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THÈSE

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Présentée par

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Contribution à l'étude de la structure-texture du lait de chamelle lors de la coagulation et du traitement thermique : comparaison avec le lait de vache

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RESUME

Le lait de chamelle constitue l'une des principales ressources alimentaires pour les peuples nomades. Malgré sa richesse en différents composants et sa production non négligeable au niveau mondial, ce produit demeure relativement peu transformé à cause du manque d'études menées sur les caractéristiques et les aptitudes technologiques de ce lait.

Dans la première partie de cette thèse, l'évolution de la structure au cours de la coagulation enzymatique et acide des laits de chamelle et de vache crus et chauffés (50 et 70 °C) enrichis en minéraux (calcium et phosphate) a été étudiée. Les cinétiques de coagulation enzymatique et acide des échantillons de lait ont été suivies aux moyens du test de cisaillement dynamique et de la spectroscopie de fluorescence frontale.

Les résultats obtenus ont permis, d'une part, de montrer des différences significatives entre les propriétés du coagulum obtenu à partir du lait de chamelle et celui du lait de vache, et d'autre part, d'évaluer l'impact de l'ajout des minéraux (calcium et phosphate) sur les propriétés du coagulum. L'analyse des spectres de fluorescence par analyse en composantes principales (ACP) a permis de caractériser sur le plan moléculaire la structure des gels, et également de discriminer les différentes conditions de coagulation. Les résultats rhéologiques ont montré que l'enrichissement de ces deux types de lait avec le calcium améliorait la fermeté du gel et diminuait le temps de gélification, alors que des effets inverses ont été observés suite à l'ajout du phosphate. L'analyse conjointe des données spectrales et rhéologiques au moyen de l'analyse en composantes communes et poids spécifiques (ACCPS) a montré une forte relation entre la structure au niveau moléculaire et la texture au niveau macroscopique.

La deuxième partie de cette thèse a porté sur l'impact du traitement thermique (55 à 75 °C à différents intervalles de temps) sur les changements moléculaires du lait de chamelle. L'application de l'ACCPS aux spectres de la vitamine A, des produits fluorescents de la réaction du Maillard (PFRM) et du NADH a montré une bonne discrimination des échantillons en fonction du couple température-temps.

Les résultats obtenus ont montré que, de par sa rapidité, la spectroscopie de fluorescence frontale couplée aux méthodes chimiométriques présentait un potentiel intéressant pour la caractérisation du lait de chamelle dans différentes conditions. En outre, le spectre de fluorescence d'un lait pourrait être considéré comme une empreinte digitale de celui-ci permettant de l'identifier.

Mots-clés : Lait de chamelle, lait de vache, coagulation, minéraux, spectroscopie de fluorescence frontale, rhéologie, chimiométrie.

ABSTRACT

Camel milk is one of the main food resources for nomadic people. Despite its richness in different components and its production in the world, this product remains poorly processed because of the lack of studies conducted on the characteristics and the technological abilities of this milk.

In the first part of this thesis, the evolution of the structure during the enzymatic and acid coagulation of raw and heated (50 and 70 °C) camel and cow's milk following the addition of minerals (calcium and phosphate) was studied. The kinetics of acid and enzymatic-induced coagulation of milk were followed using shear dynamic testing rheology at the macroscopic level and front-face fluorescence spectroscopy at the molecular scale.

The obtained results showed significant differences between the properties of the coagulum that depends on the milk species and the level of added minerals (calcium and phosphate). The analysis of spectral data by principal component analysis allowed to characterize the coagulum structure at the molecular level as well as the discrimination of different coagulation conditions. The rheological results showed that the enrichment of both types of milk with calcium improved the gels firmness and reduced the gelation time, while opposite trend was observed following the addition of phosphate. The joint analysis of fluorescence spectral data and rheological measurements by applying common components and specific weights analysis showed a strong relationship between the structure at the molecular scale and the texture at the macroscopic level.

The second part of this thesis focused on the impact of heat treatment (55 to 75 °C at different times) on the molecular changes in camel milk. The application of common components and specific weights analysis to the vitamin A, fluorescent Maillard reaction products and NADH fluorescence spectra enabled the discrimination of milk samples according to the temperature and time.

