions induced by heat treatment [21].

Karoui et al. [22] examined viscoelastic and structure characteristics of 2 hard cheeses (Comté and Emmental) and 1 semi-hard cheese (Raclette) as a function of temperature by using dynamic testing rheology and front-face fluorescence spectroscopy. They suggested that dynamic rheology data and fluorescence data could be used in determination of the melting temperature of fats and cheese-matrix. Boubellouta and Dufour [21] used dynamic testing rheology and mid-infrared and synchronous front-face fluorescence spectroscopies for investigation of structural changes in two cheeses (Comté and Racelette) during heating (from 20 to 80°C). They reported that each spectroscopic technique provided relevant information related to the cheese protein and fat structures during melting, allowing the investigation of structural changes.

There have been numerous studies on the effects of heating on viscoelastic characteristics of **cheese** varieties [23-27,5,28,29]. However, there is limited information available on changes in the viscoelastic properties during heating and cooling of blue cheeses, although these are important attributes when cheese is used in baked products. In France, a large proportion of blue cheeses can be used as an ingredient. Blue cheeses are used extensively in cooking, as an ingredient or as a condiment or as portions of grated, or to cover the meat and vegetables. A part of the blue cheeses are generally products with a Protected Denomination of Origin (**PDO**) identification.

The objectives of the present study are: *(i)* to investigate the ability of the synchronous fluorescence spectroscopy method to determine physicochemical changes in 4 categories of French-blue cheeses during heating and cooling and *(ii)* to investigate the relationship between changes in spectral characteristics and rheological characteristics of cheese during heating and cooling.

MATERIALS AND METHODS

Cheese Samples

Four different categories of Blue-veined cheeses were purchased from supermarket located in the Massif Central area of France. Cheeses were selected for their representativeness of the different cheese technologies and represent a wide range of texture. A total 13 cheeses were selected; Fourme d'Ambert (n = 4), Fourme de Montbrison (n= 2), Bleu d'Auvergne (n=3), and Bleu des Causses (n=4) which were made of pasteurized milk. Representative samples were taken from the middle of the cheese height for chemical, rheology and spectroscopy analysis.

Physicochemical Analysis

The major compositional factors interacting cheese properties are the pH, moisture, fat and protein content [25]. Grated cheese sample were analyzed in triplicate. Blue cheeses components were determined according to standards NF EN ISO 5534 (dry matter), NF V04-287 (fat), and NF EN ISO 8968-1(protein and water-soluble nitrogen contents) [30]. The pH was measured by a pH meter (Schott, CG840, Paris, France) after grating 10 g of cheese and dispersing it in 50 ml of ionized water.

Synchronous Fluorescence Spectroscopy

The broad nature of conventional fluorescence spectrum and spectral overlap can be overcome and enhanced selectivity by using synchronous fluorescence spectroscopy (SFS). In SFS, the wavelength λex (λ excitation) and λem (λ emission) are scanned simultaneously (synchronously), usually maintaining a constant wavelength interval, Δλ, between λex and λem. In this sturdy a Δλ=80 nm between excitation and emission monochromators was identified as being the most suited value after analyzing spectral shape obtained at different Δλ=20, 40, 80, 100, 120 and 140 nm (data not shown). Before measurement cheeses were sliced into thin samples (2 cm x 1 cm x 0.3 cm) and placed in a quartz cell which was then mounted on a front face accessory fitted to a FluoroMax-2 spectrofluorimeter (Spex-Jobin Yvon, Longjumeau, France), such that the incidence angle of the excitation radiation was set at 56°. The spectrofluorimeter was equipped with a thermostatically controlled cell-holder and the temperature was controlled by a Haake temperature controller (Haake, Champlan, France). Synchronous fluorescence spectra were recorded between wavelength 250-500 nm [31] at different temperatures on the same sample during heating experiment (20, 35, 50, 65, and 80°C at a rate of 3°C.min^{-1}) and during cooling experiment (80, 65, 50, 35, and 20°C at a rate of 3°C.min^{-1}). All the fluorescence measurements were performed five times for each temperature.

Viscoelastic measurements

For rheological analysis, cheeses were sliced into thin disks (2 mm thick and 20 mm diameter) with a cheese slicer. The dynamic oscillatory analyses were performed with a rheometer (CP 20, TA Instrument, Guyancourt, France) equipped with a plate geometry of 20 mm diameter and a Peltier plate that provided very accurate and rapid temperature control. To determine viscoelastic properties with dynamic tests, oscillation analyses were performed in the linear viscoelastic region by applying a constant force of 0.1 N and a constant frequency of 1 Hz (force sweep tests indicated that this was within the linear viscoelastic region, results not shown). All analyses were carried out during heating from 20 to 80°C and cooling from 80 to 20°C at a rate of $3°C.min^{-1}$. The recorded data included the elastic component G' (storage modulus), the viscous component G" (loss modulus), and Tan δ (= G"/G'). All analyses were made in triplicate.

Principal Component Analysis

Principal component analysis (PCA) is a multidimensional data treatment which provides a synthetic description of large data sets. When applied to spectral data, PCA allows similarity maps of the samples to be drawn and spectral patterns obtained [32]. PCA was applied to the synchronous spectra in order to investigate differences in the spectra. This statistical multivariate treatment made it possible to draw similarity maps of the samples and to get spectral patterns.

Partial Least Square Discriminant Analysis

PLS Discriminant Analysis (PLS-DA) is performed in order to sharpen the separation between groups of observations, by hopefully rotating PCA (Principal Components Analysis) components such that a maximum separation among classes is obtained, and to understand which variables carry the class separating information.

Canonical Correlation Analysis

The goal of CCA (Canonical Correlation Analysis) is to find the maximal correlation between the chosen linear combination of the first set of variables and the chosen linear combination of the second set of variables. Maximally correlated pairs of variables may then be identified with linear combinations and are called the canonical variables.

RESULTS AND DISCUSSION

Analysis of rheological data

The G' values of the four blue cheeses during heating and cooling is presented in figure 1. The G' values for all the blue cheeses decrease during heating from 20 to 80°C while the G' values increase during cooling from 80 to 20°C. This increase implies that during cooling cheeses changes to a more solid-like material compared with the melted cheese as indicated by tan δ (results not shown). The increase in the G' and G" values during cooling cycle could be due to an increase in the number of protein-protein interactions, such as hydrogen bonds, that increase at lower temperatures [5,33]. At temperature below ~ 35°C, we observed a large range rate of increase in G', which could describe fat crystallization (solidification) [34,35,33].

The greatest G' values were observed for 20°C after cooling. For all the bleu cheeses, the G' values were significantly higher than the G' and G" values at 20°C before heating the samples. When the G' and G" values obtained during heating and cooling were compared, hysteresis can be observed. This hysteresis was noted for all the bleu cheeses (**Figure 1**).

From **figure 1**, it appears that during temperature decrease, the classification of blue cheeses based on their viscoelastic properties previously noted during the temperature increase prevailed after heating and subsequent cooling. Indeed, the FA cheese presented the highest G' values followed by the FM, the BA and finally the BC cheese during cooling.

PLSDA was applied to rheological data (G', G" and tan δ) in order to investigate changes in the data during cooling and heating. The PLSDA results showed that the PC1 and PC2 explained more than 97% of the total variability with a large predominance of PC1 (90.27%). The similarity map obtained after PLSDA on rheological data (**Figure 2**) demonstrated that the bleu cheeses presented different viscoelastic behaviors during heating. A good discrimination between FA - FM cheeses was noted while a less marked discrimination was noted for BA-BC cheeses during heating. Moreover, this similarity map demonstrated that a good discrimination was noted between the viscoelastic characteristics of bleu cheeses during heating and cooling. This observation could suggest that the structural changes that occurred at high temperatures in the protein matrix could have been irreversible. This could also suggest that on cooling the fat slowly solidifies, but it is unlikely to be redistributed back to the original location in the matrix and this permanently alters the cheese structure. The higher variability noted between viscoelastic characteristics of cheeses during cooling compared to the ones measured during heating could imply that the structure of cheese after cooling is more heterogeneous.

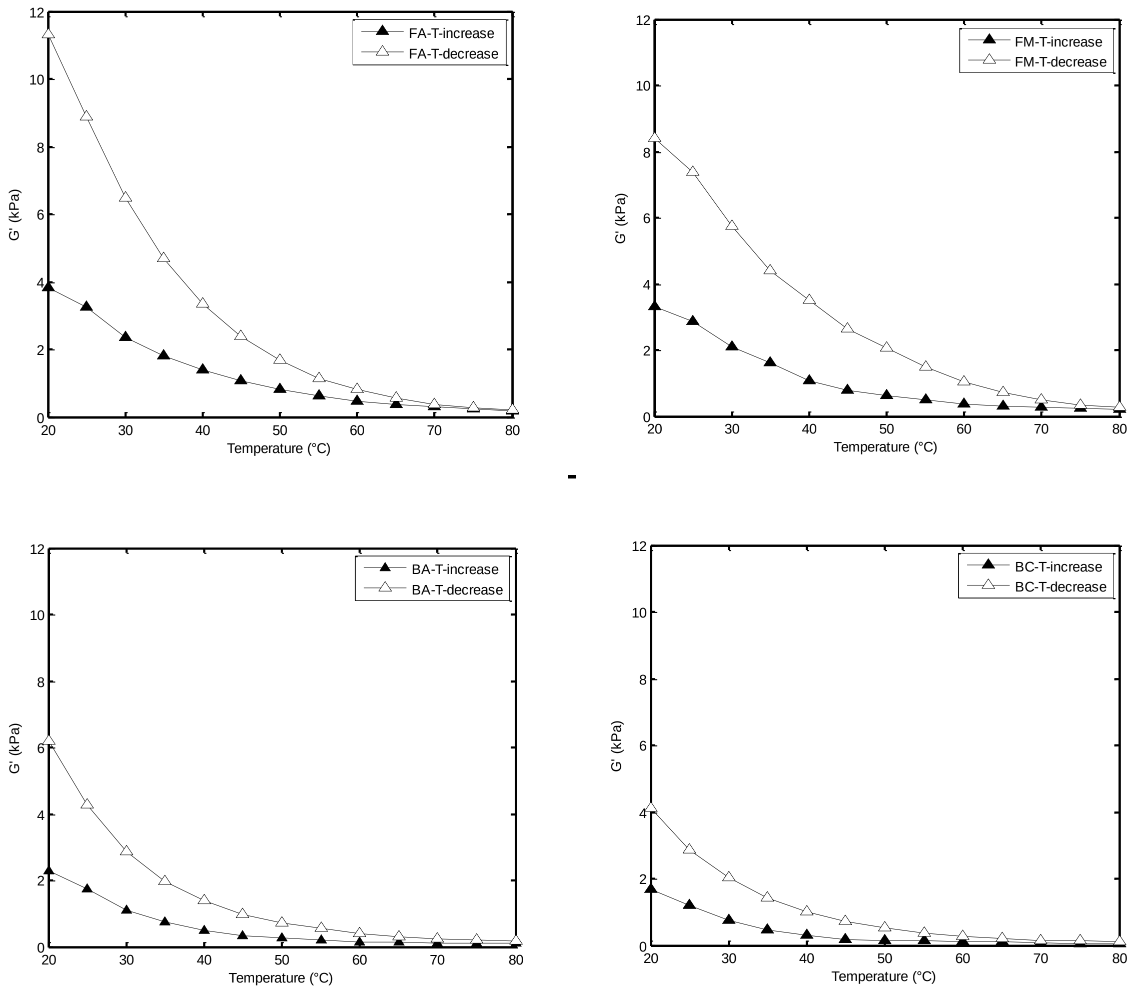


Figure 1. Viscoelastic parameters (elastic modulus: G') measured during heating (from 20 to 80°C) and subsequent cooling (from 80 to 20°C) for Blue veined cheeses obtained from pasteurized milk (FA- *Fourme d'Ambert*, FM- *Fourme de Montbrison*, BA- *Bleu d'Auvergne*; BC- *Bleu des Causses*; T – Temperature).

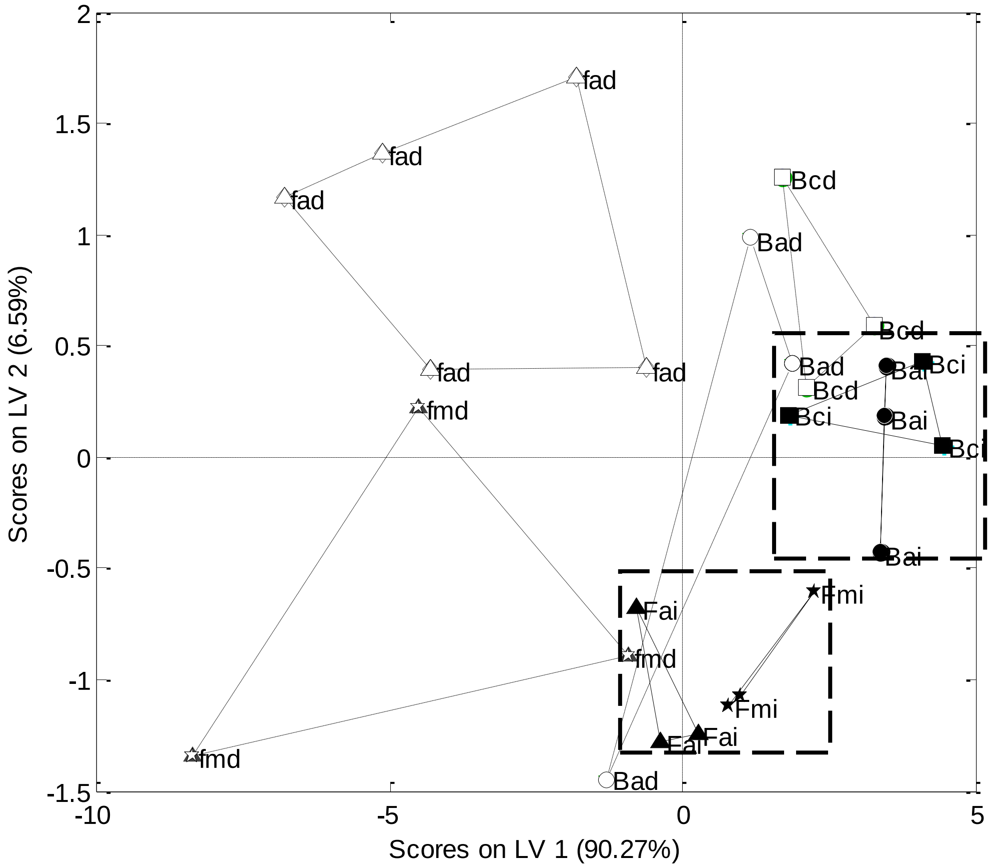


Figure 2. PC1 and PC2 Similarity maps obtained after PLSDA of SF spectra recorded during heating (from 20 to 80°C) and subsequent cooling (from 80°C to 20°C) for the different blue cheeses obtained from pasteurized milk (FA: *Fourme d'Ambert*, FM: *Fourme de Montbrison*, BA: *Bleu d'Auvergne*; BC: *Bleu des Causses;* i: measurement during heating cycle; d: measurement during cooling cycle).

Analysis of fluorescence spectra

Figure 3 presents blue cheeses SF spectra recorded during heating from 20 to 80°C and subsequent cooling from 80 to 20°C. Almost the same fluorescence bands and spectral modifications were observed from one cheese to another during heating and cooling. During cooling we observed the same fluorescence bands as previously noted during the heating cycle. Bands located at 295 nm (emission at 375 nm) and 322 nm (emission at 402 nm) were assigned to tryptophan residues of proteins and vitamin A, respectively [14,20,36,15,37]. The broader band at 355 nm (emission at 435 nm) and the small peaks around 449, 462, 467, 480 and 491 were attributed to riboflavin and coenzymes (e.g. NADH, FADH) - riboflavin - Maillard compounds [38,39,14]. During cooling from 80 to 20°C all the fluorescence bands previously mentioned increased. The band at 295 nm (emission at 375 nm), 355 nm (emission at 435 nm) and the small peaks around 449, 462, 467, 480 and 491 increase hugely, while the band located at 322 nm (emission at 402 nm) increases weakly after cooling at 20°C. Moreover, the fluorescence intensities of the bands located at 295, 355 and centered at 450 nm after cooling are closer to their respective fluorescence intensities obtained during heating. While, the fluorescence intensity of the vitamin A band during cooling are lower compared to their respective intensity during heating. Those observations could describe a better recovery of the protein matrix structure compared to the recovery of initial physical state and distribution of the triglycerides in cheese matrix. Indeed, the intensity increase of tryptophan (295 nm) and riboflavin (355 nm) bands can be attributed to the modification of their local environment [36, 41, 42]. Moreover, the tryptophan band at 20°C after cooling is less broad compared to that at 20°C of unheated cheese, which means that the tryptophan environment in cheese is modified (Figure 4). Cooling induced protein structural changes which cause less tryptophan residues to become exposed to the aqueous phase of cheese. The weak increase of the vitamin A excitation band observed during cooling probably describes a degradation of this fluorophore during heating as previously mentioned and an increase in the viscosity of the triglycerides in fat globules with the temperature decrease. Those spectral modifications could also be assigned to the difference between protein-lipid and lipid-lipid interactions since previous investigation showed that the shape of vitamin A spectra depends on the physical state of triglycerides and the interactions [16,40]. Almost the same results were observed for the other blue cheeses (**Figure 4**).

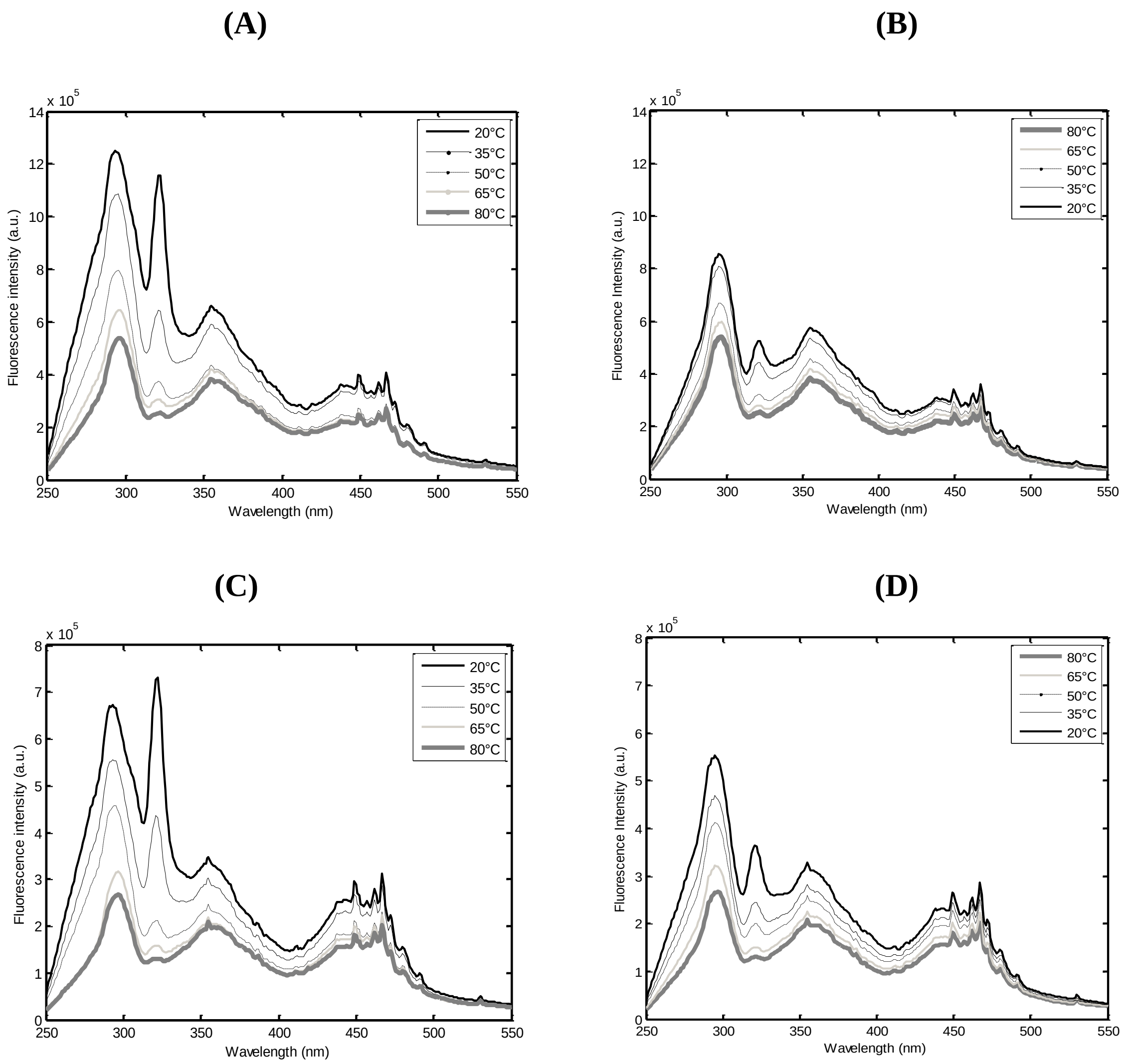


Figure 3. Synchronous fluorescence spectra (Δλ = 80 nm) recorded during heating (from 20 to 80°C) and subsequent cooling (from 80 to 20°C) of blue veined cheeses obtained from pasteurized milk. (A- SF spectra of Fourme d'Ambert cheese during heating cycle; B- SF spectra of Fourme d'Ambert cheese during cooling cycle; C - SF spectra of Bleu d'Auvergne cheese during heating cycle D- SF spectra of Bleu d'Auvergne cheese during cooling cycle).

(A) **(B)**

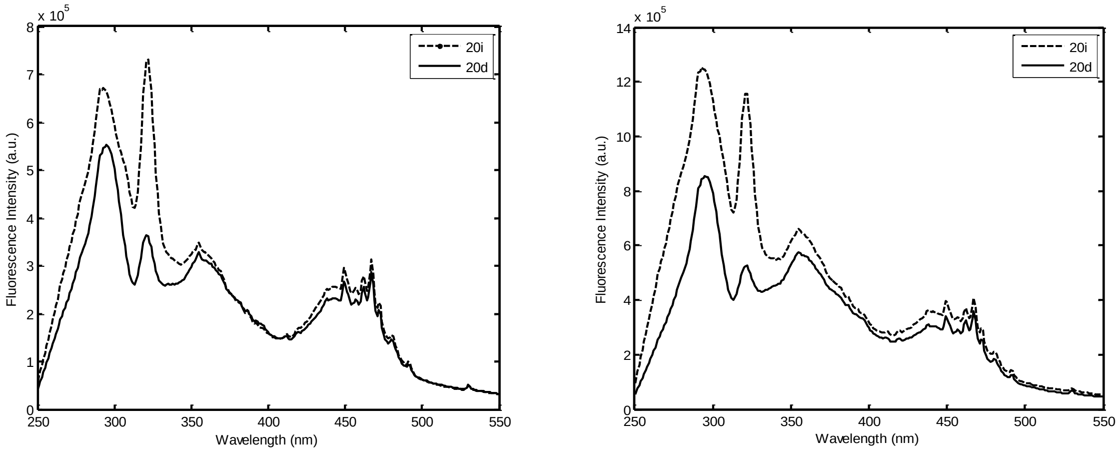


Figure 4. Synchronous fluorescence spectra (Δλ = 80 nm) recorded at 20°C before heating cycle and after heating (from 20 to 80°C) and subsequent cooling (from 80 to 20°C) of blue veined cheeses obtained from pasteurized milk. (A- Fourme d'Ambert cheese; B- Bleu d'Auvergne cheese; 20i- 20°C before temperature increase; 20d- 20°C after heating and subsequent cooling).

PCA were applied individually on each SF spectra recorded during heating and subsequent cooling for each blue cheese class to check the ability of SFS to retrieve information about the changes on the molecular structure of the cheese matrix. The **figure 5** presented similarity maps and loading spectra obtained after PCA. The PCA applied to BA cheeses allowed to obtain on the first two principal components more than 95% of the total variance explained with a large predominance of the principal component 1 (83.19%). During heating cycle, considering the map defined by principal components 1 and 2, it appeared that spectra recorded at temperatures below 50°C exhibited positive scores according to the first principal component. Whereas those recorded above 50°C exhibited negative scores (**Fig. 5A**). During cooling cycle, considering the map defined by principal components 1 and 2, it appeared that spectra recorded at temperatures below 35°C exhibited positive scores according to the first principal component, whereas those recorded above 35°C exhibited negative scores. The similarity map showed that spectra recorded during heating and cooling are not superimposed. This suggested that the structural changes that occurred at high temperatures in the cheese matrix could have been irreversible and that the microstructure of cheese is hugely modified during heating and subsequent cooling. The loading associated to the first principal component presented a high positive peak located at 322 nm (vitamin A) and small negative bands located at 299 (tryptophan), 360 (riboflavin) and 460 nm (riboflavin). This loading spectrum can be related to the modification of physical state of triglycerides during heating.

This observation is in agreement with the studies of [14,21] and our study performed on spectra recorded during heating of bleu cheeses. The loading associated to the principal component 2 presented a high positive band located at 299 nm (tryptophan) and a high negative band located at 322 nm (vitamin A). The opposition of those two bands can be related to the changes in protein–protein and protein–lipid interactions during heating and to the different network structures resulting from cheese matrix melting and solidification respectively during heating [15,17,21] and cooling.

The similarity map (**figure 5**) presented two anti-parallel phases in the changes of cheese-matrix structure with temperature according to principal component 2. During heating cycle, the first range was observed between 20 and 50°C and the second one is observed for temperatures between 50 and 80 °C as previously described. During cooling cycle, the first range was observed between 80 and 35°C and the second one is observed under 35 °C. The top of the bell-shaped curve during heating cycle was previously assigned to the melting temperature of cheese, while the top of the bell-shaped curve during cooling cycle could be assigned to the solidification of the cheese. The difference between top of the bell-shaped curve temperatures observed during heating and cooling cycles could suggest that the melting of cheese matrix during heating and its crystallization during cooling occur at different temperatures. Those observations are consistent with the rheological data and previous research on US cream cheese [33].

Almost the same results (results not shown) were obtained after PCA on the other bleu cheeses (FA, FM and BC).

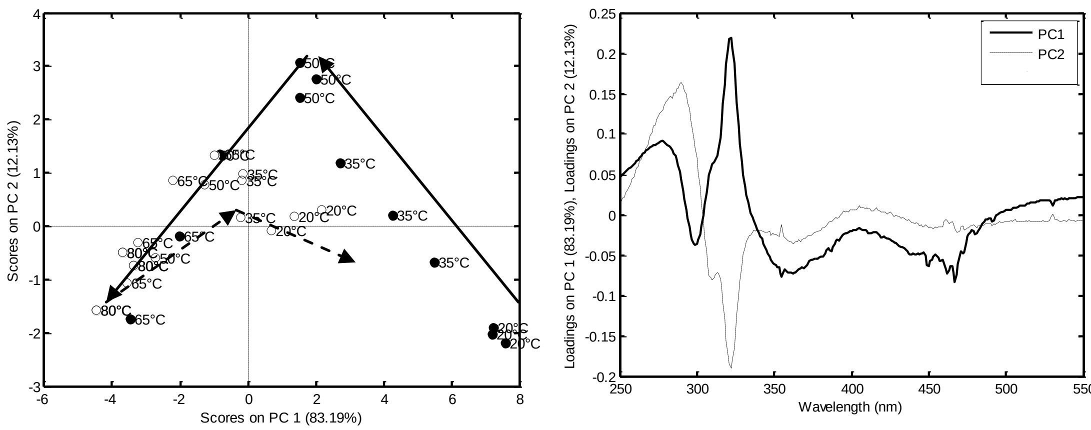


Figure 5. PC1 and PC2 Similarity maps (A) and associated spectral loadings (B) obtained after PCA of SF spectra recorded during heating (from 20 to 80°C) and subsequent cooling (from 80 to 20°C) for the *Bleu d'Auvergne* cheese.

Relation between rheological and synchronous fluorescence data

CCA was applied to the spectral data and rheological data in order to study the relationships between the two techniques used during heating and cooling of Fourme d'Ambert cheese, for example. Canonical Correlation Analysis (CCA) has been successfully applied to compare fluorescence spectra and rheological data for 2 hard cheeses (Comté and Emmental) and one semi-hard cheese (Raclette) [40] and rheology data and sensory data of Salers cheese [41].

From the similarity map (Figure 6), it appears that SFS and rheological data were highly correlated (r >0.96). These results indicated clearly that there is a relationship between the structure at a molecular level, measured by SFS, and the structure of the cheese at a microscopic level measured by dynamic rheology test. The high positive correlations observed between the results obtained from the rheology tests and the SFS could be explained by the fact that these attributes are related to the strength of the protein-protein and/ or lipids interactions in cheese matrix. For this reason, the similarity maps for the first two pairs of canonical variates performed on the SFS and the rheological data were correlated with their squared canonical correlation coefficients equal to 0.94 and 0.59. The first canonical variates discriminated the data according to temperature, while for each temperature a clustering depending on the temperature cycle (increase and decrease) was observed according to the canonical 2.

Our data strongly suggest that the structure and the interactions of micelles and fat globules, as investigated by synchronous fluorescence spectroscopy, determined the cheese texture. So, the phenomena observed at the molecular and microscopic levels were related to the changes in the cheese texture during heating and cooling.

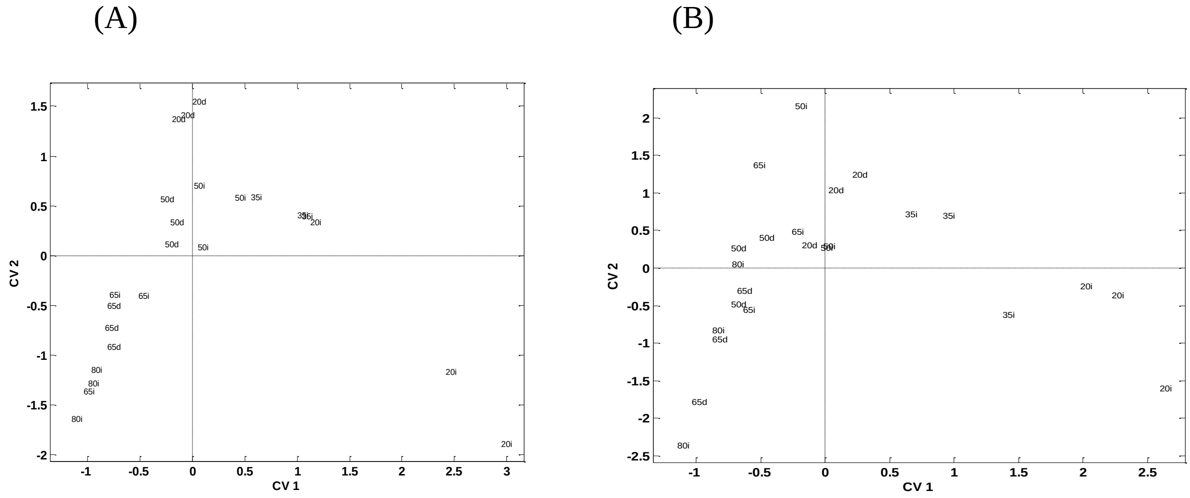


Figure 6. Canonical correlation analysis of the synchronous fluorescence spectral data and the rheology data during heating and cooling. (A) Similarity map defined by the canonical variates 1 and 2 of the CCA for the rheology data. (B) Similarity map defined by the canonical variates 1 and 2 for the synchronous fluorescence data.

CONCLUSION

This study shows that SFS and dynamic testing rheology are valuable tools to monitor the physicochemical changes of blue veined cheese-matrix during two subsequent thermal treatments, heating and cooling. These methods allowed to describe changes in protein-protein and protein-lipid interactions at a molecular and macroscopic level and to identify the melting and “solidification” points of cheese matrix. The meltability during heating and the “solidification” during cooling of cheese matrix are ones of the most important physical properties of cheese during thermal treatments. The results of canonical correlation analysis applied to blue-cheese rheological data and SF spectra collection showed that the 2 groups of variables were highly correlated. This suggests that there was a relation between the structure of the cheese at the molecular level as investigated by SFS and the texture of cheese determined by dynamic testing rheology.

This study is very promising since these two methods could be used to monitor the behavior of blue cheeses during thermal treatment (heating and cooling). It would be interesting to validate the suitability of these two methods on other blue cheeses (e.g Roquefort) to characterize their behavior during thermal treatment. This will be very benefic for the blue-cheese companies because the understanding of cheese rheological properties as a function of temperature is very important, since cheese is more and more often used as an ingredient for the manufacturing of elaborate food products.

REFERENCES

1. Mann E (2000) Cheese product innovations. Dairy Industries International 65 (10):17-18

2. Konstance RP, Holsinger VH (1992) Development of rheological test methods for cheese. Food technology 46 (1):105-109

3. Ak MM, Gunasekaran S (2001) Linear Viscoelastic Methods. In: Gunasekaran S (ed) Nondestructive food evaluation Marcel Dekker, Inc., New York, p 287

4. Steffe JF (1996) Rheological Methods in Food Process Engineering. . 2nd edition. edn. Freeman Press, East Lansing, MI.,

5. Lucey JA, Johnson ME, Horne DS (2003) Perspectives on the basis of the rheology and texture properties of cheese. Journal of dairy science 86 (9):2725-2743

6. Christensen J, Povlsen VT, Sorensen J (2003) Application of Fluorescence Spectroscopy and Chemometrics in the Evaluation of Processed Cheese During Storage. J Dairy Sci 86 (4):1101-1107

7. Christensen J, Becker E-CM, Frederiksen CS (2005) Fluorescence spectroscopy and PARAFAC in the analysis of yoghurt. Chemometrics and Intelligent Laboratory Systems 75:201-208

8. Dufour E, Frencia JP, Kane E (2003) Development of rapid method based on front face fluorescence spectroscopy for the monitoring of fish freshness. Food Res Inter 36:415-423

9. Karoui R, Martin B, Dufour É (2005) Potentiality of front-face fluorescence spectroscopy to determine the geographic origin of milks from the Haute-Loire department (France). Lait 85 (3):223-236

10. Karoui R, Pillonel L, Schaller E, Bosset JO, De Baerdemaeker J (2007) Prediction of sensory attributes of European Emmental cheese using near-infrared spectroscopy: a feasibility study. Food Chemistry 101 (3):1121-1129

11. Ruoff Kea (2006) Authentication of the botanical and geographical origin of honey by front-face fluorescence spectroscopy. J Agric Food Chem 54:6858

12. Karoui R, Cartaud G, Dufour E (2006) Front-face fluorescence spectroscopy as a rapid and nondestructive tool for differentiating various cereal products: A preliminary investigation Journal of Agricultural and Food Chemistry 54:2027–2034.

13. Dufour E, Letort A, Laguet A, Lebecque A, Serra JN (2006) Investigation of variety, typicality and vintage of French and German wines using front-face fluorescence spectroscopy. Analytica Chimica Acta 563:292-299

14. Boubellouta T, Dufour E (2008) Effects of Mild Heating and Acidification on the Molecular Structure of Milk Components as Investigated by Synchronous Front-Face Fluorescence Spectroscopy Coupled with Parallel Factor Analysis. Applied Spectroscopy 62 (5):490-496

15. Herbert S, Mouhous Riou N, Devaux MF, Riaublanc A, Bouchet B, Gallant DJ, Dufour É (2000) Monitoring the identity and the structure of soft cheeses by fluorescence spectroscopy. Le Lait 80 (6):621-634

16. Dufour E, Devaux MF, Fortier P, Herbert S (2001) Delineation of the structure of soft cheeses at the molecular level by fluorescence spectroscopy-relationship with texture. International Dairy Journal 11 (4-7):465-473

17. Mazerolles G, Devaux MF, Duboz G, Duployer MH, Mouhous Riou N, Dufour E (2001) Infrared and fluorescence spectroscopy for monitoring protein structure and interaction changes during cheese ripening. Lait 81:509-527

18. Lakowicz JR (1983) Protein fluorescence. In: Lakowicz JR (ed) Principles of fluorescence spectroscopy. Plenum Press, New York, pp 341-389

19. Karoui R, Dufour É, De Baerdemaeker J (2006) Common components and specific weights analysis: A tool for monitoring the molecular structure of semi-hard cheese throughout ripening. Analytica Chimica Acta 572 (1):125-133

20. Dufour E, Riaublanc A (1997) Potentiality of spectroscopic methods for the characterisation of dairy products. I. Front-face fluorescence study of raw, heated and homogenised milks. Le Lait 77 (6):657-670

21. Boubellouta T, Dufour E (2010) Cheese-Matrix Characteristics During Heating and Cheese Melting Temperature Prediction by Synchronous Fluorescence and Mid-infrared Spectroscopies. Food and Bioprocess Technology:1-12

22. Karoui R, Laguet A, Dufour E (2003) Fluorescence spectroscopy: A tool for the investigation of cheese melting - Correlation with rheological characteristics. Lait 83 (3):251-264

23. Foegeding EA, Brown J, Drake MA, Daubert CR (2003) Sensory and mechanical aspects of cheese texture. Int Dairy J 13:585-591

24. Foegeding EA, Drake MA (2007) Invited Review: Sensory and Mechanical Properties of Cheese Texture. Journal of Dairy Science 90 (4):1611-1624

25. Gunasekaran S, Ak M.M. (2003) Measuring cheese melt and flow properties, in Cheese Rheology and Texture In: CRC Press BR, FL, (ed). United States of America, pp 331–375.

26. Guinee TP (2002) The functionality of cheese as an ingredient: a review. Australian Journal of Dairy Technology 57 (2):79-91

27. Guinee TP, Kilcawley KN (2004) Cheese as an ingredient, in: Cheese: Chemistry, Physics and Microbiology, Vol. 2, 3rd edn., Academic Press, London, UK, 2004, pp. 394–428.

28. Guinee TP, Kilcawley KN (2001) Cheese as an ingredient, in, Cheese Chemistry, Physics and Microbiology, Vol. 1, Major Cheese Groups 3rd edn (P.F. Fox, P.L.H. McSweeney, T.M. Cogan, T.P. Guinee, eds.), pp. 395–428, Elsevier Academic Press, Amsterdam.

29. Kindstedt PS, Caric M, Milanovic S (2004) Pasta-Filata cheeses. In: Cheese Chemistry, Physics and Microbiology, Vol. 2, Major Cheese Groups, 3rd edn, pp. 251–277, Elsevier Academic Press, Amsterdam.

30. AFNOR (2002) Association francaise de normalisation. Chemical analysis.

31. Boubellouta T, Karoui R, Lebecque A, Dufour É (2010) Utilisation of attenuated total reflectance MIR and front-face fluorescence spectroscopies for the identification of Saint-Nectaire cheeses varying by manufacturing conditions. European Food Research and Technology 231 (6):873-882

32. Bertrand D, Lila L, Furtoss V, Robert P, Downey G (1987) Application of principal component analysis to the prediction of lucerne forage protein content and *in vitro* dry matter digestibility by NIR spectroscopy. Journal of the Science of Food and Agriculture 41 (4):299-307

33. Brighenti M, Govindasamy-Lucey S, Lim K, Nelson K, Lucey JA (2008) Characterization of the Rheological, Textural, and Sensory Properties of Samples of Commercial US Cream Cheese with Different Fat Contents. J Dairy Sci 91:4501-4517

34. Lopez C, Bourgaux C, Lesieur P, Bernadou S, Keller G, Ollivon M (2002) Thermal and structural behavior of milk fat: Influence of cooling rate and droplet size on cream crystallization. . J Colloid Interface Sci 254:64-78

35. Lopez C, Briard-Bion V, Camier B, Gassi J-Y (2006) Milk fat thermal properties and solid fat content in Emmental cheese: A differential scanning calorimetry study. J Dairy Sci 89 2894–2910

36. Herbert S, Riaublanc A, Bouchet B, Gallant DJ, Dufour E (1999) Fluorescence Spectroscopy Investigation of Acid-or Rennet-Induced Coagulation of Milk. J Dairy Sci 82 (10):2056-2062

37. Karoui R, Mazerolles G, Dufour É (2003) Spectroscopic techniques coupled with chemometric tools for structure and texture determinations in dairy products. International Dairy Journal 13 (8):607-620

38. Kulmyrzaev A, Dufour E (2002) Determination of lactulose and furosine in milk using front-face fluorescence spectroscopy. Lait 82 (6):725-735

39. Karoui R, Dufour E, Baerdemaeker JD (2007) Front face fluorescence spectroscopy coupled with chemometric tools for monitoring the oxidation of semi-hard cheeses throughout ripening. Food Chemistry 101 1305–1314

40. Karoui R, Dufour É (2003) Dynamic testing rheology and fluorescence spectroscopy investigations of surface to centre differences in ripened soft cheeses. International Dairy Journal 13 (12):973-985

41. Lebecque A, Laguet A, Devaux MF, Dufour E (2001) Delineation of the texture of Salers cheese by sensory analysis and physical methods. Le Lait 81:609-623

IV. Etude de l'évolution des caractéristiques sensorielles des fromages traités thermiquement

IV.1. Objectifs et méthodologie

IV.1.1. Objectifs

Ce chapitre définit, dans un premier temps, les caractéristiques sensorielles des fromages à pâte persillée à chaud. Puis ces données sont comparées à celles obtenues à froid (chapitre I méthodologie, résultats, discussion) afin de décrire l'évolution des ces qualités suite aux traitements thermiques subis par les fromages.

IV.1.2. Méthode : analyse sensorielle des fromages à chaud

Panel : Un groupe de 11 sujets qualifiés a été sélectionné et entraîné selon la norme ISO 8586-1 pour l'évaluation des descripteurs sensoriels des échantillons des fromages à pâtes persillées à chaud. La mise en place de la grille sensorielle et l'entraînement des sujets ont été conduits selon les recommandations de la norme ISO 11035. La performance du panel a été vérifiée avec les échantillons expérimentaux sur 3 répétitions. Les panélistes ont évalué au maximum 8 échantillons de fromages par session (une heure et demi), chaque fromage a été évalué 3 fois.