The obtained results showed that front-face fluorescence spectroscopy coupled with chemometric tools enabled the characterization of camel milk in different conditions. Additionally, the fluorescence spectrum recorded on milk could be considered as a fingerprint allowing its identification.

Keywords: Camel milk, cow's milk, coagulation, minerals, front-face fluorescence spectroscopy, rheology, chemometrics.

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VALORISATION DES TRAVAUX

Publications dans des revues à comité de lecture

- **Kamal, M.,** & Karoui, R. (2015). Analytical methods coupled with chemometric tools for determining the authenticity of dairy products: A review. *Trends in Food Science & Technology*, 46, 27–48.

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- Kamal, M., & Karoui, R. (2016). Monitoring mild heat treatment of camel milk by front-face fluorescence spectroscopy. 9th Euro-Global Summit & Expo on Food & Beverages, July 11-13, 2016 Cologne, Germany. *Communication par affiche* (**Obtention du deuxième prix du poster**).

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TABLE DES MATIÈRES

LISTE DES ABRÉVIATIONS

ACP : Analyse en Composantes Principales

ACC : Analyse Canonique des Corrélations

ACCPS : Analyse en Composantes Communes et Poids Spécifiques

ACSAD : Arab Center for the Studies of Arid zones and Dry Lands

AFD : Analyse Factorielle Discriminante

1,8-ANS : 1-Anilinonaphthalene-8-Sulfonic Acid

CPs : Composantes Principales

CWBP: Camel Whey Basic Protein

DPH : DiPhénylHexatriène

FAO : Organisation des Nations Unies pour l'alimentation et l'agriculture

FRV : Facteur de Réduction Volumique

GMP : Glycomacropeptide

G' : Module élastique

G" : Module visqueux

GDL : Glucono-δ-lactone

IgG : Immunoglobuline G

IgM : Immunoglobuline M

IgA : Immunoglobuline A

IRTF : Infrarouge à Transformée de Fourier

IR : Infrarouge

kDa : Kilo Dalton

MPa.s^{-1} : MégaPascal par seconde

MIR : Moyen Infrarouge

NADH : Nicotinamide Adénine Dinucléotide

NWP : Novel Whey Protein

PIR : Proche Infrarouge

PARAFAC : Parallel Factor Analysis

PFRM : Produits Fluorescents de la Réaction de Maillard

PGRP : Protéine de reconnaissance du peptidoglycane

PLS : Partial Least Squares

PP3 : Protéose Peptone-3

m/v : Masse/Volume

RTA : Réflexion Totale Atténuée

TPA : Texture Profil Analysis

Tan δ : Viscoélasticité

USDA : United States Department of Agriculture

$б_F$: Contrainte à la fracture

$ε_F$: Déformation au point de fracture

WAP : Whey Acidic Protein

W_F : Energie à la fracture

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INTRODUCTION GÉNÉRALE

Le lait est un aliment noble caractérisé par sa composition équilibrée en différents nutriments de base (protéines, glucides et lipides) et sa richesse en vitamines et en minéraux. De ce fait, il est considéré comme un élément essentiel pour certains groupes de consommateurs, particulièrement les enfants, les femmes enceintes et les personnes âgées (Souza et al. 2011).

Le lait de vache constitue le type de lait le plus consommé dans le monde entier. Selon les dernières statistiques du Centre National Interprofessionnel de l'Economie Laitière (CNIEL, 2016), sa production mondiale a progressé de 609,8 millions de tonnes en 2010 à 663,2 millions de tonnes en 2014. Cependant, dans la ceinture désertique et semi-aride d'Afrique et d'Asie, les conditions climatiques rendent difficile l'élevage des vaches (Ouajd et Kamel, 2009). Seuls les chameaux sont dotés de la capacité de vivre dans ces conditions extrêmement difficiles grâce à leurs particularités biologiques et physiologiques (Ouajd et Kamel, 2009). C'est ainsi que le lait produit par ces mammifères est vraisemblablement le plus consommé dans ces zones aussi bien à l'état frais qu'après transformation (lait fermenté, yaourt, beurre, etc.) comme le rapportent les travaux de Al-Haj et Al-Kanhal (2010).