Préparation et service des échantillons : Les échantillons de fromage ont été coupés en morceaux d'environ 20 g (5x2x3cm) et répartis dans des récipients codés avec trois chiffres aléatoires. Ce code a été modifié entre chaque séance d'évaluation. Les échantillons ont été mis au four en chaleur sèche préchauffé à 250°C pendant 3 minutes 50 secondes. A la sortie du four, les produits avaient une température de surface entre 85 et 90°C. Les produits ont été immédiatement servis et évalués. Les notations d'odeur et d'aspect se sont faites immédiatement à chaud à la sortie du four avant un refroidissement, celui-ci risquant de modifier la perception de ces paramètres. Les échantillons ont été présentés aux panélistes selon un plan d'expérience préétablis avec une présentation monadique séquentielle des échantillons.

<u>*Modalités d'évaluation*</u> : Les membres du panel ont évalué les fromages pour leurs propriétés sensorielles en utilisant une échelle continue de 10 points (à 0 : perception absente et 10 : perception très intense). Des références ont été données aux panélistes lors des entrainements.

IV.2. Résultats, discussion

IV.2.1. Caractéristiques sensorielles des fromages à chaud

27 descripteurs sensoriels (photos n°1-2) ont été générés par le jury pour décrire les 20 fromages dont :

- *aspect* : 5, couleur de la pâte, présence de persillage à la surface, capacité à fondre, exsudation d'huile,
- *Odeur* : 6, intensité globale, ammoniac, lactique, moisi, beurre fondu, biscuité
- *Saveur* : 3, salé, acide, amer
- *Arôme* : 8, intensité globale, persistance, gras, lactique, moisi, ammoniac, étable, piquant
- *Texture* : 6, filant, râpeux, caoutchouteux, onctueux, huileux, résidus

Une analyse de variance à deux facteurs réalisée sur les données montre que 24 sur 26 descripteurs sont discriminants pour les 20 fromages avec un effet significatif à 0,1%. Les descripteurs d'odeur « biscuitée, lactique » ne sont pas discriminants pour ces échantillons (tableau n°18). Une comparaison de moyenne 2 à 2 (test de fischer) a permis de préciser la nature des différences entre produit (tableau non présenté).

Comparaison des 4 catégories de fromages à pâte persillée à chaud :

Une analyse en composantes principales (figure n°19) a été réalisée sur l'ensemble des 27 descripteurs pour les 4 catégories de fromages, les fromages ont été ajoutés comme observations supplémentaires afin de les positionner par rapport à leur famille de produit. Le tableau des cosinus carré des variables et des observations issues de l'ACP (non présenté) permet une approche explicative des axes. L'axe 1 (75% de la variance totale) représente un axe de flaveur et de texture en partie, il se définit par les descripteurs suivants : arôme lactique, beurre, étable, moisi, piquant, acide, salé, amère, exsudation, râpeux, caoutchouteux. L'axe 2 (21% de la variance totale) est un axe relié à la perception de gras : huileux, onctueux, arôme huile, odeur beurre, persillage. Les axes 1 et 2 expliquent 96% de la variation. La carte des produits montre une légère différence entre les « bleus » et les « fourmes ». Dans la globalité, les bleus semblent avoir des qualités sensorielles communes.

En revanche, les fourmes de Montbrison sont plus éloignées des autres catégories de fromages et se distinguent plus fortement au niveau de certaines qualités sensorielles, notamment par des odeurs et des arômes lactique, une texture caoutchouteuse et exsudation plus significative. Les fourmes d'Ambert, quant à elles, présentent des profils plus étalés.

Caractéristiques sensorielles de chaque catégorie de fromages à chaud :

Les figures n°20 présentent les profils et récapitulent les descripteurs significatifs et particuliers de chaque catégorie de fromages.

Les *Bleus d'Auvergne* (figure n°20a) présentent des profils similaires et se démarquent par des odeurs et des arômes intenses et notamment par un arôme ammoniaqué et une sensation piquante. Les bleus sont très salés (8.2 à 8.5) et amers (5 à 6). En texture, ils se définissent par une texture râpeuse. Dans cette catégorie, certains bleus se différencient entre eux et plus particulièrement sur les arômes (arôme moisi, arôme étable, arôme beurre, arôme lactique et huileux), et sur la texture (huileux et bonne capacité à fondre).

Les *Bleus des Causses* (figure n°20b) sont des fromages également très marqués par leurs profils aromatiques intenses. Ils se caractérisent par des odeurs et des arômes intenses typés par un arôme ammoniaqué et une sensation piquante. Ce sont des fromages très salés (8.4 à 8.7), acides (5.6 à 6.5) et amères (5.9 à 6.8). En texture, ils se démarquent par une texture huileuse et râpeuse. Les bleus des Causses se différencient entre elles uniquement sur 3 descripteurs : odeur de beurre, texture onctueuse et huileuse.

Les *fourmes de Montbrison* (figure n°20c) présentent un profil très homogène. Ce sont des fromages ayant un profil aromatique moins intense que les bleus. Les fourmes sont moyennement salées et peu amères (2.17 à 3.71). En revanche, certaines exsudent beaucoup d'huile (3.38 à 5.86) et ont une texture caoutchouteuse. Ces fromages sont très peu filants.

Les profils des *fourmes d'Ambert* (figure n°20d) sont plus complexes sachant que 2 paramètres technologiques sont intervenus (i) le traitement thermique : lait cru et lait pasteurisé et (ii) le stade d'affinage : 30 jours et 45 jours. De ce fait, les profils sont différents et les fourmes entre elles différent sur plusieurs descripteurs.

Effet du traitement thermique du lait sur le comportement à chaud des fourmes d'Ambert :

Une analyse de variance au sein de la catégorie FA a montré que 18 descripteurs sont discriminants pour décrire ces fromages. Une ACP (figure n°21) a permis de mettre en évidence l'effet de la pasteurisation du lait comparé au lait cru sur ces descripteurs. Nous avions choisi 2 temps d'affinage mais aucun effet du temps d'affinage n'a été mis en évidence. Les fourmes au lait cru se caractérisent par des odeurs et des arômes intenses tels que : l'odeur de moisi, ammoniaquée mais aussi par une texture plus huileuse. Les fourmes au lait pasteurisé sont plus onctueuses, mais aussi plus lactiques et biscuitées.

IV.2.2. Comparaison des fromages à froid et à chaud

13 descripteurs sont communs aux 2 modes de chauffage. Ces descripteurs sont essentiellement reliés à l'odeur et la flaveur. Un seul descripteur de texture est commun aux 2 approches : résidus en bouche, ce qui semble logique compte tenu du fait que les matrices fromagères sont fondues à chaud. Les descripteurs communs sont :

- Odeur : 4, intensité globale, lactique, moisi, ammoniac
- Arôme : 5, intensité globale, lactique, moisi, ammoniac, piquant
- Saveur : 3, salé, acide, amer
- Texture : 1, résidus

Le tableau suivant récapitule les descripteurs significatifs caractérisant chaque catégorie de fromage les unes par rapport aux autres en fonction des traitements thermiques (O : Odeur, A : Arôme)

Bleu				**Fourmes**			
Bleu des Causses chaud	**Bleu des Causses froid**	**Bleu d'auvergne chaud**	**Bleu d'Auvergne froid**	**Fourme d'Ambert chaud**	**Fourme d'Ambert froid**	**Fourme de Montbrison chaud**	**Fourme de Montbrison froid**
Intensité odeur			O- ammoniac				
O-ammoniac O-moisi	O-ammoniac O-moisi						
Amère Salée Acide	Acide	Amère Salée Acide	Salée				
Intensité arôme A-moisi A-ammoniac Piquant	Intensité arôme Piquant	Intensité arôme A-moisi A-ammoniac Piquant	Intensité arôme Piquant	A-ammoniac Piquant		A- lactique	A- lactique

Les bleus, au profil aromatique affirmé à froid, amplifient ces caractéristiques avec le traitement thermique appliqué. Les fourmes restent beaucoup plus discrètes et le traitement thermique n'enrichit pas significativement les flaveurs pour la plupart de ces fromages excepté pour les arômes ammoniaqués et piquants.

Afin de voir globalement si l'effet des traitements thermiques agit de la même façon sur les 4 catégories, une classification hiérarchique ascendante (dissimilarité, méthode de Wardd) a été appliquée à l'ensemble de ces données (figure n°22).

Les fromages se répartissent dès la racine en 2 grandes classes puis la deuxième classe en 2 sous classes distinctes :

- Classe 1 : ceux sont tous les bleus à chaud plus 2 bleus à froid et 3 fourmes d'Ambert à chaud. Parmi ces 5 derniers, 4 sont des fromages au lait cru et 1 au lait thermisé
- Classe 2 : toutes sont des fourmes d'Ambert et de Montbrison à chaud
- Classe 3 : ceux sont les bleus et fourmes à froid

Nous pouvons donc en déduire que la majorité des fromages se distingue bien entre les 2 modes de traitement thermique mais de façon différente entre les bleus et les fourmes. Tous les fromages à froid se retrouvent dans la même classe (sauf 2). Une attention particulière peut être donnée aux fromages au lait cru qui semblent développer plus intensément ses caractéristiques sous l'effet de la chaleur que les mêmes fromages au lait pasteurisé. Par ailleurs, il est possible que le mode de chauffage que nous avons choisi, ait une influence sur les résultats. Pour cette étude, nous nous sommes rapprochés du mode de préparation culinaire préconisé par les industriels à destination des consommateurs.

Concernant les descripteurs de texture, les 2 modes de traitement à froid et à chaud aboutissent à des textures totalement différentes qui ne peuvent pas être comparées. Toutefois il serait intéressant par la suite de vérifier si certaines caractéristiques à froid peuvent être prédictibles des caractéristiques à chaud.

V. Etude des relations entre les données sensorielles, rhéologiques et spectrales

Ce paragraphe est présenté sous forme d'un article « Heat-induced textural characteristics of Blue-veined cheeses : instrumental and sensory methods» Khaled ABBAS, Cécile BORD, Shaïmaa OTHMAN, Abderrahmane AÏT KADDOUR, Annick LEBECQUE (Article n° 5 non publié).

V.1. Objectifs

Après avoir montré l'intérêt des mesures spectrale et rhéologique pour suivre les changements structuraux avant et après chauffage, nous avons appliqué les deux techniques développées à la caractérisation de fromages à pâte persillée commerciaux et pu ainsi tester les aptitudes de chaque technique pour la description et la discrimination de ces fromages.

Dans ce troisième chapitre, nous avons voulu étudier les relations entre la description des échantillons à partir de mesures spectrale et rhéologique et celle obtenues à l'aide des analyses sensorielles pour le fromage chauffé. Dans cette optique, l'analyse canonique des corrélations a pu être utilisée pour rechercher les corrélations entre deux domaines de mesures en tenant compte de l'ensemble des variables de chaque domaine étudié. Ainsi, l'étude par analyse canonique permet de prendre en compte l'ensemble du profil sensoriel plutôt que de considérer les variables sensorielles une à une.

Dans cette étude, nous avons comparé deux à deux les résultats des différentes techniques par analyse canonique des corrélations. Pour chaque analyse, des coefficients canoniques des corrélations ont été calculés entre les différents variables des deux domaines. Ces coefficients permettent d'évaluer le niveau de relation entre les deux domaines étudiés.

V.2. Résultats et discussion

L'analyse de corrélation canonique peut être appliquée lorsque les mêmes échantillons ont été caractérisés par deux techniques différentes. Cette méthode permet à la fois une mesure globale de la liaison entre le groupe de variables et une représentation graphique de la corrélation révélée.

V.2.1. Relation entre les données rhéologiques et sensorielles

La corrélation observée entre les données rhéologiques et sensorielles ont été prises en compte afin d'obtenir un meilleur aperçu de la relation entre les caractéristiques macroscopiques et microscope du fromage étudiés.

Une ACC a été appliquée entre les données rhéologiques et les données sensorielles pour tous les fromages étudiés, quelle que soit la température (de 80 à 35°C). Les trois premiers coefficients de corrélations canoniques au carré sont respectivement de 0,95, 0,63 et 0,41 ont été évalués pour décrire cette relation. Les cartes canoniques (figure 7, article 5) montrent que les données sensorielles (crémeux et caoutchouteux) étaient positivement corrélés à G' et G" variables rhéologiques et étaient situées du côté négatif selon le CV1. En ce qui concerne Tan δ, il a été positivement corrélé avec le filant, la capacité à fondre et le râpeux, tandis que les autres attributs sensoriels étaient corrélés négativement en fonction de CV1.

V.2.2. Relation entre les données spectrales et sensorielles

Une CCA a été appliquée sur les données sensorielles et fluorescence pour tous les fromages étudiés, quelle que soit la température (de 80 à 35°C). Les trois premières paires de variants canoniques ont été évalués pour décrire les relations entre les données sensorielles et les données de fluorescence synchrones. Les coefficients de corrélation au carré canonique pour les CV1, 2, 3 sont égales à 1, 0,97 et 0,86, respectivement (tableau 4, article 5).

Les cartes canoniques (figure 8, article 5) montrent que le tryptophane est positivement corrélé avec 3 attributs de texture sensorielle (caoutchouteux, onctueux et huileux) et il a été corrélé négativement avec d'autres attributs de texture sensorielle. La vitamine A a été corrélée positivement avec l'attribut onctueux et a été corrélée négativement avec tous les autres attributs sensoriels texture.

V.3. Conclusion

Les attributs de texture des fromages à pâte persillée ont été caractérisés et bien séparés par analyse sensorielle, mesures rhéologiques et de fluorescence synchrones. Les résultats de l'ACC ont montré une forte corrélation entre les méthodes instrumentales et sensorielles avec un coefficient de corrélation canonique carré à 0,93%. En ce qui concerne les liens entre les données sensorielles et les autres données, ce sont les données de spectroscopie de fluorescence synchrone qui sont les mieux corrélées aux données sensorielles, puis celles de rhéologie.

ARTICLE N° 5: HEAT-INDUCED TEXTURAL CHARACTERISTICS OF BLUE-VEINED CHEESES: INSTRUMENTAL AND SENSORY METHODS

Khaled ABBAS, Cécile BORD, Shaimaa OTHMAN,

Abderrahmane AIT KADDOUR, Annick LEBECQUE*

U.R CALITYSS, Vetagro Sup, Campus agronomique de Clermont,

89 avenue de l'Europe -BP 35- 63370 Lempdes, France.

*Corresponding author: annick.lebecque@vetagro-sup.fr

Tel: 0033.(0)4.73.98.13.41

Introduction

Blue-veined cheeses are the most popular cow's milk cheese produced and consumed in France. In the southern France (state of Cantal), the cantalise communities manufacture have four Blue-veined cheeses (Fourme d'Ambert, Fourme de Montbrison, Bleu d'Auvergne, Bleu des Causses). The textural properties of Blue cheeses are critical to its usefulness as a food ingredient.

Texture is a group of physical properties that derives from the structure of cheese and the way its constituent ingredients interact. It is a reflection of its structure at the microscopic and molecular levels (Dufour *et al.*, 2001). Understanding the textural properties of a food product can be achieved by examination of its rheological behaviour and microstructure, so long as they can be related to the perception of texture by consumers. Dynamic rheological testing is a useful approach used to assess viscoelastic characteristics and also to provide information about cheese texture and such this information have been used to characterize and differentiate cheese varieties (Ak & Gunasekaran, 2001). It is implemented within the linear viscoelastic region of the material and therefore, is designed to be non-destructive to the basic structure of the material.

Fluorescence spectroscopy has been successfully utilized to evaluate molecular-level interactions between fat and proteins in various food, as well as monitor structural changes in cheese and Maillard browning in milk and dairy products (Genot et al., 1992; Herbert, 1999; Herbert et al., 2000; Boubellouta & Dufour, 2010). Caseins in cheese contain the amino acid tryptophan, which is a naturally occurring fluorescent substance. The fluorescent properties of tryptophan in a hydrophobic environment are different from its fluorescent properties when it is in a hydrophilic environment (Lakowicz, 1983). Consequently, researchers have utilized fluorescence spectroscopy to measure the spectra of tryptophan and predict the microstructure of cheese (Karoui *et al.*, 2003). On another note, the shape of vitamin A excitation spectrum (located in fat membrane globules in milk), as an intrinsic fluorescent probe, is correlated with physical state (viscosity) of triglycerides in the fat globule and protein-lipid interaction (Dufour *et al.*, 2000).

Sensory evaluation of texture is very important in food quality because no instrumental determinations are adequately developed to replace human evaluation. The goal of sensory analysis is to create a profile that describes textural properties of cheeses. Textural attributes of heated cheese focus on chewdown characteristics (mouth evaluation) and aspect evaluation terms (hand evaluation). Traditionally, trained sensory panels were used to evaluate textural characteristics of cheese melted. The cheese evaluator uses defined attributes, then evaluates each attribute and assigns intensity levels.

Multivariate analysis has been used for cheese characteristic evaluation. Partial least square (PLS) and principal component regression (PCR) are frequently employed statistical analyses and have been applied to sensory and instrumental results. These methods allow only to study the correlations between the two sets of data, variable by variable. However, it is interesting to study the correlations between the all variables of two data sets (Herbert, 1999). Therefore, the approach chosen in this study was to consider the variables as a whole rather than one after another. In this context, the canonical correlation analysis can be used to find correlations between two areas of data with taking into account all the variables in each area studied.

Correlations are generally used to assess the relationship between instrumental measurement and sensory perception of food texture in order to predict consumer responses or to develop instrumentation that will accurately replicate sensory evaluations (Szczesniak, 1987)**.** Relationships between sensory and instrumental measurements are poorly defined (Walstra & Peleg, 1991) despite their importance (Szczesniak, 1987). Up to now, no information on the correlation of sensory textural properties of heated cheese with instrumental measurements is available. Due to the limited amount of research on the textural properties of heated cheese after cooling, the instrumental and sensory protocol in this experiment offers a tool to evaluate these properties as textural attributes.

Therefore, the objectives of this research were (1) to find the textural difference between the Blue-veined cheeses using instrumental and sensory evaluation during cooling from 80 to 35°C, (2) to asses the relationships between the sensory data and the instrumental data (rheological, spectroscopy) obtained for the cheeses investigated using CCA. This study evaluated a wide range of Blue-veined cheeses in order generalize the sensory-instrumental correlations for Blue-veined cheeses. Multivariate analysis techniques such as PCA and CCA were used to extract relevant information regarding the relationships at molecular (SFS), microscopic (rheology) and macroscopic (sensory) levels of the investigated cheeses.

Materials and methods

Cheese Samples

Four categories Blue-veined cheeses were purchased from local market (Massif Central area of France). A total 13 cheeses were selected; Fourme d'Ambert (n = 4), Fourme de Montbrison (n= 2), Bleu d'Auvergne (n=3), and Bleu des Causses (n=4) and were made from pasteurized milk. Representative samples were taken from the centre of each cheese. The samples were evaluated by a trained sensory panel for heated texture attributes and the same samples were also instrumentally tested for the corresponding parameters using SFS and dynamic test rheology.

Instrumental Evaluation

Dynamic Test Rheology

The evaluation of the cheeses' textural properties was measured with a rheometer (CP 20, TA Instrument, Guyancourt, France) with plate geometry of 20 mm diameter and a thermostat-controlled. A temperature-sweep dynamic test was used to determine the viscoelastic properties of cheese at a constant frequency of 1 Hz and constant force of 0.1 N within the linear viscoelastic region. Values of storage modulus (G'), loss modulus (G''), and phase angle (Tan δ), were obtained under two conditions. First, temperature sweeps were run on all the samples to determine changes in the viscoelastic properties during heating (from 20 to 80°C in 20 min). Second, temperature sweeps were done on all the samples to characterize changes in the viscoelastic behavior during cooling (from 80 to 20°C in 20 min). All analyses were made in triplicate.

Synchronous Fluorescence Spectroscopy

Synchronous fluorescence spectra were obtained on a FluoroMax-2 spectrofluorimeter (Spex-Jobin Yvon, Longjumeau, France), and the incidence angle of the excitation radiation was set at 56°. The spectrofluorimeter was equipped with a thermostatically controlled cell-holder and the temperature was controlled by a Haake temperature controller (Haake, Champlan, France). Synchronous fluorescence were gathers spectra in the 250-500 nm range using offset of $\Delta\lambda$ = 80 nm between excitation and emission monochromators (Boubellouta & Dufour, 2008). Synchronous fluorescence spectra were recorded at different temperatures on the same sample from 20 to 80°C in 20 min and back to 20°C in 20 min. All the fluorescence measurements were performed three times for each sample.

Sensory Evaluation

Panel and sensory texture attributes

Panelists (n =11 female) were trained for 2 weeks in 7 sessions of 2 h in order to determine the terminology and the definition of the 7 descriptors in texture terms (Table 1). Cheeses textures were evaluated using hand and mouth. Three to four cheese samples were evaluated by session of an hour and half in duration. Panel members evaluated cheese for texture terms (Table 1) using a 10-point scale with 1 being no perception and 10 the perception very intensely. Panelists were provided with cheese references (Brown et al., 2003) during evaluations to minimize variability.

Sample Preparation

Cheese sample was cut into pieces about 20 gm and placed on aluminum plates coded with three-digit random numbers in an oven (at 250°C/3 min 30 s) and were presented to the panelists in a random order for testing (Figure 1). The evaluation and degustation of cheese samples were performed between ~ 80- 35°C after heating (250°C/3 min 30 s) a slice of cheese (20 gm) in an oven. Evaluations were conducted in a sensory laboratory with isolated booths and suing normal lighting. Data were recorded and stored using the Stragraphics 3.1 (Statistical Graphics Vorp., Rockville, USA) and Tastel (ABT Informatique, Paris, France) statistical packages. All cheeses samples were analyzed in triplicate by each assessor.

Statistical Analysis

Sensory texture and instrumental data were analysed using analysis of variance (ANOVA) under XLSTAT software (2007). Fisher's least significant difference (LSD) test was used to find significant difference among the different melted textural properties of Blue cheese ($P < 0.05$). Later, the principal component analysis (Bertrand & Scotter, 1992) was used to make visual comparisons of how the instrumental and sensory variables differentiated the cheeses. Finally, the relationships between rheological, spectral data and sensory texture terms were also evaluated by canonical correlation analysis (Saporta, 1990). This method make it possible to assess new variables, called canonical variates, as linear combinations of the variables of each data set so that these new variables exhibit the highest correlation that may be found between the two groups of data.

Table 1: Texture attributes used by trained assessors to characterise the textural properties of heated Blue-veined cheeses.

Texture attributes	**Definition**
Aspect evaluation terms (visual)	
Stretch quality (Filant)	Tendency of heated cheese to form fibrous strands that extend under tension
Melting capacity (Capacité à Fondre)	Tendency of cheese to soften or flow on heating
Oiling-off (Huileuse)	Tendency of heated cheese to exude oil on heating
Chewdown evaluation terms (Mouth)	
Mouth-coating (Râpeuse)	The degree of coating on the langue and palate during mastication, Perception in the mouth, which is grainy on the tongue
Rubbery (Caoutchouteuse)	Property related to the elasticity, reflecting the ability of cheese to return to its initial shape after biting
Smooth (Onctueuse)	Property related to the viscosity, the degree to which the chewed mass surface is smooth
Oily (Exsudation)	Oily, fatty, greasy mouthful of any kind

Fourme d'Ambert (FA) **Fourme de Montbrison (FM)** **Bleu d'Auvergne (BA)** **Bleu des Causses (BC)**

Figure 1: Schematic representation of four Blue-veined cheeses after heating used to determine sensory texture terms.

Results and discussion

Instrumental Evaluation

Cheese discrimination from their rheological data

Temperature-sweep tests were used to determine the viscoelastic characteristics of the cheeses. These properties were measured within the linear viscoelastic range previously determined by stress amplitude sweeps at a frequency of 1 Hz. Parameters describing the viscoelastic characteristics of the cheeses include G' (the storage modulus or elastic rigidity) related to the molecular events of elastic nature; G" (the loss modulus or viscous rigidity) related to the viscous character of material, and Tan δ corresponding to G"/G' (values closer to 0 are characteristic of solid-like behavior).

Mean values and standard deviation of these parameters for four Blue-veined cheeses during cooling from 80°C to 35°C for 20 min are outlined in Table (2). As observed from this table there was a variation in the dynamic moduli values among the four Blue-veined cheeses investigated. Since, every cheese variety has its characteristic structural features that reflect the chemical and biological changes in the cheese (Abd El-Salam & El-Shibiny, 1973). From Table (2), it appears that FA and FM cheeses exhibited the highest value of dynamic moduli values, whereas BA and BC cheeses showed the lowest ones whatever the temperature. This indicated that fourme cheeses have a compact protein matrix than Blue cheeses. This variability could be explained by the structural properties of the cheese matrix, protein-protein interactions and water-protein interactions and thus to the cheese-making conditions.

The G' was greater than G" at any temperature for all cheeses, which indicates a dominant contribution of the elastic component to the viscoelasticity (Kahyaoglu & Kaya, 2003), reflecting the typical behavior for a solid viscoelastic material (Ustunol *et al.*, 1995). During cooling, lowering the temperature results in marked increases in the values of G′ and G″ and vice versa in Tan δ for all the four cheeses investigated (see Table 3). This indicated that there was recovery of the total number or strength of bonds as the matrix cooled down. As the cheese mass cools, the viscosity decreases, the fat crystallizes and interaction occur between the protein strands that lead to the build up of new matrix. The new matrix formed during cooling is thus influenced by the solidification (crystallization) of fat, protein-protein interactions, and the interactions between protein and fat (Fox & Mc Sweeney, 2004).

Therefore, the changes in dynamic parameters during cooling reflected changes in molecular interactions related to mechanical properties. Since, the liquefied fat caused by heating may coalesce and separate out from the protein network, thus changing the matrix properties compared to the original unheated structure. Upon cooling the fat slowly solidifies, but it is unlikely to be redistributed back to the original location in the matrix and this permanently alters the cheese structure.

The possibility of discriminating different Blue-veined cheeses on the basis of their rheological data (G', G'' and Tan δ) was investigated by using PCA. PCA was applied to mean rheological data for all 4 cheeses investigated whatever the temperature.

The PCA showed that, the first two principal components took into account 98.61% of the total variation. A discrimination of the cheese samples was observed according to principal component 1 (80.78% of the inertia): FA and FM cheeses, exhibited the highest values, were located on the position side; while BA and BC cheeses with the lowest values showed negative scores (figure 2). It means that cheeses which showed the highest values of PC_1, they were firm but not hard and had less elasticity to its matrix. It is indicated that Blue cheeses tend to be more elastic and more adhesive. Thus, principal component one may be related most to the viscous and elastic components of the cheeses (G' and G'') since it was positively dominated by the G' and G'' variables and negatively by the Tan δ.

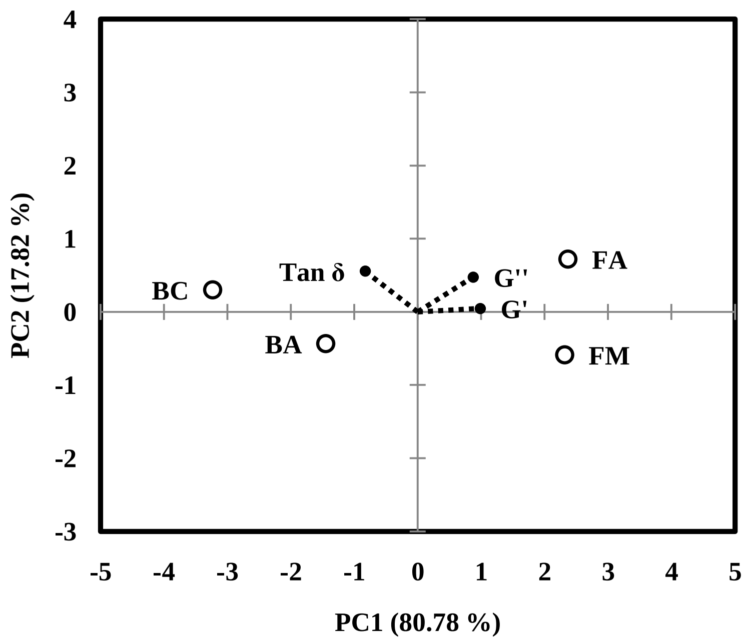


Figure 2: Principal components analysis similarity map of the rheological data used to differentiate heated Fourme d'Ambert (FA), Fourme de Montbrison (FM), Bleu d'Auvergne (BA) and Bleu des Causses (BC) cheeses.

Table 2: Mean values (± standard deviation) of rheological parameters (G', G'' and Tan δ) measured for heated Bleu-veined cheeses (FA: Fourme d'Ambert, FM: Fourme de Montbrison, BA: Bleu d'Auvergne; BC: Bleu des Causses) during cooling from 80 to 35°C.

Parameters	Temperature	Cheeses			
		FA	FM	BA	BC
G' (kPa)	80°C	0.19 (±0.04)[a]	0.20(±0.09)[a]	0.10(±0.01)[b]	0.06 (±0.02)[b]
	65°C	0.56 (±0.16)[ab]	0.70(±0.26)[a]	0.32(±0.15)[bc]	0.17(±0.04)[c]
	50°C	1.73 (±0.53)[a]	2.05(±0.88)[a]	0.72(±0.22)[b]	0.47(±0.13)[b]
	35°C	4.68 (±1.47)[a]	4.41(±1.80)[a]	1.97(±0.41)[b]	1.47(±0.46)[b]
G" (kPa)	80°C	0.23(±0.05)[a]	0.23(±0.05)[a]	0.09(±0.02)[b]	0.07(±0.02)[b]
	65°C	0.56 (±0.15)[a]	0.67(±0.25)[a]	0.29 (±0.12)[a]	0.18(±0.04)[a]
	50°C	1.28 (±0.35)[a]	1.28(±0.51)[a]	0.64(±0.17)[a]	0.45(±0.12)[a]
	35°C	2.75 (±0.72)[a]	2.14(±0.80)[a]	1.55(±0.33)[a]	1.22(±0.34)[a]
Tan δ (G''/G')	80°C	1.21 (±0.02)[a]	1.15(±0.12)[a]	1.11(±0.01)[a]	1.16(±0.08)[a]
	65°C	1.00 (±0.06)[a]	0.95(±0.09)[a]	0.94(±0.07)[a]	1.06(±0.00)[a]
	50°C	0.74 (±0.05)[b]	0.63(±0.03)[c]	0.89 (±0.03)[a]	0.96(±0.03)[a]
	35°C	0.59(±0.05)[b]	0.49(±0.02)[c]	0.78(±0.00)[a]	0.83(±0.02)[a]

[a,b,c] Values followed by the same letter in the same row are not significantly different ($P < 0.05$).

Cheese discrimination from their synchronous fluorescence spectra

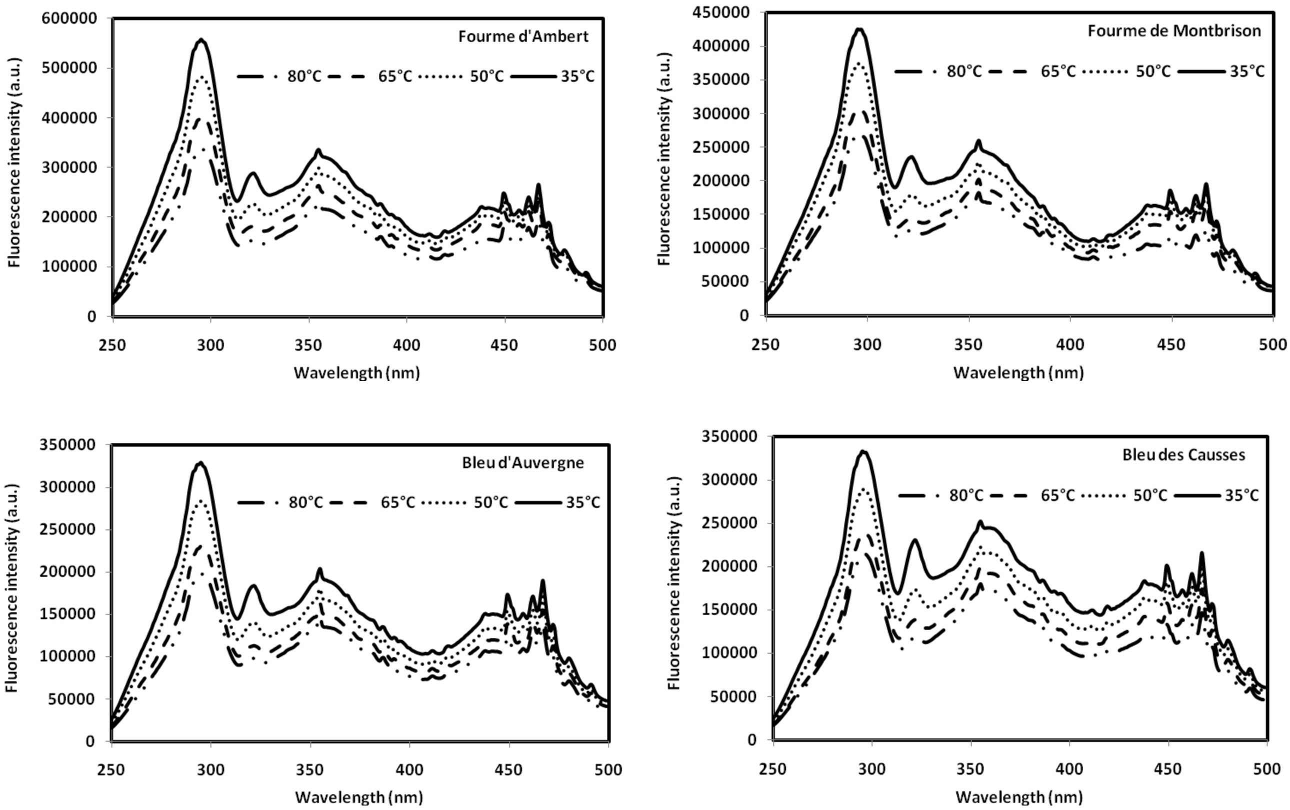


Figure 3: Synchronous fluorescence spectra of heated Fourme d'Ambert (FA), Fourme de Montbrison (FM), Bleu d'Auvergne (BA) and Bleu des Causses (BC) cheeses during cooling from 80 to 35°C.

The synchronous fluorescence spectra characteristics at a wavelength interval of 80 nm of heated Fourme d'Ambert, Fourme de Montbrison, Bleu d'Auvergne and Bleu des Causses cheeses during cooling from 80 to 35°C are depicted in Figure (3). Three sharp and intense bands were observed at 295 nm (attributed to tryptophan), 322 nm (attributed to vitamin A) and 355 nm (attributed to riboflavin). And some small peaks around 449, 462, 467, 480 and 491 nm can also be observed. The tryptophan spectra of four cheeses investigated exhibited a maximum at ~ 295 nm and the location varied slightly from one cheese to another. This variation in tryptophan spectra could be explained by the structural properties of the cheese matrix, protein-protein interactions and water-protein interactions and thus to the cheese-making conditions. It is well recognized that, temperature induced a drastic modification of the fluorescence intensities in all bands, since the fluorescence intensities decreased with the increase of temperature. Conversely, the fluorescence intensities increased with the decrease of temperature. This could be explained by the changes of cheese-matrix structure with temperature. Indeed, it has been shown that the fluorescent properties of fluorophore are very sensitive to the changes in the solvent viscosity (Dufour & Riaublanc, 1997) and physical state of the triglyceride. As observed from figure (3), the maximum intensity of fluorophores existent in Fourme d'Ambert cheese was higher than those of three other cheeses. These differences probably reflect cheese-making processing condition and cheese-matrix structure.

Due to the large number of variables and observations, a PCA analysis was carried out on the mean SFS data of all four cheeses investigated so as to extract information related to structural changes in cheese from their florescence spectra. SFS data for all four cheeses was gathered in one matrix whatever the temperatures and after that PCA was applied to data matrix.

The first two principal components (PC) were obtained, explaining 99.92% of the total variance. Figure (4) shows the PCA biplot of the spectral data set for PC_1 vs. PC_2. It can be observed that, FA and FM cheeses are clustered to the left of the negative PC_1 axis whereas the others cheeses (BA and BC) are positioned in the positive side of PC_1 axis. This grouping indicates that this component (PC_1) explains that the textural changes occur in heated Fourme cheese (FA and FM) are different than that in the other cheeses (BA and BC).). It means that cheeses which showed the highest values of PC_1, they were higher in fluorescence intensity of tryptophan and were lower in lower in fluorescence intensity of vitamin A. It is indicated that fourme cheeses tend to be more viscous texture and less compact texture than the blue cheeses.

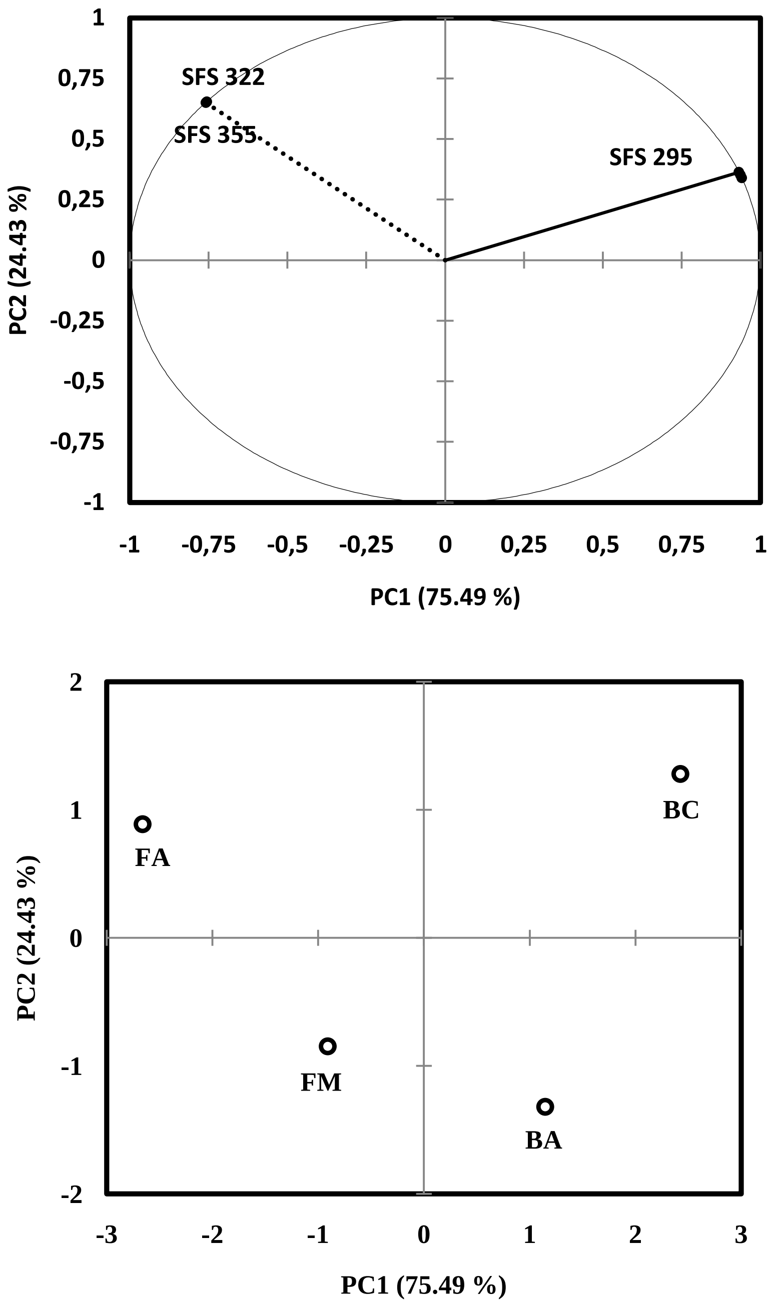


Figure 4: Principal component analysis map of the spectral data used to differentiate heated Fourme d'Ambert (FA), Fourme de Montbrison (FM), Bleu d'Auvergne (BA) and Bleu des Causses (BC) cheeses.

Cheese discrimination from their sensory evaluation

For this study, the judges were only asked to evaluate the textural properties of heated Blue-veined cheeses. Table (1) showed a list of defined texture terms that have been applied by trained panelists to document texture attributes of different Blue-veined cheeses. Among these attributes, we can distinguish at least seven sensory textures attributes which are shown in Table (3) for Blue-veined cheeses.

Table 3: Mean scores (± standard deviation) of sensory texture attributes for heated Blue-veined cheeses: Fourme d'Ambert (FA), Fourme de Montbrison (FM), Bleu d'Auvergne (BA) and Bleu des Causses (BC) cheeses.

Cheeses	Sensory texture attributes						
	Aspect evaluation (hand)			Chewdown evaluation (mouth)			
	Stretch quality	Melting capacity	Oiling-off	Mouth-coating	Rubbery	Smoothness	Oily
FA	3.8(±2.22)^{c}	3.3 (±2.61)^{a}	2.6(±2.56)^{c}	2.7(±2.54)^{b}	1.2(±1.99)^{b}	5.6(±3.08)^{a}	2.8(±3.20)^{b}
FM	2.8 (±2.06)^{d}	1.8 (±2.12)^{b}	5.3(±2.61)^{a}	1.1(±2.13)^{c}	7.3(±2.34)^{a}	2.2(±2.61)^{c}	6.6(±3.30)^{a}
BA	4.5 (±2.32)^{b}	3.6 (±2.82)^{a}	3.4(±3.01)^{b}	4.8(±2.91)^{a}	0.7(±1.54)^{c}	4.2(±3.20)^{b}	0.7(±1.68)^{c}
BC	5.2 (±2.01)^{a}	3.3(±2.80)^{a}	5.6(±2.83)^{a}	5.4(±2.57)^{a}	0.3(±1.18)^{c}	3.9(±3.34)^{b}	0.5(±1.47)^{c}

^{a,b,c,d} means with the same letter, in the same column, do not differ significantly from each other (P <0.05)

Firstly, one-way Anova was performed on the sensory data of cheeses groups. For the multiple comparison tests, the Fisher's test was used. One-way Anova results indicated that there were significant differences ($P < 0.05$) among the investigated cheeses for all texture attributes. More particularly, the group Fourme cheese (FA and FM) tends to be having a compact protein matrix. This group presented stretching and melting properties but they had the lower score than the other group. The other group (Bleu des Causses and Bleu d'Auvergne cheeses) seems to be similar and tends to be more elastic and more adhesive. They presented a higher stretching and melting scores. In mouth, Bleu des Causses and Bleu d'Auvergne cheeses were defined by rough and oiling-off characteristics. They had the lowest values concerning the rough texture (0.3 and 0.5, respectively) and were characterized by an oily appearance.