Ces dernières années, le lait de chamelle a fait l'objet de quelques travaux de recherche en vue de sa caractérisation grâce à ses intérêts nutritionnels et ses vertus thérapeutiques (Boughellout et al. 2016). En effet, l'orientation d'une partie des consommateurs vers ce type de lait s'explique principalement par sa haute valeur nutritionnelle. Comparé au lait de vache, le lait de chamelle se singularise par sa teneur élevée en vitamine C qui est trois (Farah et al. 1992) à cinq (Stahl et al. 2006) fois plus grande que celle du lait de vache. De surcroît, le lait de chamelle est doté d'une haute teneur en agents antimicrobiens, lié à sa richesse en lysozyme, lactoperoxydase, lactoferrine et en bactériocines produites par les bactéries lactiques (El-Agamy et al. 2009). Cette composition particulière lui permet de garder sa stabilité pendant 5 jours à 30 °C, alors que le lait de vache est complètement déstabilisé après 48 heures à la même température (Yagil et al. 1984 ; Al-Haj et Al-Kanhal, 2010).

Certaines études ont également souligné les vertus thérapeutiques du lait de chamelle, principalement son pouvoir anti-cancérigène (Magjeed, 2005), anti-diabétique (Agrawal et al. 2007) et anti-hypertensif (El-Agamy et al. 2009). Malgré ces caractéristiques, la production du lait de chamelle reste bien inférieure à celle du lait de vache même si les données de la littérature montrent que la production de lait de chamelle ne cesse d'augmenter. En effet, selon les dernières statistiques de l'Organisation des Nations Unies pour l'alimentation et l'agriculture (FAO, 2014), la production mondiale du lait de chamelle s'élève à 2 928 188 tonnes en 2013 ; la Somalie était le premier producteur mondial de lait de chamelle avec une production annuelle

de 1 100 000 tonnes. Bien qu'il n'existe pas de données sur la consommation réelle du lait de chamelle au niveau mondial, Faye et al. (2014) ont rapporté une consommation moyenne de 0,11, 0,21, 0,34 et 0,43 litre par personne et par an, respectivement en Tunisie, au Maroc, en Algérie et en Libye.

En dépit de ces propriétés nutritionnelles, la consommation des produits laitiers issus de la transformation du lait de chamelle reste toujours incomparable à celle du lait de vache à cause de son aptitude limitée aux transformations technologiques. Face à cette contrainte, une connaissance approfondie de la structure de ses constituants et de ses caractéristiques physico-chimiques est indispensable aux développements de nouveaux produits dérivés. Dans ce cadre, des travaux de recherche menés ces dernières années ont montré que les difficultés rencontrées au cours de la transformation du lait de chamelle sont liées principalement à sa composition particulière en micelles de caséines (Farah et Bachmann, 1987). En effet, la faible teneur en caséine-κ (~ 3,47 % de la caséine totale *versus* 13 % pour le lait de vache) et la grande taille de la micelle de caséines (~ 380 nm contre 150 nm concernant le lait de vache) constituent des verrous technologiques comme le rapportent quelques travaux de recherche (Kappeler et al. 2003 ; Bornaz et al. 2009 ; Al-Haj et Al-Kanhal, 2010).

Des études portant sur la coagulation du lait de chamelle et du lait de vache traités dans les mêmes conditions ont montré que : i) le temps de coagulation du lait de chamelle est deux à trois fois plus long (10 - 15 minutes) que celui du lait de vache qui avoisine les 5 minutes (Farah et Bachmann, 1987) ; et ii) la fermeté du gel du lait de vache est supérieure à celle du lait de chamelle (Ramet, 2001).

Il est bien connu que la texture finale du produit dépend de sa structure à l'échelle moléculaire et de l'organisation de ses constituants (Karoui et al. 2003a). Le type et la nature des interactions (protéines-protéines, protéines-eau, protéines-lipides et lipides-lipides) confèrent au produit final sa texture spécifique. Pour appréhender l'effet de la structure sur la texture, différents niveaux peuvent être investigués allant du niveau moléculaire au niveau macroscopique. Les spectroscopies infrarouge et de fluorescence, mais aussi le test de cisaillement dynamique et la microscopie confocale à balayage laser ont montré leurs potentiels à étudier la structure et la texture des produits laitiers (Herbert et al. 1997, 1999 ; Laporte et al. 1998 ; Lucey et al. 2001 ; Lucey, 2002 ; Karlsson et al. 2007 ; Cecchinato et al. 2009 ; Boubellouta et al. 2011 ; Cipolat-Gotet et al. 2012 ; Blecker et al. 2012 ; Ramasubramanian et al. 2014).