Secondly, in order to examine the relationships between cheeses groups, mean data of the sensory analysis were processed by PCA. The results of the PCA showed in the figure (5). This plot represented the projection of cheeses on the plane defined by PC_1 and PC_2. The total variance was explicated by 97.30%. The first PC (PC_1) accounted by 87.86% of the variation among the different groups of cheeses, and the second PC (PC_2) accounted 21.04%. The first component was represented all sensory texture attributes. The second axis represented two attributes: oily and rubbery.

The 4 Blue-veined cheeses were well-separated. Fourme cheeses (FA and FM) were positioned in the negative PC_1 values, and was mainly separated from the others groups. FM and FA cheeses were characterized by a rubbery texture and an oiling-off appearance. On the other hand, BA and BC cheeses can de described as higher smoothness, meltability and stretchability products. Furthermore, BA and BC cheeses were distinguished by a low oily texture. Principal component analysis illustrated the diversity of Blue-veined cheeses and more particularly about the texture attributes. Moreover, this plot mainly indicated a difference between Fourme cheeses and the other group. Variations in these functional properties could be attributed to differences in pH, moisture, fat contents. In addition, the amount and distribution of fat in protein matrix probably affects flow property of cheese upon heating. The net results, panelist's observations of textural attributes were agreed with instrumental technique.

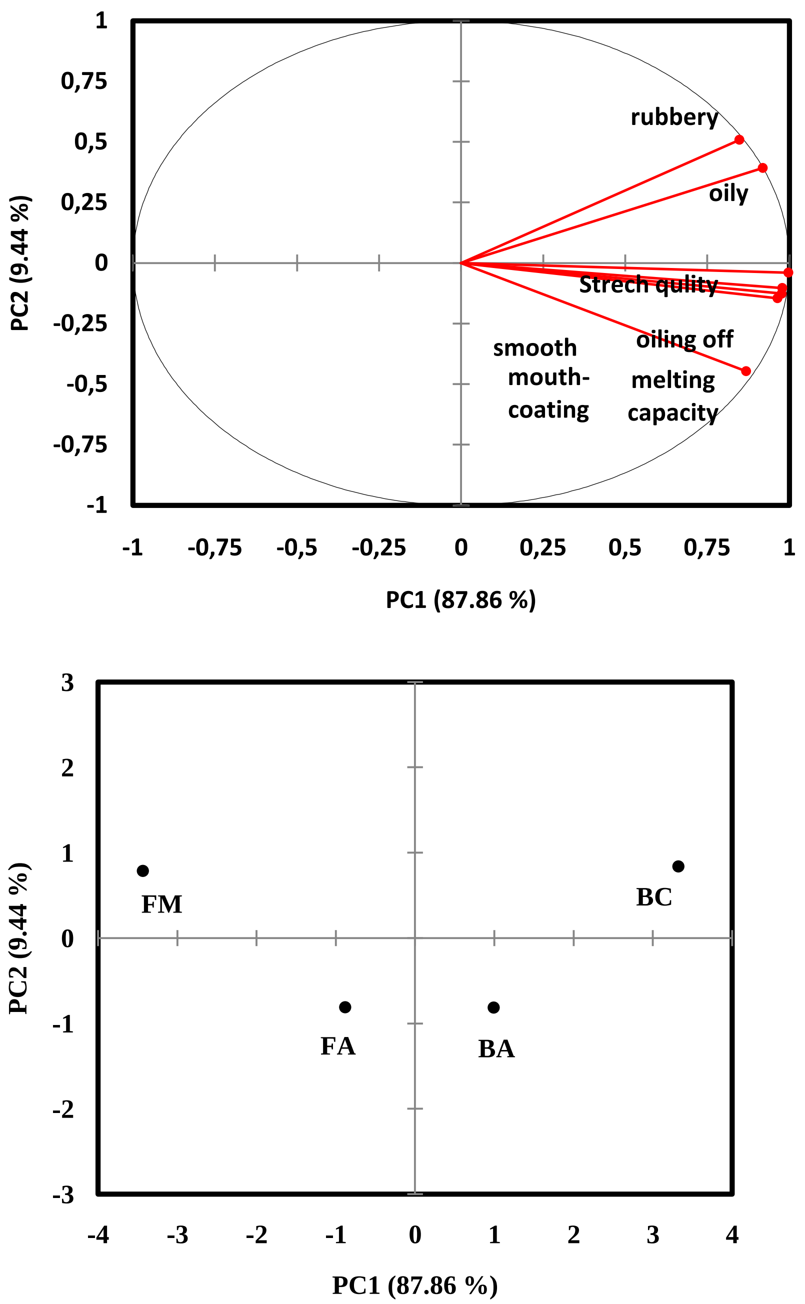


Figure 5: Principal component analysis (maps 1-2) of sensory texture data used to differentiate heated Fourme d'Ambert (FA), Fourme de Montbrison (FM), Bleu d'Auvergne (BA) and Bleu des Causses (BC) cheeses.

Cheese discrimination from their instrumental and sensory data

Principal component analysis was also done on the instrumental and sensory variables together in order to explore characterizing capabilities of the combination of both methods. The first two principal components were able to define 88.39 % of the total variation among these cheeses. Figure (6) shows principal component one (PC_1) and two (PC_2) from this analysis. Principal component one (PC_1) accounted for 66.6% of the total variation in the samples and was able to differentiate the cheeses by variety. It was positively driven by the variable Tan δ, tryptophan spectra and all the sensory texture attributes. Principal component two (PC_2), accounting for 22.34 % of the total sample variation, mainly determined by the rheological variables (G' and G'') and vitamin A spectra.

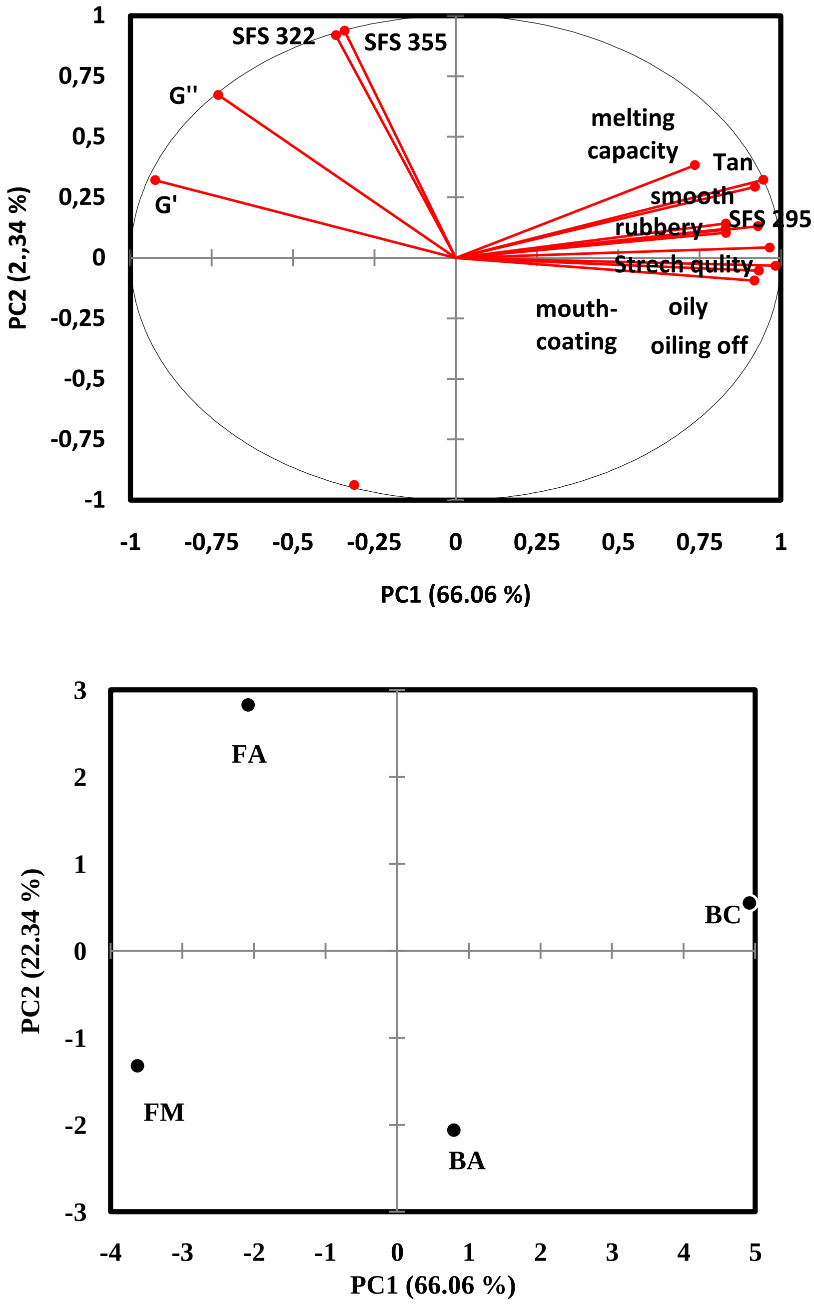


Figure 6: Principal component analysis biplot of sensory and instrumental parameters used to differentiate Fourme d'Ambert (FA), Fourme de Montbrison (FM), Bleu d'Auvergne (BA) and Bleu des Causses (BC) cheeses.

Instrumental-Sensorial Canonical Correlations

Rheological-Sensorial Canonical Correlations

The correlation observed between the rheology measurement and the sensory data have been taken into account in order to get a better insight into the correlation between the macroscopic and microscope characteristics of the investigated Blue-veined cheeses. Canonical correlation analysis may be applied when the same samples have been characterized by two different techniques. This method provides both global measure of the link between the group of variables and a graphical representation of the correlation revealed.

For the CCA analysis, the sensory and the rheological data sets recorded at all temperature (from 80 to 35°C) for each cheese samples were considered. The first 3 pairs of canonical variates were assessed to describe this relation. The sensory and the rheological data were correlated with squared canonical correlation coefficients equal to 0.95, 0.63 and 0.41 (Table 4). It can be seen from CVA biplot of sensory and instrumental data (Figure 7) that the cheeses, were 'rubbery' and 'oily' (sensory), and were positively correlated to G' and G'' variables (instrumental) and were located at the negative side according to the first canonical variate. These sensory attributes ('rubbery' and 'oily') and instrumental parameters (G' and G'') might give a perception of moistness, softness and stickiness to cheese-like products during chewing and thus, it might be concluded that products, which scored high on these sensory attributes, were softer in texture. With regard to Tan δ, it was positively correlated with stretch quality, melting capacity and mouth-coating, while the other sensory attributes was negatively correlated according to CV_1.

Table 4: Squared canonical correlation coefficient between sensory attributes and the instrumental data (rheology and SFS) used to define cheese texture attributes.

	Canonical variates		
Instrumental data	**1**	**2**	**3**
Rheology	0.95	0.63	0.41
SFS	0.99	0.97	0.86

Table 5: Correlation coefficients from the CCA analysis between sensory data (Y-matrix) and instrumental data (X-matrix) used to define cheese texture attributes.

R^2					
	Independent variables (instrumental)				
Dependent variables (Sensory)	SFS 295 nm	SFS 322 nm	G'	G"	Tan δ
Stretch quality	-0,342	-0,278	**-0,804**	-0,153	**0,755**
Melting capacity	-0,120	-0,144	-0,488	-0,065	**0,529**
Oiling off	-0,336	-0,173	-0,369	-0,112	0,330
Mouth-coating	-0,450	-0,272	**-0,806**	-0,480	**0,780**
Rubbery	0,132	0,035	**0,630**	0,253	**-0,691**
Smooth	**0,512**	**0,495**	-0,008	0,084	0,062
Oily	0,320	0,172	**0,633**	0,395	**-0,708**

From Table (5), positive correlations were observed between G' and rubbery and oily. G' is a measurement of the elastic component in a viscoelastic material and is an indication of the rigidity of the network. Thus, cheeses having a higher G' were perceived as firmer (less rubbery). Conversely, G' was highly negatively correlated with stretch quality and mouth-coating. However the melting characteristics were negatively correlated with textural characteristics. The only textural property with good correlation to melt and flow was Tan δ, which had a positive correlation with stretch, melting and mouth-coating.

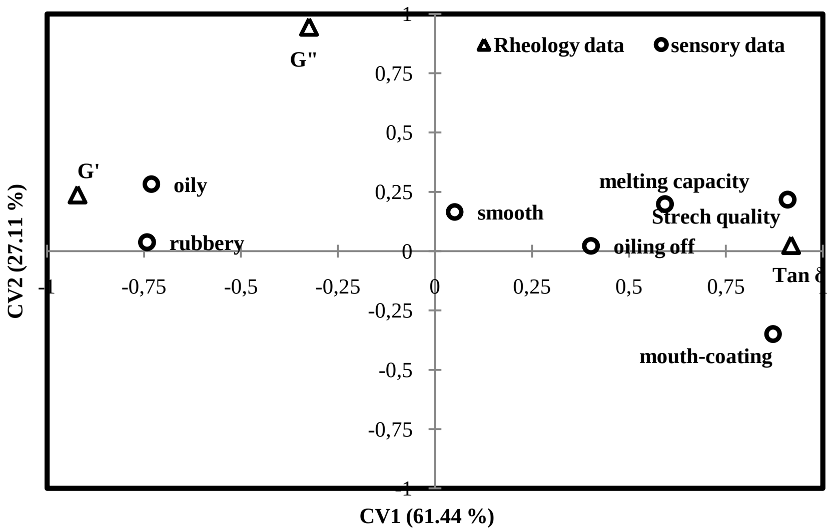


Figure 7: Similarity map of CCA applied to the rheology data and the sensory data sets of Blue veined cheeses.

Spectral-Sensorial Canonical Correlations

The correlation observed between sensory and synchronous fluorescence data sets have been taken into account in order to get a better insight into the correlation between the characteristics of the investigated heated Blue-veined cheeses at macroscopic and molecular levels. For the CCA analysis, the sensory and the fluorescence spectra data sets recorded at the same temperature (from 80 to 35°C) for each cheese were considered.

The first 3 pairs of canonical variates were assessed to describe correlation between the sensory data and the synchronous fluorescence data. The sensory and the synchronous fluorescence data were correlated with squared canonical correlation coefficients for canonical variate 1, 2, 3 and were equal to 0.99, 0.97, and 0.86, respectively (Table 4). It can be seen from CVA biplot of the sensory data and the fluorescence data (Figure 8) that tryptophan was positively correlated with 3 sensory texture attributes (rubbery, smooth and oily) and it was negatively correlated with other sensory texture attributes (see table 5). This negative correlation indicates that an increase in tryptophan excitation may be attributed to the movement of tryptophan residues to a hydrophilic environment as a result of changes in environment. Consequently, the characteristics of cheese may be related to the fluorescence properties of tryptophan. Considering vitamin A was only positively correlated with smooth attribute ($r^2 = 0.49$) and was negatively correlated with 4 out of 7 sensory texture attributes (see table 5) indicating that broadening of the curve occurs with a decrease in viscosity of triglycerides.

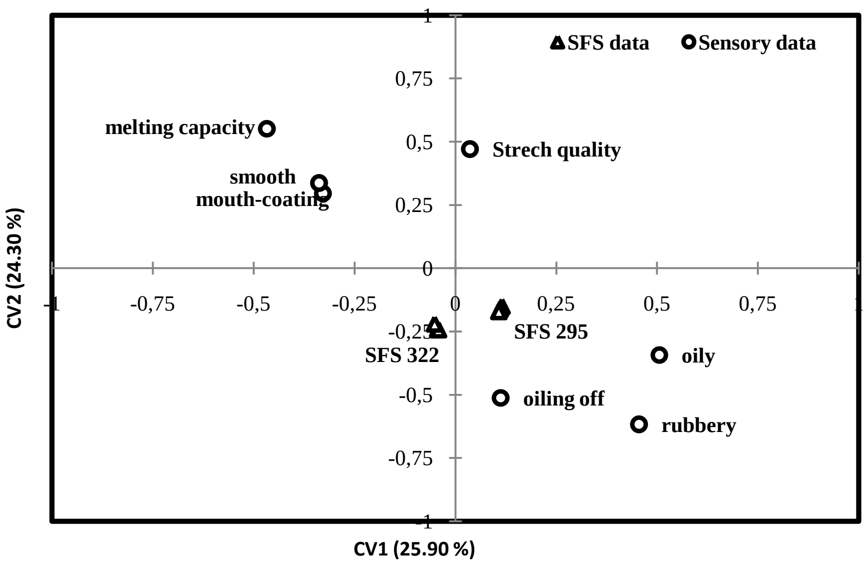


Figure 8: Similarity map of CCA applied to the fluorescence data and the sensory data sets of blue-veined cheeses.

Conclusion

Textural attributes of heated Blue-veined cheeses were characterized and well-separated by sensory analysis, rheological measurement and intrinsic synchronous fluorescence (tryptophan and vitamin A) during cooling from 80 to 35°C. Since, the obtained similarity maps of PCA illustrated that general variability in texture among the investigated heated Blue-veined cheeses during cooling from 80 to 35°C. Fourme cheeses were characterized by a rubbery, viscous texture and an oiling-off appearance, while blue cheeses tend to be more elastic and more adhesive texture. CCA results showed that strong correlation between the instrumental and sensory methods with a squared canonical correlation coefficient above to 0.93%. The textural changes in cheeses matrices at molecular (SFS), microscopic (rheology) and macroscopic (sensory) were interrelated with each others.

References

Abd El-Salam, M.H. & El-Shibiny, S. (1973) An electron-microscope study of the structure of Domiati cheese J. Dairy Res 40, 113-115.

Ak, M.M. & Gunasekaran, S. (2001) Linear Viscoelastic Methods. In nondestructive food evaluation p. 287. Edited by S. Gunasekaran. New York: Marcel Dekker, Inc.

Bertrand, D. & Scotter, C.N.G. (1992) Application of Multivariate Analyses to NIR Spectra of Gelatinized Starch Applied Spectroscopy 46, 1420–1425.

Boubellouta, T. & Dufour, E. (2008) Effects of Mild Heating and Acidification on the Molecular Structure of Milk Components as Investigated by Synchronous Front-Face Fluorescence Spectroscopy Coupled with Parallel Factor Analysis. Applied Spectroscopy 62(5), 490-496.

Boubellouta, T. & Dufour, E. (2010) Cheese-Matrix Characteristics During Heating and Cheese Melting Temperature Prediction by Synchronous Fluorescence and Mid-infrared Spectroscopies. Food and Bioprocess Technology, 1-12.

Brown, J.A., Foegeding, E.A., Daubert, C.R., Drake, M.A. & Gumpertz, M. (2003) Relationships Among Rheological and Sensorial Properties of Young Cheeses. J. Dairy Sci. 86(10), 3054-3067.

Dufour, E., Devaux, M.F., Fortier, P. & Herbert, S. (2001) Delineation of the structure of soft cheeses at the molecular level by fluorescence spectroscopy—relationship with texture. International Dairy Journal 11(4-7), 465-473.

Dufour, E., Mazerolles, G., Devaux, M.F., Duboz, G., Duployer, M.H. & Mouhous Riou, N. (2000) Phase transition of triglycerides during semi-hard cheese ripening. International Dairy Journal 10(1-2), 81-93.

Dufour, E. & Riaublanc, A. (1997) Potentiality of spectroscopic methods for the characterisation of dairy products. I. Front-face fluorescence study of raw, heated and homogenised milks. Le Lait 77(6), 657-670.

Fox, F. & Mc Sweeney, L.H. (2004) Cheese: Chemistry, physics and microbiology, 3rd ed. England: Elsever Academic Press

Genot, C., Tonetti, F., Montenay-Garestier, T., Marion, D. & Drapon, R. (1992) Front-face fluorescence applied to structural studies of proteins and lipidprotein interactions of visco-elastic food products.2-Application to wheat gluten Sciences des Aliments 12, 687-704.

Herbert, S. (1999) Caractérisation de la structure moléculaire et microscopique de fromages à pâte molle, Analyse multivariée des données structurales en relation avec la texture Vol. Thèse: École Doctorale Chimie Biologie de l'Université de Nantes, France.

Herbert, S., Mouhous Riou, N., Devaux, M.F., Riaublanc, A., Bouchet, B., Gallant, D.J. & Dufour, É. (2000) Monitoring the identity and the structure of soft cheeses by fluorescence spectroscopy. Le Lait 80(6), 621-634.

Kahyaoglu, T. & Kaya, S. (2003) Effects of heat treatment and fat reduction on the rheological and functional properties of Gaziantep cheese. International Dairy Journal 13(11), 867-875.

Karoui, R., Mazerolles, G. & Dufour, É. (2003) Spectroscopic techniques coupled with chemometric tools for structure and texture determinations in dairy products. International Dairy Journal 13(8), 607-620.

Lakowicz, J.R. (1983) Protein fluorescence. In Principles of fluorescence spectroscopy, pp. 341-389. Edited by J.R. Lakowicz. New York: Plenum Press.

Saporta, G. (1990) Probabilités – Analyse des données statistique, Technip édn., Paris.

Szczesniak, A., S. (1987) Review paper: Correlating sensory with instrumental texture measurements - An overview of recent developments. J. Texture Studies 18, 1-15.

Ustunol, Z., Kawachi, K. & Steffe, J. (1995) Rheological properties of Cheddar cheese as influenced by fat reduction and ripening time. J. Food Sci. 60, 1208–1210.

Walstra, P. & Peleg, M. (1991) General considerations. Chap 1. In: Rheological and fracture properties of cheese. Bullint Dairy Fed 268, 3-4.

CONCLUSION GENERALE ET PERSPECTIVES

CONCLUSION GENERALE ET PERSPECTIVES

Les fromages AOP français représentent une part non négligeable de la production française (16% des fromages affinés), la majorité est fabriquée à base de lait cru ce qui leur confère leur spécificité. Ces productions liées à des terroirs et des savoir-faire doivent rester durables dans le temps mais pour cela elles doivent également s'adapter aux nouveaux consommateurs et modes de consommation. Ces fromages sont destinés principalement à être dégustés. Toutefois ces dernières années voient apparaitre une stagnation voire un affaiblissement des ventes. Les professionnels inquiets face à cette évolution cherchent de nouveaux débouchés innovants pour valoriser leurs fromages AOP. L'utilisation des fromages en tant qu'ingrédient dans des préparations froides ou chaudes est un secteur en pleine expansion. Cette forme de préparation pourrait correspondre à un nouveau débouché pour les fromages AOP dans la mesure où ils répondent aux critères requis pour cet usage. Les industriels utilisent depuis un certain temps des fromages spécifiquement fabriqués pour répondre à certaines fonctionnalités technologiques. Les fonctionnalités recherchées par les industriels sont liées à la fois à la macro et micro structure fromagère, à la composition et aux caractéristiques sensorielles des fromages. Certains termes professionnels sont utilisés pour décrire certains critères : fondant, filant, étalement, exsudation, gratinant…Ils sont souvent estimés par des méthodes empiriques de contrôle et décrits par des termes sensoriels.

L'objet de cette étude était d'étudier les potentialités de fromages AOP, ici des pâtes persillées, à être utilisés sans transformation particulière comme ingrédient dans différentes préparations et d'étudier également les potentialités de certaines techniques à se substituer aux méthodes classiques (test de Schreiber, test à la fourchette…). Une connaissance approfondie du comportement des différents constituants de la matrice fromagère et de leurs interactions lors du chauffage et/ou du refroidissement est indispensable pour maîtriser la qualité finale du fromage.

Nous avons abordé le sujet en analysant l'effet du chauffage et du refroidissement sur les fonctionnalités des fromages à pâtes persillées. Cette première étude a été conçue avec une approche exploratoire tant au niveau de la caractérisation des fonctionnalités des fromages qu'à celui des méthodes utilisées. C'est dans cet esprit que nous avons choisi de travailler sur un large échantillonnage de fromages du commerce correspondant à 4 catégories de pâtes persillées. Les techniques chimiques, rhéologiques sont classiquement utilisées pour ce type d'étude mais les observations sont souvent limitées à quelques critères. Nous avons testé 2 méthodes innovantes dans ce secteur des fromages ingrédients, la spectroscopie de fluorescence synchrone et l'analyse sensorielle, pour caractériser et observer l'évolution de ces produits au cours du chauffage et du refroidissement. Nous avons également étudié la potentialité de la méthode spectrale à servir de méthode rapide de substitution aux méthodes classiques (méthodes d'analyses biochimiques) ou aux méthodes plus lentes (analyse sensorielle), cette technique étant particulièrement intéressante pour sa rapidité de mesure et son faible coût de fonctionnement.

La première partie de l'étude a consisté à décrire les fromages à froid (20°C) par des méthodes d'analyses de composition physicochimique, d'analyse sensorielle, et rhéologique. Certaines propriétés fonctionnelles à chaud ont également été étudiées telles que les capacités à l'étalement, l'exsudation, le gratinage ainsi que les températures de ramollissement et de goutte. Ces dernières méthodes utilisées sont simples voire empiriques pour certaines et souvent utilisées par les professionnels. Ces premiers résultats ont permis de discriminer les fromages entre eux et de caractériser les 4 catégories de fromages. Les qualités des matrices fromagères reposent essentiellement sur l'organisation des composants tels que les protéines et azote soluble, les matières grasses, les minéraux (calcium, phosphore) qui vont conférer aux fromages des critères de texture et d'aspect, de potentialité à s'étendre, à exsuder…Certaines de ces mesures sont partiellement complémentaires (rhéologie et physicochimie), d'autres sont partiellement corrélées et donc redondantes. L'analyse sensorielle est la méthode qui reflète le mieux les perceptions des consommateurs, généralement les attributs de texture sont reliés aux fonctionnalités technologiques recherchées. Cependant, l'analyse sensorielle enrichit ici les informations apportées par les mesures instrumentales en décrivant certaines perceptions non mesurées telles que « granuleux, collant, résidus, filant ». Les mesures empiriques présentent de nombreux défauts particulièrement leur manque de sensibilité et leur mauvaise répétabilité mais elles sont couramment utilisées dans les industries comme indicateur qualité.

Dans l'industrie, une grande partie du contrôle des matières premières, des produits en cours de transformation et des produits finis repose sur l'analyse chimique des aliments. Ces méthodes sont longues et onéreuses. L'industrie est très sensible aux nouvelles méthodes d'analyse rapide. Les données spectrales enregistrées sont souvent définies comme une empreinte du produit à un moment donné pouvant être reliée à la composition chimique des produits étudiés. Par ailleurs, les fromages à pâte persillée étudiés sont très hétérogènes du fait de leur technologie particulière liée à la présence et au développement de moisissures. La deuxième partie de l'étude vise à analyser la capacité de la spectroscopie de fluorescence à prédire les caractéristiques physicochimiques des fromages à pâtes persillées avant tout traitement thermique. Cette étude nous démontre, malgré la forte hétérogénéité des échantillons qu'il est possible de prédire certains paramètres physicochimiques mais pas la totalité.

Dans une troisième partie, nous avons testé les potentialités de la fluorescence synchrone pour la caractérisation de la microstructure des fromages à chaud. L'étude a porté dans un premier temps sur 4 fromages pasteurisés "modèles" représentant les 4 catégories de fromages. Cette technique permet effectivement de suivre l'évolution de la microstructure des fromages à chaud. Les observations de l'évolution spectrales (modifications de largeur des pics et de leur hauteur, modification de la position des pics en longueur d'onde) sont concordantes avec les observations physicochimiques, elles sont également fortement corrélées aux données rhéologiques de viscoélasticité (fonte de la matière grasse et de la matrice fromagère). Les mesures (fluorescence et test de compression dynamique) réalisées sur les 4 fromages ont été étendues à l'ensemble des 20 fromages pour vérifier si l'approche ainsi que les conclusions étaient généralisables. Les résultats sont confirmés, les données spectrales suivent bien les données rhéologiques et les observations physicochimiques malgré l'hétérogénéité au sein des fromages. En effet, l'analyse conjointe des différents jeux de données (rhéologie et spectroscopie) aux moyens des outils chimiométriques que sont l'ACC et la PLS ont mis en évidence de fortes corrélations entre les données de rhéologie et les données de fluorescence synchrone. Les fortes corrélations obtenues indiquent qu'il existe une relation entre la structure au niveau moléculaire et les propriétés rhéologiques des fromages à pâtes persillées. L'approche semble donc pertinente pour décrire et suivre les phénomènes généralement observés au cours de la montée en température (fonte de la matière grasse et fonte de la matrice fromagère).

Dans la quatrième partie, l'objectif était de mettre en évidence la pertinence des outils rhéologique (test de compression dynamique) et spectral (spectroscopie de fluorescence) pour la caractérisation de la macro et micro structure des fromages à pâtes persillés au cours du chauffage mais également du refroidissement. Le test de compression dynamique utilisé permet de suivre les modifications de la macrostructure au cours du refroidissement avec une bonne discrimination entre les 4 types de fromages. Cependant, on peut observer une dispersion plus importante des échantillons dans le cas du refroidissement comparé à la montée en température traduisant une plus grande hétérogénéité dans la structure des échantillons après refroidissement (externalisation de la MG du réseau protéique formé par les caséines) La spectroscopie de fluorescence suit également l'évolution de la microstructure au cours du refroidissement. Les variations observées sont concordantes avec les modifications rhéologiques et physicochimiques. En effet, l'analyse conjointe des différents jeux de données (rhéologie et spectroscopie) enregistrées au cours du chauffage et du refroidissement aux moyens de l'ACC a mis en évidence de fortes corrélations entre ces données. Ces résultats confortent nos résultats préliminaires obtenus uniquement lors du chauffage des fromages. Les fortes corrélations obtenues indiquent qu'il existe toujours une relation entre la structure au niveau moléculaire et les propriétés rhéologiques des fromages à pâte persillée après refroidissement.

Après avoir testé les potentialités des différentes techniques à représenter les phénomènes d'évolution de la macro et microstructure de la matrice fromagère au cours du traitement thermique, la cinquième partie présente l'intérêt de l'analyse sensorielle pour le suivi de l'évolution des caractéristiques sensorielles des fromages après chauffage. Une étude comparative a été menée entre les fromages dégustés à froid et après chauffage. Cette analyse concerne toutes les caractéristiques sensorielles afin de mettre en évidence les différents changements perçus par le consommateur non seulement au niveau de la texture mais également sur des critères olfactogustatifs. A chaud, les fromages se distinguent sur des critères de flaveur, de texture et de perception de gras. Certains critères de texture sont spécifiques à certaines catégories de fromages (râpeux, caoutchouteux). Les 4 catégories de fromages sont bien discriminées par cette méthode. On observe également des discriminations internes à la catégorie portant sur l'utilisation ou non du lait cru ou traité thermiquement. La comparaison des résultats à froid et à chaud permet de constater qu'environ 50% des descripteurs ne sont pas communs, les changements portant uniquement sur l'aspect et la texture ce qui semble logique. Les critères olfactogustatifs restent les mêmes et ne se sont pas enrichis, seule leur intensité a varié, ceci en fonction des 2 grandes catégories bleu/fourme. La méthode des profils sensoriels est particulièrement pertinente et sensible mais longue et onéreuse, il serait intéressant de trouver des techniques pouvant se substituer en partie à l'analyse sensorielle.

L'objet du dernier chapitre porte sur la possibilité de trouver des corrélations entre les données sensorielles et les mesures instrumentales et spectrales, ce qui permettrait d'envisager des mesures plus rapides. Les résultats montrent qu'uniquement certains attributs peuvent être remplacés par l'une des 2 autres méthodes. Les 3 méthodes permettent bien de discriminer les échantillons entre eux mais avec des approches différentes : suivi de l'évolution de la macro et micro structure de la matrice fromagère en parallèle à l'évolution des perceptions. Chaque méthode peut être considérée comme une empreinte ou une cartographie du produit dans des conditions prédéfinies. Certaines approches peuvent se substituer à d'autres pour décrire l'évolution du phénomène au cours du chauffage et du refroidissement mais ceci de façon partielle. Nous pouvons considérer que ces méthodes sont complémentaires, elles permettent de discriminer les produits entre eux, elles donnent une identité aux produits et leur sensibilité est nettement plus importante comparée aux méthodes traditionnelles utilisées par les professionnels.

Ainsi ces fromages à pâte persillée ont des comportements au cours des traitements thermiques très diversifiés. Il est possible d'en déduire les potentialités de chacun à être utilisé comme ingrédient selon l'usage et les fonctionnalités recherchées. Les méthodes envisagées sont aptes à bien décrire et suivre les phénomènes observés, certaines méthodes peuvent se substituer aux méthodes professionnelles tout en apportant un enrichissement et une meilleure précision sur les phénomènes observés.

Toutefois, cette étude est exploratoire et donne des perspectives de développement de notre recherche. Les méthodes utilisées ont montré leur intérêt, la rhéologie pour caractériser la fonte de la matière grasse et de la matrice fromagère, l'analyse sensorielle pour caractériser les perceptions, la spectroscopie de fluorescence pour donner une empreinte spectrale des produits et suivre les évolutions de la microstructure. Cependant les méthodes sont évidement perfectibles. A titre d'exemple, le suivi spectral n'était pas suffisamment précis, une meilleure maitrise de la montée en température aurait permis de mieux encadrer les phénomènes de fonte de matière grasse.

Il sera indispensable de vérifier nos résultats sur un nombre plus important d'échantillons dont on pourrait maitriser les paramètres de fabrication ce qui permettrait de développer des modèles prédictifs et de donner des éléments de réponses aux professionnels concernant leur potentialité d'utilisation en tant qu'ingrédient. Par ailleurs, il serait intéressant de voir si certains critères à froid sont prédictibles du comportement à chaud pour ces fromages.

Lors de ce projet de thèse, nous avons utilisé les performances d'une seule méthode de mesure spectrale, la SFS. L'étude des fonctionnalités lors de traitements thermiques des fromages à pâte persillée pourrait être envisagée à l'aide d'autres méthodes de spectroscopies (moyen infrarouge, proche infrarouge, Raman, visible…). Ces techniques pourraient donner des informations complémentaires pour l'analyse des modifications physico-chimiques et chimiques observées lors de traitements thermiques. En effet, ces méthodes spectroscopiques font intervenir différentes régions du spectre électromagnétique mettant en jeu différents types de transitions atomiques ou moléculaires. L'acquisition de ces différentes informations permettrait de construire des modèles de connaissance sur les propriétés de structuration de produits fromages à pâtes persillés et de mettre en place des outils de prédictions permettant d'améliorer la compréhension des réactions impliquées dans la structuration des produits au cours de leur mise en forme. Ces différentes connaissances pourraient s'avérer particulièrement utiles au contrôle en ligne, notamment sur les chaînes de production industrielles. En effet, seule une approche globale s'intéressant aux différents niveaux d'organisation de la matrice fromagère est à même de nous donner les clés qui permettront de modéliser la structure de la matrice fromagère et de préciser les relations entre structure et texture. C'est là un élément incontournable pour qui envisage de maîtriser la texture des fromages et de développer de nouveaux produits aux propriétés recherchées.

RÉFÉRENCES BIBLIOGRAPHIQUES

RÉFÉRENCES BIBLIOGRAPHIQUES

Addeo, F. & Masi, P. (1992) Production of Pasta cheese, In 3rd Cheese Symposium National Dairy Products Research Center, Moorepark, ed. T.M., Cogan Teagasc, Fermoy, Co. Cork, , 31-40.

AFNOR (2002) Association francaise de normalisation. Chemical analysis.

AFNOR (2004) Association Française de Normalisation. Chemical analysis.

Agnar, H. (1988) PLS regression methods. Journal of Chemometrics 2(3), 211-228.

Aït Kaddour, A., Barron, C., Morel, M.H. & Cuq, B. (2007) Dynamic Monitoring of Dough Mixing Using Near-Infrared Spectroscopy: Physical and Chemical Outcomes. Cereal Chemistry 84 (1), 70-79.

Ak, M.M., Bogenrief, D., Gunasekaran, S. & Olson, N.F. (1993) Rheological evaluation of Mozzarella cheese by uniaxial horizontal extension. J. Texture Stud., 437–453.

Ak, M.M. & Gunasekaran, S. (1992) Stress-Strain Curve Analysis of Cheddar Cheese under Uniaxial Compression. Journal of Food Science 57(5), 1078-1081.

Ak, M.M. & Gunasekaran, S. (1995) Measuring elongational properties of Mozzarella cheese J. Texture Stud 26, 147–160.

Ak, M.M. & Gunasekaran, S. (2001) Linear Viscoelastic Methods. In Nondestructive food evaluation p. 287. Edited by S. Gunasekaran. New York: Marcel Dekker, Inc.

Alexander, M. & Corredig, M. (2007) Spectroscopic methods to determine inÂ situ changes in dairy systems â€“ ultrasonic and light scattering. Lait 87(4-5), 435-442.

Alison, A.S. & Michael, W.A.M. (2000) Evaluation of helical viscometry for assessing the functional properties of Mozzarella cheese, International J. Dairy Tech. 53, 57-62.

Alvarez, R.J. (1986) Expectations of Italian cheese in the pizza industry. In Proc. 23rd Annu. Marschall Invit. Ital. Cheese Sem., Madison, WI, .

Amiot, J., Fournier, S., Lebeuf, Y., Paquin, P. & Simpson, R. (2002) Composition, propriétés physicochimiques, valeur nutritive, qualité technologique et techniques d’analyse du lait. In Science et technologie du lait, pp. 1-74. Edited by C.L. Vignola. Montréal: Presses internationales polytechnique.

AOAC (1995) Chapter 33,Dairy products. In:Official methods of analysis of Association of Official Analytical Chemistry. 16th ed. Virginia: AOAC. p 60–1.

Apostolopoulos, C. (1994) Simple empirical and fundamental methods to determine objectively the stretchability of Mozzarella cheese, . J. Dairy Res. 61, 405–413.

Arnott, D.R., Morris, H.A. & Combs, W.B. (1957) Effect of Certain Chemical Factors on the Melting Quality of Process Cheese. Journal of dairy Science 40(8), 957-963.

Banon, S. & Hardy, J. (1991) Study of acid milk coagulation by an optical method using light reflection. . J. Dairy Res. 58, 75-84.

Banon, S. & Hardy, J. (1992) A colloidal approach of milk acidification by glucono-delta-Iactone. . J. Dairy Sci. 75, 935-941.

Barry, A.L. & Tamine, A.Y. (2010) Technology of cheesemaking. London, UK.

Belton, P. (2000) Spectroscopic methods for authentication – an overview. Biotechnol. Agron. Soc. Environ. 4(4), 204-207.

Bertola, N.C., Bevilacqua, A.E. & Zaritzky, N.E. (1992) Proteolytic and Rheological Evaluation of Maturation of Tybo Argentino Cheese. J. Dairy Sci. 75(12), 3273-3281.

Bertrand, D. (2006) Les méthodes d'analyse rapides dans les industries agroalimentaires. In La spectroscopie infrarouge et ses applications analytiques, pp. 4-28. Edited by D. Bertrand & E. Dufour. Paris, France: Lavoisier.

Bertrand, D., Courcoux, P. & Qannari, M. (2006) Méthodes exploratoires. In La spectroscopie infrarouge et ses applications analytiques, pp. 317-345. Edited by D. Bertrand & E. Dufour. Paris, France: Lavoisier.

Bertrand, D. & Dufour, É. (2006) La spectroscopie infrarouge est ses applications analytiques. paris, France: Tec & Doc, Lavoisier

Bertrand, D., Lila, L., Furtoss, V., Robert, P. & Downey, G. (1987) Application of principal component analysis to the prediction of lucerne forage protein content and *in vitro* dry matter digestibility by NIR spectroscopy. Journal of the Science of Food and Agriculture 41(4), 299-307.

Bertrand, D. & Scotter, C.N.G. (1992) Application of Multivariate Analyses to NIR Spectra of Gelatinized Starch Applied Spectroscopy 46, 1420–1425.

Birlouez-Aragon, I., Nicolas, M., Metais, A., Marchond, N., Grenier, J. & Calvo, D.A. (1998) Rapid Fluorimetric Method to Estimate the Heat Treatment of Liquid Milk. International Dairy Journal 8, 771–777.

Birlouez-Aragon, I., Sabat, P. & Gouti, N. (2002) A new method for discriminating milk heat treatment. International Dairy Journal 12(1), 59-67.

Blumenthal, A., Weymuth, H. & Hansen, W. (1976) Flow and drip points of Raclette cheese. Schweiz. Milchztg 102, 391–395.

Boubellouta, T. (2008) Appoets des spectroscopies infrarouge et de fluorescence couplées à la chimiométrie pour la caractérisation de la structure de matrices fromagères et des realtions structure-texture. In Ecole doctorale des sciences de la vie et de la sante, Vol. thèse clermont-ferrand: Blaise pascal

Boubellouta, T. & Dufour, E. (2008) Effects of Mild Heating and Acidification on the Molecular Structure of Milk Components as Investigated by Synchronous Front-Face Fluorescence Spectroscopy Coupled with Parallel Factor Analysis. Applied Spectroscopy 62(5), 490-496.

Boubellouta, T. & Dufour, E. (2010) Cheese-Matrix Characteristics During Heating and Cheese Melting Temperature Prediction by Synchronous Fluorescence and Mid-infrared Spectroscopies. Food and Bioprocess Technology, 1-12.