L'objectif de cette thèse est d'étudier les changements de structure du lait de chamelle et des gels obtenus suite à la coagulation acide et enzymatique sous différentes conditions expérimentales (ajout de minéraux, traitement thermique) en comparaison avec le lait de vache. Les changements structuraux au niveau moléculaire ont été étudiés grâce à la spectroscopie de fluorescence frontale en vue du développement d'une méthode d'analyse rapide du lait de chamelle transformé ou non.

La première partie de ce manuscrit est dédiée à un état de l'art sur le sujet, alors que la deuxième partie est consacrée à la présentation des résultats et de leur discussion.

Dans un premier temps, les potentialités de la spectroscopie de fluorescence et du test de cisaillement dynamique à suivre les changements des constituants du lait de chamelle en comparaison avec le lait de vache suite à l'ajout des minéraux (calcium et phosphate) au lait cru et à des laits ayant subi un chauffage à 50 et 70 °C pendant 10 minutes seront exposées.

Dans un second temps, l'impact du traitement thermique (55 à 75 °C) appliqué au lait de chamelle pendant des temps variables en utilisant la spectroscopie de fluorescence frontale couplée à l'analyse en composantes communes et poids spécifiques (ACCPS) sera investigué.

Les conclusions et perspectives de ce travail de thèse seront présentées dans une dernière partie.

SYNTHÈSE BIBLIOGRAPHIQUE

Tableau 1 : Évolution de la production de lait de chamelle dans le monde (A) et les principaux pays producteurs de lait de chamelle (B) (FAO, 2014).

(A)

Année	Production mondiale (tonnes)
2003	1 812 813
2004	2 000 199
2005	1 814 829
2006	1 880 027
2007	2 520 218
2008	2 860 182
2009	2 760 340
2010	2 981 506
2011	2 920 573
2012	2 790 372
2013	2 928 188

(B)

Principaux pays producteurs de lait de chamelle	Quantité produite par pays en 2013 (tonnes)
Somalie	1 100 000
Kenya	937 000
Mali	242 911
Éthiopie	170 000
Niger	105 000
Arabie Saoudite	105 000
Soudan	60 000
Émirats Arabes Unis	47 000
Mauritanie	27 125
Tchad	24 600
Algérie	15 000

Tableau 2 : Évolution du nombre de têtes de chameaux en Syrie (ACSAD, 2013).

Année	Nombre de têtes de chameaux
2000	13 368
2001	12 169
2002	12 477
2003	15 232
2004	20 393
2005	23 442
2006	26 712
2007	27 358
2008	27 536
2009	32 494
2010	50 202

1. Importance des camélidés dans les zones arides

Les camélidés (chameaux et dromadaires) jouent un rôle socio-économique primordial puisqu'ils ont été toujours associés aux modes de vie dans les régions arides et semi-arides. Ils sont considérés comme une bonne source de lait et de viande ; ils sont également utilisés comme un moyen de transport (Al-Haj et Al-Kanhal, 2010). Selon les dernières statistiques de la FAO en 2014, la population mondiale de chameaux est estimée à environ 27 777 346 têtes, le plus grand cheptel se trouvant en Somalie (7 150 000 têtes). Les dromadaires (*Camelus dromedarius*) représentent la majorité de cette population (84 %) et se trouvent dans les zones arides du Nord et du Nord-Est de l'Afrique. La population restante (16 %) est représentée par les chameaux à deux bosses appelés aussi chameaux de Bactriane (*Camelus bactrianus*) peuplant les déserts froids d'Asie Centrale jusqu'au Nord-Ouest de la Chine et de la Mongolie (Faye, 1997 ; Zhang et al. 2005 ; Fouzia, 2015). Les dromadaires sont principalement utilisés pour la production de lait et les chameaux de Bactriane pour le travail et la production de laine (Zhang et al. 2005).