Boubellouta, T., Galtier, V. & Dufour, É. (2009) Effects of Added Minerals (Calcium, Phosphate, and Citrate) on the Molecular Structure of Skim Milk as Investigated by Mid-Infrared and Synchronous Fluorescence Spectroscopies Coupled with Chemometrics. Appl. Spectrosc. 63(10), 1134-1141.

Bourne, M.C. (1978) Texture profile analysis. Food Technol 32, 62–66.

Bouroche, J.M. & Saporta, G. (1998) L'analyse des données. ed. Presse universitaire de France, Paris, coll "que sais-je?".

Bowland, E.L. & Foegeding, E.A. (1999) Factors determining large-strain (fracture) rheological properties of model processed cheese, . Journal of dairy science 82, 1851-1859.

Brandt, M.A., Skinner, E.Z. & Coleman, J.A. (1963) Texture profile method. Journal of Food Science 29, 404-409.

Breene, W.A., Price, W.V. & Ernstrom, C.A. (1964) Manufacture of Pizza cheese without starter,. J. Dairy Sci. 47, 1173_1180.

Brighenti, M., Govindasamy-Lucey, S., Lim, K., Nelson, K. & Lucey, J.A. (2008) Characterization of the Rheological, Textural, and Sensory Properties of Samples of Commercial US Cream Cheese with Different Fat Contents. J Dairy Sci 91, 4501-4517.

Bro, R. (1997) PARAFAC. Tutorial and applications. Chemometrics and Intelligent Laboratory Systems 38(2), 149-171.

Bro, R., Van den Berg, F., Thybo, A., Andersen, C.M., Jorgensen, B.M. & Andersen, H. (2002) Multivariate Data Analysis as a Tool in Advanced Quality Monitoring in the Food Production Chain. . Trends in Food Science and Technology 13, 235-244.

Brown, J.A., Foegeding, E.A., Daubert, C.R., Drake, M.A. & Gumpertz, M. (2003) Relationships among rheological and sensorial properties of young cheeses. Journal of dairy science 86(10), 3054-3067.

Brulé, G., Maubois, J.L. & Fauquant, J. (1974) Étude de la teneur en éléments minéraux des produits obtenus lors de "ultrafiltration du lait sur membrane. . Lait 54, 600-615.

Bryant, A., Ustunol, Z. & Steffe, J. (1995) Texture of Mozzarella cheese as influenced by fat reduction,. J. Food Sci. 60, 1216–1219.

Bryant, C.M. & McClements, D.J. (1998) Molecular basis of protein functionality with special consideration of cold-set gels derived from heat-denatured whey. Trends Food Sci. Technol. 9, 143-151.

Campanella, O.H., Popplewell, L.M., Rosenau, J.R. & Peleg, M. (1987) Elongational Viscosity Measurements of Melting American Process Cheese. Journal of Food Science 52(5), 1249-1251.

Casiraghi, E.M., Bagley, E.B. & Christianson, D.D. (1985) Behavior of mozzarella, cheddar and processed cheese spread in lubricated and bonded uniaxial compression. Journal of Texture Studies 16(3), 281-301.

Cavella, S., Chemin, S. & Masi, P. (1992) Objective measurement of the stretchability of Mozzarella cheese. Journal of Texture Studies 23(2), 185-194.

Cerning, J., Gripon, J.C., Lamberet, G. & Lenoir, J. (1987) Les activités biochimiques des PeniciUium utilisés en fromagerie. Le Lait 67(1), 3-39.

Christensen, J., Becker, E.-C.M. & Frederiksen, C.S. (2005) Fluorescence spectroscopy and PARAFAC in the analysis of yoghurt. Chemometrics and Intelligent Laboratory Systems 75, 201-208.

Christensen, J., Povlsen, V.T. & Sorensen, J. (2003a) Application of Fluorescence Spectroscopy and Chemometrics in the Evaluation of Processed Cheese During Storage. J. Dairy Sci. 86(4), 1101-1107.

Christensen, J., Povlsen, V.T. & Sørensen, J. (2003b) Application of fluorescence spectroscopy and chemometrics in the evaluation of processed cheese during storage. Journal of Dairy Science 86, 1101-1107.

Christensen, J., Povlsen, V.T. & Sørensen, J. (2003c) Application of fluorescence spectroscopy and chemometrics in the evaluation of the stability of processed cheese,. J. Dairy Sci 86, 1101-1107.

Civille, G.V. & Liska, I.H. (1975) Modifications and applications to foods of the General Foods Sensory Texture Profile Technique. J. Texture Stud. 6, 19-32.

Civille, G.V. & Szczesniak, A., S. (1973) Guidelines to Training a Texture Profile Panel. Journal of Texture Studies 4, 204-223.

CNIEL (2009) In: L'économie laitière en chiffres. Paris, France.

Corrieu, G. (1996) Capteurs de measures de la coagulation et du raffermissement des caillés de fromageries Nice, France: Abstract of Mediterranean Dairy Conference

Coulon, J.B., Delacroix-Buchet, A., Martin, B. & Pirisi, A. (2004) Relationships between ruminant management and sensory characteristics of cheeses: a review. lait 84, 221–241.

Creamer, L.K. (1985) Water absorption by renneted casein micelles. . Milchwissenschaft 40, 589-591.

Creamer, L.K. & Olson, N.F. (1982) Rheological evaluation of maturing Cheddar. Journal of Food Science 47, 631-646.

Dalgleish, D.G. & Law, A.J.R. (1988) pH-induced dissociation of bovine casein micelles. I. Analysis of liberated caseins. . J. Dairy Res. 55, 529-538.

Dalibart, M. & Servant, S. (2000) Spectroscopie dans l'infrarouge. Techniques de l'Ingénieur 2845, 1-26.

Darling, D.F. & Dickson, J. (1979) The determination of the zeta potential of casein micelles. Journal of Dairy Research 46, 329-332.

Desmazeaumd, J., Griponj, C., Le barsd & Bergerej, L. (1976) Etude du rôle des microorganismes et des enzymes au cours de la maturation des fromages. III. Influence desmicro-organismes. Le Lait 56, 379-396.

Devaux, M.F., Robert , P., Qannari, E.M., Safar, M. & Vigneau, E. (1993) Canonical correlation analysis of mid and near infrared oil spectra. Appl. Spectrosc. 47, 1024-1028.

Dillard, C.J. & Tappel, A.L. (1971) Fluorescent Products of Lipid Peroxidation of Mitochondria and Microsomes Lipids 6, 715-721.

Diplock, A.T., Aggett, P.J., Ashwell, M., Bornet, F., Fern, E.B. & Roberfroid, M.B. (1999) Scientific concepts of functiional foods in Europe : consensus document. Br J Nutr, 81 suppl1 : S1-S28.

Downey, G. (2006) Discrimination et authentification des aliments et des ingrédients alimentaires par spectroscopie dans l'infrarouge proche et moyen. In La spectroscopie infrarouge et ses applications analytiques, pp. 479-504. Edited by D. Bertrand & E. Dufour. Paris, France: Lavoisier.

Downey, G., Sheehan, E., Delahunty, C., O'Callaghan, D., Guinee, T. & V.Howard (2005) Prediction of maturity and sensory attributes of Cheddar cheese using near-infrared spectroscopy. Int. Dairy J. 15, 701–709.

Drake, M.A., Gerard, P.D., Truong, V.D. & Daubert, C.R. (1999) Relationship between instrumental and sensory measurements of cheese texture. Journal of Texture Studies 30(4), 451-476.

Dufour, É. (2010) Recent advances in the analysis of dairy product quality using methods based on the interactions of light with matter. International Journal of Dairy Technology 64(2), 153-165.

Dufour, E., Devaux, M.F., Fortier, P. & Herbert, S. (2001) Delineation of the structure of soft cheeses at the molecular level by fluorescence spectroscopy-relationship with texture. International Dairy Journal 11(4-7), 465-473.

Dufour, E., Frencia, J.P. & Kane, E. (2003) Development of rapid method based on front face fluorescence spectroscopy for the monitoring of fish freshness. Food Res. Inter. 36, 415-423.

Dufour, E., Genot, C. & Haertlé, T. (1994) β-lactoglobulin binding properties during its folding changes studied by fluorescence spectroscopy. Biochimica et Biophysica Acta 1205, 105-112.

Dufour, E., Lopez, C., Riaublanc, A. & Mouhous Riou, N. (1998) La spectroscopie de fluorescence frontale : une approche non invasive de la structure et des interactions entre les constituants des aliments. Agoral 10, 209-215.

Dufour, E., Mazerolles, G., Devaux, M.F., Duboz, G., Duployer, M.H. & Mouhous Riou, N. (2000) Phase transition of triglycerides during semi-hard cheese ripening. International Dairy Journal 10(1-2), 81-93.

Dufour, E. & Riaublanc, A. (1997a) Potentiality of spectroscopic methods for the characterisation of dairy products. I. Front-face fluorescence study of raw, heated and homogenised milks. Le Lait 77(6), 657-670.

Dufour, E. & Riaublanc, A. (1997b) Potentiality of Spectroscopic Methods for the Characterisation of dairy Products. II. Mid Infrared Study of the Melting Temperature of Cream Triacylglycerols and of the Solid Fat Content in Cream. . Le lait 77, 671-681.

Duggan, D.E., Bowman, R.L., Brodie, B.B. & Udenfriend, S. (1957) A Spectrophotofluorometric Study of Compounds of Biological Interest Arch. Biochem. and Biophys 16, 1-14.

Eberhard, P., Moor, U., Ruegg, M. & Fluckiger, E. (1986) Objective measurement of the softening and dropping point of Raclette cheese Schweiz Milchwirtschaftliche Forsch 15, 93-96.

Famelart, M.-H., Le Graet, Y., Michel, F., Richoux, R. & Riaublanc, A. (2002) Evaluation of the methods of measurement for functional properties of Emmental cheeses from the west of France. Lait 82(2), 225-245.

Fenelon, M.A. & Guinee, T.P. (2000) Primary proteolysis and textural changes during ripening in Mozzarella cheeses manufactured to different fat contents. Int. Dairy J.10 151-158.

Fife, R.L., McMahon, D.J. & Oberg, C.J. (1996) Functionality of low fat Mozzarella cheese. Journal of Dairy Science 79(11), 1903-1910.

Fife, R.L., McMahon, D.J. & Oberg, C.J. (2002) Test for Measuring the Stretchability of Melted Cheese. Journal of dairy science 85(12), 3539-3545.

Foegeding, E.A., Brown, J., Drake, M.A. & Daubert, C.R. (2003) Sensory and mechanical aspects of cheese texture. Int. Dairy J 13, 585-591.

Foegeding, E.A. & Drake, M.A. (2007) Invited Review: Sensory and Mechanical Properties of Cheese Texture. Journal of Dairy Science 90(4), 1611-1624.

Fox, J.B. & Thayer, D.W. (1998) Radical oxidation of riboflavin. Int. J. Vitam. Nutr. Res. 68, 174–180.

Fox, P.F. (1989) The milk protein system. In Developments in dairy chemistry, pp. 1-54. Edited by P.F. Fox. New York: Elsevier Applied Science.

Fox, P.F., Guinee, T.P., T.M., C. & McSweeney, P.H. (2000) Fundamentals of cheese science. ASPEN Publishers, Inc., Gaithersburg, Maryland.

Garimella, S.K.P., Prow, L.A. & Metzger, L.E. (2005) Utilization of Front-Face Fluorescence Spectroscopy for Analysis of Process Cheese Functionality. . J. Dairy Sci. 88, 470-477.

Gatellier, P., Gomez, S., Gigaud, V., Berri, C., Bihan-Duval, E.L. & Santé-Lhoutellier, V. (2007) Use of a fluorescence front face technique for measurement of lipid oxidation during refrigerated storage of chicken meat. Meat Science 76(3), 543-547.

Geladi, P. & Kowalski, B.R. (1986) Partial least-squares regression: a tutorial. Analytica Chimica Acta 185, 1-17.

Genot, C., Tonetti, F., Montenay-Garestier, T., Marion, D. & Drapon, R. (1992) Front-face fluorescence applied to structural studies of proteins and lipid-protein interactions of visco-elastic food products.1-Designing of front-face adaptor and validity of front-face fluorescence measurements Sciences desAliments 12, 199-212.

Gerry, P.S. & Theodore, P.L. (2006) Evaluation of Front-face Fluorescence for Assessing Thermal Processing of Milk. Journal of Food Science 71(2), C69-C74.

Grappin, R., Lefier, D. & Mazerolles, G. (2006) Analyse du lait et des produits laitiers. In La spectroscopie infrarouge et ses applications analytiques, pp. 583-626. Edited by D. Bertrand & E. Dufour. Paris, France: Lavoisier.

Guinee, T. (2002a) Cheese rheology. Pages 341–349 in Encyclopedia of Dairy Science. H. Roginski, J. W. Fuquay, and P. F. Fox, ed. Academic Press, London, UK. .

Guinee, T.P. (2002b) The functionality of cheese as an ingredient: a review. Australian Journal of Dairy Technology 57(2), 79-91.

Guinee, T.P., Aut, M.A.E. & Fenelon, M.A. (2000) The effect of fat on heat rheology, microstructure and heat induced functional characteristics of cheddar cheese. Int. Dairy J. 10: 277-288.

Guinee, T.P., Auty, M.A.E. & Mullins, C. (1999) Observations on the microstructure and heat-induced changes in the viscoelasticity of commercial cheeses. Australian J. Dairy Technol. 54, 84 - 89.

Guinee, T.P. & Kilcawley, K.N. (2001) Cheese as an ingredient, in, Cheese Chemistry, Physics and Microbiology, Vol. 1, Major Cheese Groups 3rd edn (P.F. Fox, P.L.H. McSweeney, T.M. Cogan, T.P. Guinee, eds.), pp. 395–428, Elsevier Academic Press, Amsterdam.

Guinee, T.P. & Kilcawley, K.N. (2004) Cheese as an ingredient, in: Cheese: Chemistry, Physics and Microbiology, Vol. 2, 3rd edn., Academic Press, London, UK, 2004, pp. 394–428.

Guinee, T.P. & O'Callaghan, D.J. (1997) The use of a simple empirical method for objective quantification of the stretchability of cheese on cooked pizza pies. Journal of Food Engineering 31(2), 147-161.

Gunasekaran, S. & Ak M.M. (2003) Measuring cheese melt and flow properties, in Cheese Rheology and Texture pp. 331–375. Edited by B.R. CRC Press, FL, . United States of America.

Gunasekaran, S., Hwang, C.H. & Ko, S. (2002) Cheese melt/flow measurement methods: recent developments. Australian Journal of Dairy Technology 57(2), 128-133.

Hardy, J. (1997) L'activité de l'eau et le salage des fromages. In Le fromage, pp. 63-83. Edited by A. Eck & J.C. Gillis. Paris, France: Lavoisier.

Hardy, J. & Scher, J. (1997) Les propriétés physiques et organoleptiques du fromage. In Le fromage, pp. 479-492. Edited by A. Eck & J.C. Gillis. Paris, France: Lavoisier.

Heertje, I., Visser, J. & Smits, P. (1985) Structure formation in GDL milk gels. Food Microstructure 4, 267-277.

Herbert, S. (1999) Caractérisation de la structure moléculaire et microscopique de fromages à pâte molle, Analyse multivariée des données structurales en relation avec la texture Vol. Thèse: École Doctorale Chimie Biologie de l'Université de Nantes, France.

Herbert, S., Mouhous Riou, N., Devaux, M.F., Riaublanc, A., Bouchet, B., Gallant, D.J. & Dufour, É. (2000) Monitoring the identity and the structure of soft cheeses by fluorescence spectroscopy. Le Lait 80(6), 621-634.

Herbert, S., Riaublanc, A., Bouchet, B., Gallant, D.J. & Dufour, E. (1999) Fluorescence Spectroscopy Investigation of Acid-or Rennet-Induced Coagulation of Milk. J. Dairy Sci. 82(10), 2056-2062.

Hort, J. & Le Grys, G. (2000) Rheological models of Cheddar cheese texture and their application to maturation. J. Texture Stud. 31, 1-24.

IDF (2003) Determination of calcium, sodium, potassium and magnesium contents–Atomic absorption spectroscopic method. 119:2003. Brussels, Belgium: International Dairy Federation.

Jack, F.R., Paterson, A. & Piggott, J.R. (1993) Relationships between rheology and composition of Cheddar cheeses and texture as perceived by consumers. International Journal of Food Science and Technology 28(3), 293-302.

Jollife, I.T. (2002) Principal component analysis. New York: Springer.

Karoui, R. & Blecker, C. (2010a) Fluorescence Spectroscopy Measurement for Quality Assessment of Food Systems-a Review. Food and Bioprocess Technology.

karoui, R. & Blecker, C. (2010b) Fluorescence Spectroscopy Measurement for Quality Assessment of Food Systems—a Review. Food and Bioprocess Technology DOI 10.1007/s11947-010-0370-0.

Karoui, R., Cartaud, G. & Dufour, E. (2006a) Front-face fluorescence spectroscopy as a rapid and nondestructive tool for differentiating various cereal products: A preliminary investigation Journal of Agricultural and Food Chemistry 54, 2027–2034.

Karoui, R. & De Baerdemaeker, J. (2007) A review of the analytical methods coupled with chemometric tools for the determination of the quality and identity of dairy products. Food Chemistry 102(3), 621-640.

Karoui, R., De Baerdemaeker, J. & Dufour, E. (2008a) Utilisation of front face fluorescence spectroscopy as a tool for the prediction of some chemical parameters and the melting point of semi-hard and hard cheeses: A preliminary study Eur. Food Res. Technol. 226, 119-1126.

Karoui, R. & Dufour, É. (2003) Dynamic testing rheology and fluorescence spectroscopy investigations of surface to centre differences in ripened soft cheeses. International Dairy Journal 13(12), 973-985.

Karoui, R. & Dufour, É. (2006) Prediction of the rheology parameters of ripened semi-hard cheeses using fluorescence spectra in the UV and visible ranges recorded at a young stage. International Dairy Journal 16(12), 1490-1497.

Karoui, R., Dufour, E. & Baerdemaeker, J.D. (2007a) Front face fluorescence spectroscopy coupled with chemometric tools for monitoring the oxidation of semi-hard cheeses throughout ripening. Food Chemistry 101 1305–1314.

Karoui, R., Dufour, E. & Baerdemaeker, J.D. (2007b) Monitoring the molecular changes by front face fluorescence spectroscopy throughout ripening of a semi-hard cheese. Food Chemistry 104, 409–420.

Karoui, R., Dufour, E., Bosset, J.O. & De Baerdemaeker, J. (2007c) The use of front face fluorescence spectroscopy to classify the botanical origin of honey samples produced in Switzerland. Food Chemistry 101(1), 314-323.

Karoui, R., Dufour, É. & De Baerdemaeker, J. (2006b) Common components and specific weights analysis: A tool for monitoring the molecular structure of semi-hard cheese throughout ripening. Analytica Chimica Acta 572(1), 125-133.

Karoui, R., Dufour, E., Pillonel, L., Picque, D., Cattenoz, T. & Bosset, J.O. (2004a) Determining the geographic origin of Emmental cheeses produced during winter and summer using a technique based on the concatenation of MIR and fluorescence spectroscopic data. Eur. Food Res. Technol. 219, 184-189.

Karoui, R., Dufour, E., Pillonel, L., Picque, D., Cattenoz, T. & Bosset, J.O. (2004b) Fluorescence and infrared spectroscopies: a tool for the determination of the geographic origin of Emmental cheeses manufactured during summer. Lait 84, 359-374.

Karoui, R., Dufour, E., Schoonheydt, R. & Baerdemaeker, J.D. (2007d) Characterisation of soft cheese by front face fluorescence spectroscopy coupled with chemometric tools: Effect of the manufacturing process and sampling zone. Food Chemistry 100, 632–642.

Karoui, R., Kemps, B., Bamelis, F., De Ketelaere, B., Merten, K., Schoonheydt, R., Decuypere, E. & De Baerdemaeker, J. (2005a) Development of a rapid method based on front-face fluorescence spectroscopy for the monitoring of egg freshness: 2-evolution of egg yolk. Eur Food Res Technol.

Karoui, R., Laguet, A. & Dufour, E. (2003a) Fluorescence spectroscopy: A tool for the investigation of cheese melting - Correlation with rheological characteristics. Lait 83(3), 251-264.

Karoui, R., Laguet, A. & Dufour, É. (2003b) Fluorescence spectroscopy: A tool for the investigation of cheese melting-Correlation with rheological characteristics. Lait 83, 251-264.

Karoui, R., Lefur, B., Grondin, C., Thomas, E., Demeulemester, C., Baerdemaeker, J.D. & Guillard, A.-S. (2007e) Mid-infrared spectroscopy as a new tool for the evaluation of fish freshness. International Journal of Food Science & Technology 42(1), 57-64.

Karoui, R., Martin, B. & Dufour, É. (2005b) Potentiality of front-face fluorescence spectroscopy to determine the geographic origin of milks from the Haute-Loire department (France). Lait 85(3), 223-236.

Karoui, R., Mazerolles, G. & Dufour, É. (2003c) Spectroscopic techniques coupled with chemometric tools for structure and texture determinations in dairy products. International Dairy Journal 13(8), 607-620.

Karoui, R., Mouazen, A.M., Dufour, É., Pillonel, L., Schaller, E., Picque, D., De Baerdemaeker, J. & Bosset, J.O. (2006c) A comparison and joint use of NIR and MIR spectroscopic methods for the determination of some parameters in European Emmental cheese. European Food Research and Technology 223(1), 44-50.

Karoui, R., Mouazen, A.M., Dufour, E., Schoonheydt, R. & Baerdemaeker, J.D. (2006d) Utilisation of front-face fluorescence spectroscopy for the determination of some selected chemical parameters in soft cheeses. Lait 86(2), 155-169.

Karoui, R., Mouazen, A.M., Dufour, E., Schoonheydt, R. & De Baerdemaeker, J. (2006e) Utilisation of front-face fluorescence spectroscopy for the determination of some selected chemical parameters in soft cheeses. Le Lait 86(2), 155-169.

Karoui, R., NicolaÃ¯, B. & De Baerdemaeker, J. (2008b) Monitoring the Egg Freshness During Storage Under Modified Atmosphere by Fluorescence Spectroscopy. Food and Bioprocess Technology 1(4), 346-356.

Karoui, R., Pillonel, L., Schaller, E., Bosset, J.O. & De Baerdemaeker, J. (2007f) Prediction of sensory attributes of European Emmental cheese using near-infrared spectroscopy: a feasibility study. Food Chemistry 101(3), 1121-1129.

Karoui, R., Schoonheydt, R., Decuypere, E., Nicolaï, B. & De Baerdemaeker, J. (2007g) Front face fluorescence spectroscopy as a tool for the assessment of egg freshness during storage at a temperature of 12.2°C and 87% relative humidity Analytica Chimica Acta 582, 83-91.

Karoui, R., Thomas, E. & Dufour, E. (2006f) Utilisation of a rapid technique based on front-face fluorescence spectroscopy for differentiating between fresh and frozen–thawed fish fillets Food Research International 39, 349-355.

Kindstedt, P.S. (1993) Effect of manufacturing factors, composition, and analysis on the functional characteristics of Mozzarella cheese. Food Sci. Nutr 33, 167-187.

Kindstedt, P.S., Caric, M. & Milanovic, S. (2004) Pasta-Filata cheeses. In: Cheese Chemistry, Physics and Microbiology, Vol. 2, Major Cheese Groups, 3rd edn, pp. 251–277, Elsevier Academic Press, Amsterdam.

Kindstedt , P.S. & Fox, P.F. (1991) Modify Gerber test for free oil in Mozzarella cheese. J. Dairy Sci. 56, 1115-1116.

Kindstedt, P.S. & Rippe, J.K. (1990) Raid quantitive test for free oil (oiling-off) in melted Mozzarella cheese. J. Dairy Sci. 73, 867-873.

Kindstedt, P.S., Rippe, J.K. & Duthie, C.M. (1989) Measurement of Mozzarella cheese melting properties by helical viscometry. Journal of Dairy Science 72(12), 3117.

Konstance, R.P. & Holsinger, V.H. (1992) Development of rheological test methods for cheese. Food technology 46(1), 105-109.

Kosikowski, F. (1982) Process cheese and related type. in cheese and fermented milk foods, 2nd ed., p 405-406. Edwards Brothers, Inc., Ann Arbor, MI.

Kristensen, D., Hansen, E., Arndal, A., Trinderup, R.A. & Skibsted, L.H. (2001) Influence of light and temperature on the colour and oxidative stability of processed cheese. int. Dairy J. 11, 837-843.

Kristensen, D., Orlien, V., Mortensen, G., Brockhoff, P. & Skibsted, L.H. (2000) Light-induced oxidation in sliced Havarti cheese packaged in modified atmosphere. International Dairy Journal 10(1-2), 95-103.

Kulmyrzaev, A., Bertrand, D. & Dufour, E. (2008) Characterization of different blue cheeses using a custom-design multispectral imager. Dairy Sci. Technol. 88(4-5), 537-548.

Kulmyrzaev, A. & Dufour, E. (2002) Determination of lactulose and furosine in milk using front-face fluorescence spectroscopy. Lait 82(6), 725-735.

Kulmyrzaev, A., Dufour, E., Noël, Y., Hanafi, M., Karoui, R., Qannari, E.M. & Mazerolles, G. (2005a) Investigation at the molecular level of soft cheese quality and ripening by infrared and fluorescence spectroscopies and chemometrics—relationships with rheology properties. International Dairy Journal 15(6-9), 669-678.

Kulmyrzaev, A.A., Levieux, D. & Dufour, E. (2005b) Front-Face Fluorescence Spectroscopy Allows the Characterization of Mild Heat Treatments Applied to Milk. Relations with the Denaturation of Milk Proteins. Journal of Agricultural and Food Chemistry 53(3), 502-507.

Lahner, B. (1991) Spectrométrie infrarouge à transformée de Fourrier et analyse multidimensionnelle de données spectrales : Application à la quantification et au contrôle de procédés dans le domaine des produits laitiers. Vol. Thèse de doctorat Sciences des aliments Dijon: Université de Bourgogne.

Lakowicz, J.R. (1983a) Fluorophores. In Principles of Fluorescence Spectroscopy,. New York: Meyers, R.A.; Eds.; Plenum Press:.

Lakowicz, J.R. (1983b) Protein fluorescence. In Principles of fluorescence spectroscopy, pp. 341-389. Edited by J.R. Lakowicz. New York: Plenum Press.

Lawless, H.T. & Heymann., H. (1999) Sensory Evaluation of Food. Aspen Publishers, Gaithersburg, MD.

Lawlor, J.B., Delahunty, C.M., Sheehan, J. & Wilkinson, M.G. (2003) Relationships between sensory attributes and the volatile compounds, non-volatile and gross compositional constituents of six lue-type cheeses. International Dairy Journal 13(6), 481-494.

Le Graet, Y. & Brulé, G. (1993) Effects of pH and ionic strength on distribution of mineral salts in milk. Le Lait 73(1), 51-60.

Lebart, L., Morineau, A. & Tabard, N. (1977) Technique de la description statistique, méthodes et logiciels pour l'analyse des grands tableaux. Paris: Dunod.

Lebecque, A., Laguet, A., Devaux, M.F. & Dufour, E. (2001) Delineation of the texture of Salers cheese by sensory analysis and physical methods. Le Lait 81, 609-623.

Lefevere, I., Dewettinck, K. & Huyghebaert, A. (2000) Cheese fat as driving force in cheese flow upon melting. Milchwissenschaft 55, 563-565.

Lenoir, J., Lambert, G., Schmidt, J.L. & Tourneur, C. (1985) La maîtrise du bioréacteur fromage. Biofutur 41, 23-50.

Liu, X. & Metzger, L.E. (2007) Application of Fluorescence Spectroscopy for Monitoring Changes in Nonfat Dry Milk During Storage. J. Dairy Sci. 90(1), 24-37.

Lloyd, J.B.F. (1971) The Nature and Evidential Value of the Luminescence of Automobile Engine Oils and Related Materials: Part I. Synchronous Excitation of Fluorescence Emission. Journal of the Forensic Science Society 11(2), 83-94.

Lopez, C., Bourgaux, C., Lesieur, P., Bernadou, S., Keller, G. & Ollivon, M. (2002) Thermal and structural behavior of milk fat: Influence of cooling rate and droplet size on cream crystallization. . J. Colloid Interface Sci 254, 64-78.

Lopez, C., Briard-Bion, V., Camier, B. & Gassi, J.-Y. (2006) Milk fat thermal properties and solid fat content in Emmental cheese: A differential scanning calorimetry study. J. Dairy Sci. 89 2894–2910.

Lucey, J.A. (2008) Some perspectives on the use of cheese as a food ingredient. Dairy Sci. Technol., 1-22.

Lucey, J.A., Johnson, M.E. & Horne, D.S. (2003) Perspectives on the basis of the rheology and texture properties of cheese. Journal of dairy science 86(9), 2725-2743.

Ma, L., Darke, M.A., Barbosa-Canovas, G.V. & Swanson, B.G. (1996) Viscoelastic properties of reduced-fat and full-fat Cheddar cheese. Journal of Food Science 61, 821-823.

Mann, E. (2000) Cheese product innovations. Dairy Industries International 65(10), 17-18.

Marshall, R. (1993) Chemical and physical methods. In: Standard methods for the examination of dairy products. 16th ed.Washington, D.C.: American Public Health Assn. p 4333–529.

Martın-del-Campo, S.T., Picque, D., Cosı´o-Ramı´rez, R. & Corrieu, G. (2007) Evaluation of Chemical Parameters in Soft Mold-Ripened Cheese During Ripening by Mid-Infrared Spectroscopy. J. Dairy Sci 90, 3018–3027.

Matzdorf, B., Cuppett, S.L., Keeler, L. & Hutkins, R.W. (1994) Browning of Mozzarella cheese during high temperature pizza baking. Journal of Dairy Science 77(10), 2850.

Mazerolles, G., Devaux, M.F., Duboz, G., Duployer, M.H., Mouhous Riou, N. & Dufour, E. (2001) Infrared and fluorescence spectroscopy for monitoring protein structure and interaction changes during cheese ripening. Lait 81, 509-527.

Mazerolles, G., Devaux, M.F., Dufour, E., Qannari, E.M. & Courcoux (2002) Chemometric methods for the coupling of spectroscopic techniques and for the extraction of the relevant information contained in the spectral data tables. Chemometrics and Intelligent Laboratory Systems 63(1), 57-68.

McMahon, D.J. (1996) Measuring Stretch of Mozzarella Cheese. in Proc.12th Biennial Cheese Ind. Conf., Utah State University, Logan, .

McSweeney, P.L.H. (2007) Cheese problems solved. Woodhead Publishing Limited, Cambridge, England. p.18.

McSweeney, P.L.H. & Sousa, M.J. (2000) Biochemical pathways for the production of flavour compounds in cheese during ripening Le Lait 80, 293-324.

Miquel Becker, E., Christensen, J., Frederiksen, C.S. & Haugaard, V.K. (2003) Front-face fluorescence spectroscopy and chemometrics in analysis of yogurt: rapid analysis of riboflavin. Journal of dairy Science 86, 2508-2515.

Muller, K.E. (1982) Understanding canonical correlation through the general linear model and principal components. American statistician 36, 342-354.

Olson, N.F. & Price, W.V. (1958) A Melting Test for Pasteurized Process Cheese Spreads. J. Dairy Sci. 41(7), 999-1000.

Patra, D. & Mishra, A.K. (2002) Recent developments in multi-component synchronous fluorescence scan analysis. Trends in Analytical Chemistry 21(12), 787-798.

Payne, F. (1995) Automatic control of coagulum cutting in cheese manufacturing. Applied engineering in Agriculture 11(5), 691-697.

Payne, F.A., Hicks, C.L. & Shen, P.-S. (1993) Predicting Optimal Cutting Time of Coagulating Milk Using Diffuse Reflectance. J. Dairy Sci. 76(1), 48-61.

Pearson, K. (1901) On lines and planes of closest fit to systems of points in shape. Philosophical Magazine 2, 559-572.

Peyron, M.A., Mioche, L. & Culioli, J. (1994) Bite force and sample deformation during hardness assessment of viscoelastic models of foods. J. Text. Stud. 25, 59-76.

Pillonel, L., Badertscher, R., Bütikofer, U., Casey, M., M., D.T., Lavanchy, P., Meyer, J., Tabacchi, R. & Bosset, J.O. (2002) Analytical methods for the determination of the geographic origin of Emmentaler cheese. Main framework of the project; chemical, biochemical, microbiological, colour and sensory analyses. European Food Research and Technology 215(3), 260-267.

Pinho, O., Mendes, E., Alves, M.M. & Ferreira, I.M.P.L.V.O. (2004) Chemical, Physical, and Sensorial Characteristics of "Terrincho" Ewe Cheese: Changes During Ripening and Intravarietal Comparison. J. Dairy Sci. 87(2), 249-257.

Poulli, K.I., Mousdis, G.A. & Georgiou, C.A. (2007) Rapid synchronous fluorescence method for virgin olive oil adulteration assessment. Food Chemistry 105(1), 369-375.

Purna, S.K.G., Prow, L.A. & Metzger, L.E. (2005) Utilization of front-face fluorescence spectroscopy for analysis of process cheese functionality. Journal of dairy science 88(2), 470-477.

Reparet, J.M. (2000) Les propriétés fonctionnelles évaluées à chaud : des fromages aux imitations fromagères. p. 156. France: Institut National Agronomique Paris-Grignon.

Reparet, J.M. & Noël, Y. (2003) Relation between a temperature-sweep dynamic shear test and functional properties of cheeses. Le lait 83, 321-333.

République Française (1988) Décret du 30 décembre 1988 relatif à défintion de fromages

Richardson, N.J. & Booth, D.A. (1993) Multiple physical patterns in judgements of the creamy texture of milks and creams. Acta Psychol. 84: 93-101.

Richoux, R., Roset, G., Famelart, M.H. & Kerjean, J.R. (2001) Diversité de quelques propriétés fonctionnelles à chaud de l'Emmental français. Le Lait 81, 547-559.

Roefs, S., Walstra, P., Dalgleish, D. & Horne, O. (1985) Preliminary note on the change in casein micelles caused by acidification. Neth Milk Dairy J. 39, 119-122.

Rollema, H.S. & Brinkhuis, J.A. (1989) A H-NMR study of bovine casein micelles; influence of pH, temperature and calcium ions on micellar structure. Journal of Dairy Research 56, 417-425.

Romain, R., Grégory, R., Marie-Hélène, F. & Jean-René, K. (2001) Diversité de quelques propriétés fonctionnelles à chaud de l'Emmental français. Lait 81, 547-559.

Rosenberg, M., Wang, Z., Chuang, S.L. & Shoemaker, C.F. (1995) Viscoelastic property changes in cheddar cheese during ripening. Journal of Food Science 60(3), 640-644.

Rowney, M., Roupas, P., Hickey, M.W. & Everett, D.W. (1999) Factors affecting the functionality of Mozzarella cheese. Australian Journal of Dairy Technology 54(2), 94-102.

Ruoff, K.e.a. (2006) Authentication of the botanical and geographical origin of honey by front-face fluorescence spectroscopy. J. Agric. Food Chem. 54, 6858.

Sanchez, C., Beauregard, J.L., Chassagne, M.H., Bimbenet, J.J. & Hardy, J. (1996) Effects of processing on rheology and structure of double cream cheese. Food Research International 28(6), 547-552.

Saporta, G. (1990) Probabilités – Analyse des données statistique, Technip édn., Paris.

Schmidt, D. & Poli, J. (1986) Electrokinetic measurements on unheated and heated casein micelle systems. . Neth Milk Dairy J. 40, 269-280.

Schmidt, D.G., Editor (1982) Association of caseins and casein micelle structure. In Developments in dairy chemistry 1-Proteins, pp. 61-85. Edited by P.F. Fox. London: Applied Science Publishers.

Snoeren, T., Klok, H., Van Hooydonk, A. & Damman, A. (1984) The voluminosity of casein micelles. . Milchwissenschaft 39, 461-463.

Sørensen, H.H. (2001) The world market for cheese. IDF Bulletin 359. 5th ed. Brussels, Belgium: Intl. Dairy Federation 4-62.

Stapelfeldt, H. & Skibsted, L.H. (1994) Modification of ß-lactoglobulin by aliphatic aldehydes in aqueous solution. J. Dairy Res. 61, 209-219.

Steffe, J.F. (1996) Rheological Methods in Food Process Engineering. . Freeman Press, East Lansing, MI.

Stone , H., Sidel, J., Oliver, S., Woolsey, A. & Singleton, R.C. (1974) Sensory evaluation by qualitative descriptive analysis. Food Technology 28, 24-32.

Sun, D.W. (2006) Food Processing New Technology and Quality Issues. .

Szczesniak, A.S. & Kleyn, D.H. (1963) Consumer awareness of texture and other food.

Tarodo, Fuente, B. & Alais, C. (1975) Solvation of casein in bovine milk. . J. Dairy Sci 58, 293-300.

Thomas, P.R. & Robert, E. (1994) Opportunities in the nutrition and food sciences. Washington DC, National Academy Press.

TTunick, M.H., P.H. Cooke, E.L. Malin, P.W. Smith and V.H. Holsinger (1997) Reorganization of casein submicelles in Mozzarella cheese during storage. Int. Dairy J. 7:149-155.

Tunick, M.H. (2000) Rheology of Dairy Foods that Gel, Stretch, and Fracture. J. Dairy Sci. 83(8), 1892-1898.

Van Hooydonk, A., Hagedoorn, H. & Boerrigter, I. (1986) pH-induced physico-chemical changes of casein micelles in milk and their effect on renneting. 1. Effect of acidification on physico-chemical properties. Neth Milk Dairy J. 40, 281-296.

Van Vliet, T. (1991) Terminolgy to be used in cheese rheology. In Bull. IDF, Vol. 268, pp. 5-15.

Vassal, L., Monnet, V., Le Bars, D., Roux, C. & Gripon, J.C. (1987) Relation entre le pH, la composition chimique et la texture des fromages de type Camembert. Le Lait 67(2), 173-185.

Veberg, A., Sørheim, O., Moan, J., Iani, V., Juzenas, P., Nilsen, A.N. & Wold, J.P. (2006) Measurement of lipid oxidation and porphyrins in high oxygen modified atmosphere and vacuum-packed minced turkey and pork meat by fluorescence spectra and images. Meat Science 73(3), 511-520.

Vétier, C., Banon, S., Ramet, J.P. & Hardy, J. (2000) Hydratation des micelles de caséine et structure fractale des agrégats et des gels de lait. Le Lait 80, 237-246.

Vigneau, E., Qannari, M. & Devaux, M.F. (2000) Méthodes prédictives. In La spectroscopie infrarouge et ses applications analytiques. D. Bertrand, & E. Dufour, ed. Tec & Doc, Paris, France. pp 295-332.

Vigneau, E., Qannari, M., Jaillais, B., Mazerolles, G. & Bertrand, D. (2006) Méthodes prédictives. In La spectroscopie infrarouge et ses applications analytiques, pp. 347-401. Edited by D. Bertrand & E. Dufour. Paris, France: Lavoisier.

Visser, J. (1991) Factors affecting the rheological and fracture properties of hard and semi-hard cheese. Bulletin of the International Dairy Federation 268, 49-61.

Visser, J., Minihan, A., Smits, P., Tjan, S. & Heertje, I. (1986) Effects of pH and temperature on the milk salts system. . Neth Milk Dairy j. 40, 351-368.

Waagner, N.E. (1993) North European varieties of cheese. In P. F. Fox (Ed.).Cheese, chemistry, physics and microbiology (Vol. 2). London: Chapman and Hall. pp. 253.

Walstra, P. (1999) Casein sub-micelles: do they exist? International Dairy Journal 9(3-6), 189-192.

Walstra, P., Van Dijk, H.J.M. & Geurts, T.J. (1985) The syneresis of curd. 1. General consideration and literature review. Netherlands Milk and Dairy Journal 39, 209-246.

Walstra, P. & Van Vliet, T. (1986) The physical chemistry of curd making. . Neth. Milk Dairy J. 40, 241-259.

Walstra, P., Wouters, J.T.M. & Geurts, T.J. (2005) Dairy Science and technology. Floride, USA: Taylor & Francis.

Whited, L.J., Hammond, B.H., Chapman, K.W. & Boer, K.J. (2002) Vitamin A Degradation and Light-Oxidized Flavor Defects in Milk. J. Dairy Sci. 85, 351–354.

Williams, P. (2003) Near-Infrared Technology-Getting the Best Out of Light. PDK Grain, Nanaimo, Canada.

Wold, H. (1966) Estimation of principal components and related models by iterative least squares. In Multivariate Analysis, pp. 391-420. Edited by P.R. Krishnaiah. New-York: Academic Press.

Wold, J.P., Jorgensen, K. & Lundby, F. (2002) Nondestructive Measurement of Light-induced Oxidation in Dairy Products by Fluorescence Spectroscopy and Imaging. J. Dairy Sci. 85(7), 1693-1704.

Wold, J.P., Veberg, A., Nilsen, A., Iani, V., Juzenas, P. & Moan, J. (2005) The role of naturally occurring chlorophyll and porphyrins in light-induced oxidation of dairy products. A study based on fluorescence spectroscopy and sensory analysis. International Dairy Journal, 343-353.

Wold, S., Sjöström, M. & Eriksson, L. (2001) PLS-regression: a basic tool of chemometrics. Chemometrics and Intelligent Laboratory Systems 58(2), 109-130.

Woodcock, T., Fagan, C.C., O'Donnell, C.P. & Downey, G. (2008) Application of Near and Mid-Infrared Spectroscopy to Determine Cheese Quality and Authenticity. Food and Bioprocess Technology 1(2), 117-129.

Yamaki, S., Kato, T. & Kikugawa, K. (1992) Characteristics of fluorescence formed by the reaction of proteins with unsaturated aldehydes: Possible degradation products of lipid radicals. . Chem. Pharm. Bull. 40, 2138–2142.

ANNEXES