Le lait de chamelle, comme celui des autres mammifères (vache, chèvre, brebis, etc.) fait partie du régime alimentaire des bédouins, bergers et autres tribus nomades. En effet, le lait de chamelle est même souvent la seule ressource alimentaire d'origine animale pour les peuplades nomades. Dans la plupart des zones pastorales sahariennes, il est considéré comme l'aliment de base tout au long de l'année (Al-Haj et Al-Kanhal, 2010).

La production mondiale du lait de chamelle est passée de 1 812 813 tonnes en 2003 à 2 928 188 tonnes en 2013 comme le montre le **Tableau 1 A** ; les principaux pays producteurs de lait de chamelle dans le monde en 2013 sont présentés dans le **Tableau 1 B**. La production laitière de chamelle varie d'une région à l'autre et d'une race à l'autre. Alors que, la production moyenne est de 1500 litres par an, la production individuelle de lait varie de 1000 à 2700 litres par lactation en Afrique, et peut atteindre les 7000 à 12000 litres en Asie pour une durée de lactation variant de 8 à 18 mois (Faye, 2003). Concernant la race, il a été observé que le maximum de production laitière par jour est de 14 litres pour la race Wadha et peut atteindre 18,3 litres pour la race Malha en Arabie Saoudite (Faye, 2003). Un minimum de production laitière de 3,7 litres par jour a été quant à lui observé chez la race Jaisalmeri en Inde (Sahani et al. 1998).

En Syrie, les données portant sur le lait de chamelle sont très limitées. Selon les travaux du Centre Arabe pour l'étude des zones arides et des terres sèches (ACSAD, 2013), le nombre de chameaux en Syrie est passé de 13 368 têtes en 2000 à 50 202 têtes en 2010 (**Tableau 2**),

Tableau 3 : Composition globale du lait de chamelle et du lait de vache.

	Composition (%)						Références
	Protéines	**Matière grasse**	**Lactose**	**Cendre**	**Matière sèche**	**Eau**	
Lait de chamelle	3,45	3,70	4,62	0,74	12,63	-	Jardali, (1988)
	3,35	3,24	4,52	0,80	11,91	-	Mehaia et Al-Kanhal, (1989)
	3,19	5,22	5,00	0,80	14,50	-	Taha et Kielwein, (1989)
	3,27	3,60	5,53	0,80	13,20	-	Bayoumi, (1990)
	3,10	3,90	4,47	0,80	12,36	-	Farag et Kabary, (1992)
	2,54	3,90	4,71	0,79	11,94	88,00	Mehaia, (1993)
	2,52	2,85	4,46	0,80	10,63	-	Mehaia et al. (1995)
	3,42	5,60	3,65	0,86	12,14	-	Karue, (1998)
	3,31	4,81	4,28	0,83	13,44	-	Wangoh et al. (1998a)
	2,79	3,39	4,81	0,77	11,5	-	Guliye et al. (2000)
	2,81	1,20	5,40	0,99	9,61	-	Attia et al. (2001)
	3,53	4,47	4,95	0,70	13,64	-	Indra, (2003)
	3,25	2,65	4,05	0,83	10,80	-	Kouniba et al. (2005)
	3,50	3,26	3,60	0,67	11,03	-	Abdoun et al. (2007)
	3,30	3,78	5,85	0,70	15,06	-	Kamal et al. (2007)
	2,69	2,95	3,92	0,82	12,30	-	Haddadin et al. (2008)
	2,93	2,64	3,12	0,73	9,56	-	Shuiep et al. (2008)
	3,40	3,40	3,60	0,80	10,90	89,30	Bakheit et al. (2008)
	2,06	2,35	4,41	0,94	9,78	90,20	Omer et Eltinay, (2009)
	2,50	2,90	4,91	1,30	-	88,20	Meiloud et al. (2011)
	3,19	3,30	4,43	0,82	-	91,80	Medhammar et al. (2012)
	3,96	5,32	4,50	0,83	14,52	-	Zhao et al. (2015)
Lait de vache	3,20	3,50	4,50	0,80	13,00	87,00	Lindmark-Maansson et al. (2003) ; Haug et al. (2007) ; Pereira, (2014)
	3,30	3,40	5,26	0,72	-	90,90	Medhammar et al. (2012)

(-) : valeurs non déterminées.

soit une augmentation de 12,7 % par an. La répartition régionale de ces animaux en 2010 a montré que 40 % provenaient de la province de Deir ez-Zor, 19,2 % de Rif Dimachq, 16,7 % de Homs, 13 % de la province de Raqqa et 11,1 % dans le reste du pays.

Les chameaux sont de race Alshamia, seule race qui existe exclusivement en Syrie. Et selon les données d'ACSAD en 2013, la production de lait de chamelle par an fut de 2000 tonnes en 2010, représentant environ 0,09 % de la production totale du lait en Syrie. En dépit de la faible quantité de lait de chamelle produite, elle représente la principale source d'alimentation pour les bédouins qui le consomme souvent à l'état frais.

2. Composition du lait

Le lait est un liquide sécrété par les glandes mammaires des mammifères après la parturition pour assurer la nourriture du nouveau-né. Il correspond à la fois à une solution aqueuse constituée essentiellement de lactose, de protéines sériques, de sels minéraux et de vitamines, à une émulsion de matière grasse sous forme de globules gras (huiles dans l'eau) et à une suspension de micelles constituées de caséines et de sels minéraux (Walstra et al. 2005). Le lait est une bonne source de nutriments, et est donc important pour la croissance (Gurmessa et Melaku, 2012). La composition physico-chimique du lait varie en fonction de nombreux facteurs zoologiques et d'élevage tels que l'espèce, la race, le stade de lactation, les conditions environnementales et l'alimentation (Caroli et al. 2009 ; Kalač et Samková, 2010).

Le lait de chamelle est, comme les autres types de lait, de couleur blanche, relativement pauvre en β-carotène (0,32 µg.100 mL^{-1} contre 99,6 µg.100 mL^{-1} dans le lait de vache), de goût sucré ou salé selon le type d'aliment ingéré et la disponibilité en eau (Sawaya et al. 1984 ; Farah, 1993 ; Wangoh et al. 1998a ; Stahl et al. 2006 ; Sisay et Awoke, 2015). Le pH du lait de chamelle varie entre 6,30 et 6,57, alors que sa densité décrit une variation de 1,029 à 1,037 $g.cm^{-3}$; son acidité est de l'ordre de 15° Dornic (Hassan et al. 1987 ; Farah, 1996 ; Zhao et al. 2015). Le lait de chamelle est moins visqueux (1,72 $MPa.s^{-1}$ à 20 °C) que le lait de vache (2,04 $MPa.s^{-1}$ à 20 °C) (Kherouatou et al. 2003). Cette différence est probablement due au diamètre des micelles de caséines qui est relativement plus large dans le lait de chamelle (Kherouatou et al. 2003).

La composition chimique du lait de chamelle, en relation avec sa valeur nutritionnelle, a fait l'objet de plusieurs travaux de recherche. Malgré des résultats discordants obtenus par différents auteurs (**Tableau 3**) et qui pouvaient être expliqués par des variations de la race, du type d'alimentation, de la saison, etc. ces études ont montré que les teneurs en protéines, en

Tableau 4 : Répartition des différentes formes d'azote dans le lait de chamelle et le lait de vache.

Type de lait	Composition de l'azote total (%)			Références
	Azote caséinique	Azote sérique	Azote non protéique	
Lait de chamelle	76,0	17,0	7,0	Farah et Rüegg, (1989)
	72,0	22,0	6,0	Abu-Lehia, (1987)
	71,0	23,0	6,0	Bayoumi, (1990)
	65,4	24,5	10,1	Mehaia et al. (1995)
	68,7	19,8	11,5	Mehaia, (1996)
	68,0	20,8	11,2	Shamsia, (2009)
Lait de vache	76,0	17,5	5,5	Mietton et al. (1994)
	78,4	16,6	5,0	Mehaia et al. (1995)

Tableau 5 : Concentration moyenne en acides aminés des protéines du lait de chamelle et du lait de vache.

Acides aminés (% protéine)		Lait de chamelle (Shamsia, 2009)	Lait de vache (Shahein et Soliman, 2014)
Acides aminés essentiels	Lysine	6,60	8,10
	Thréonine	5,30	2,31
	Valine	4,80	7,25
	Méthionine	2,60	3,20
	Isoleucine	4,90	5,62
	Leucine	9,00	9,18
	Phénylalanine	3,70	5,40
	Histidine	2,90	3,20
Acide aminés non essentiels	Acide Aspartique	7,20	7,40
	Acide Glutamique	18,09	23,00
	Glycine	1,20	1,92
	Sérine	3,00	6,62
	Tyrosine	3,00	5,81
	Arginine	5,10	4,15
	Alanine	3,30	3,41
	Proline	13,00	11,28
	Cystéine	1,50	0,38

matière grasse et en lactose variaient de 2,06 à 3,96 %, de 1,2 à 5,6 % et de 3,12 à 5,85 %, respectivement.

2.1. Fraction azotée et protéines

Comme pour les autres types de lait, la fraction azotée du lait de chamelle est composée de deux sous-fractions : l'azote protéique et l'azote non protéique (**Tableau 4**). Les différentes concentrations en acides aminés essentiels et non-essentiels des laits de chamelle et de vache ont été étudiées (Shamsia, 2009 ; Shahein et Soliman, 2014). À l'exception de la thréonine, l'arginine, la proline et la cystéine, les résultats obtenus ont montré que les teneurs en acides aminés essentiels et non essentiels dans le lait de chamelle sont sensiblement plus faibles que dans le lait de vache (**Tableau 5**).

La teneur totale en protéines du lait de chamelle varie de 2,15 à 4,90 % (Konuspayeva et al. 2009) contre une composition moyenne de 3 % pour le lait de vache. Aussi bien pour le lait de chamelle que pour le lait de vache, cette teneur dépend de divers facteurs comme la race, la saison, etc. Par exemple, le lait de race Majaheim présente une teneur en protéines plus élevée (2,91 %) que celui collecté de la race Wadah (2,36 %) (Mehaia et al. 1995). D'autre part, une chute de l'extrait sec total durant la période estivale, résultant de la diminution du taux de matière azotée et plus particulièrement des caséines a été rapportée par Kamoun (1995). Cette diminution s'explique par le stress hydrique subi par les animaux induisant l'augmentation de la teneur en eau du lait (Ramet, 1994a).

2.1.1. Azote non protéique

Sa teneur est d'environ 10 % de l'azote total dans le lait de chamelle, alors qu'elle est seulement de 5 à 6 % dans le lait de vache (DePeters et Ferguson, 1992 ; Mehaia et Al-Kanhal, 1992). L'azote non protéique est caractérisé par sa haute valeur biologique due à sa richesse en acides aminés libres, en vitamines B et en nucléotides (Mehaia et Al-Kanhal, 1992 ; Mehaia et al. 1995).

Les acides aminés libres les plus abondants dans le lait de chamelle sont l'acide glutamique, l'alanine, la phosphosérine, la glutamine et la phénylalanine (Taha et Kielwein, 1990 ; Mehaia et Al-Kanhal, 1992), alors que dans le lait de vache, les acides aminés libres les plus abondants sont l'acide glutamique, la taurine, la thréonine et l'histidine (Ferreira, 2003).

Tableau 6 : Concentration moyenne en protéines sériques du lait de chamelle et du lait de vache.

Protéine ($mg.L^{-1}$)	**Lait de chamelle**	**Lait de vache**	**Références**
α-lactalbumine	3800	1260	Kappeler et al. (2003)
	2200	2040	El-Hatmi et al. (2007)
	2010	1080	Omar et al. (2016)
β-lactoglobuline	-	3500	Kappeler et al. (2003)
	-	5970	Omar et al. (2016)
Sérum albumine	400	360	Omar et al. (2016)
Lactophorine (PP3)	950	300	Kappeler et al. (2003)
	1100	-	Girardet et al. (2000)
Lactoferrine	95	140	Kappeler et al. (2003)
Lactoperoxydase	-	30	
Lysozyme	0,15	0,07	El-Agamy et al. (1996)
	0,42	0,37	El-Agamy, (2009)
Immunoglobulines	1640	760	El-Agamy et Nawar, (2000); El-Agamy, (2009)

(-) : valeurs non déterminées.

2.1.2. Azote protéique

Cette fraction représente 90 à 95 % de l'azote total du lait de chamelle et 95 à 97 % dans le lait de vache (Ruska et Jonkus, 2014). Elle est constituée de protéines micellaires appelées caséines et de protéines sériques qui représentent 75 % et 25 % des protéines totales, respectivement. Dans le lait de vache, les pourcentages des protéines micellaires et sériques sont respectivement de 80 % et 20 % (Khaskheli et al. 2005 ; Haug et al. 2007). Les caséines du lait de vache se caractérisent par leur précipitation pour un pH de 4,6, alors que celles du lait de chamelle précipitent pour un pH de 4,3 (Wangoh et al. 1998b).

Tenant compte de l'importance de cette fraction dans notre étude, nous l'aborderons, dans ce qui suit, de façon plus détaillée.

2.1.2.1. Protéines sériques

Les protéines sériques représentent la fraction soluble des protéines du lait qui ne précipitent pas à pH = 4,6 pour le lait de vache et constituent les protéines du lactosérum. Ces protéines ont des propriétés nutritionnelles et fonctionnelles très intéressantes dans le domaine agroalimentaire. Les protéines du lactosérum sont composées de l'α-lactalbumine, la β-lactoglobuline, la lactoferrine, la lactoperoxydase, le lysozyme, la sérum-albumine, les immunoglobulines, la transferrine et la lactophorine (Protéose-peptones-3 ou PP3) (Merin et al. 2001 ; Kappeler et al. 2004 ; Miciński et al. 2013).

La composition des protéines du lactosérum dans le lait de chamelle est différente de celle du lait de vache (El-Agamy, 2000a ; Merin et al. 2001 ; Kappeler et al. 2003 ; Laleye et al. 2008 ; Hinz et al. 2012). En effet, Boughellout et al. (2016) ont souligné que la β-lactoglobuline, représentant la principale protéine dans le lactosérum du lait de vache, est complètement absente dans le lait de chamelle. Les différences en concentrations des protéines du lactosérum sont présentées dans le **Tableau 6**.

L'α-lactalbumine est la principale protéine soluble du lait de chamelle (Ochirkhuyag et al. 1998), sa concentration qui varie de 2,01 à 3,8 $g.L^{-1}$ est supérieure à celle du lait de vache (1,08 - 2,04 $g.L^{-1}$) (**Tableau 6**). Cette protéine possède 123 résidus d'acides aminés et a une masse moléculaire de 14,6 kDa (Beg et al. 1985 ; Al-Haj et Al-Kanhal, 2010). La séquence complète de l'α-lactalbumine du lait de chamelle a été déterminée par Beg et al. (1985) et se différencie de son homologue du lait de vache par 39 résidus d'acides aminés.

La β-lactoglobuline est la principale protéine soluble du lait de vache (5,97 $g.L^{-1}$), ayant une masse moléculaire de 18,2 kDa alors qu'elle est totalement absente dans le lait de chamelle comme dans le lait humain (Merin et al. 2001 ; Kappeler et al. 2003 ; Laleye et al. 2008 ; El-Agamy et al. 2009 ; Omar et al. 2016). L'absence de la β-lactoglobuline dans le lait de chamelle, qui est un composant allergène dans le lait de vache, conduit à l'utilisation de ce lait comme une nouvelle source de protéines pour l'alimentation des enfants allergiques au lait de vache (Boughellout et al. 2016).

La sérum-albumine a une concentration dans le lait de chamelle de 0,40 $g.L^{-1}$, quasi-similaire que dans le lait de vache (0,36 $g.L^{-1}$). Cette protéine a une masse moléculaire de 66 et 66,4 kDa dans le lait de chamelle et de vache, respectivement (Hinz et al. 2012 ; Omar et al. 2016).

Le lysozyme dont la masse moléculaire est de 14,4 kDa, est présent dans le lait de chamelle à une teneur de 0,15 $mg.L^{-1}$, soit le double de celle dans le lait de vache (0,07 $mg.L^{-1}$) comme le rapportent les travaux d'El-Agamy et al. (1996). Cette protéine inhibe la croissance de certains germes pathogènes comme *Micrococcus luteus, Salmonella typhimuriumles, Bacillus subtilis, Bacillus cereus,* etc. en détruisant leurs parois bactériennes suite à l'hydrolyse des peptidoglycanes (El-Agamy et al. 1992, 1996).

La lactoferrine est une glycoprotéine qui présente deux sites capables chacun de fixer un ion ferrique (Fe^{3+}). Cette capacité à fixer le fer explique en partie son rôle dans le contrôle de la croissance de certaines bactéries pathogènes (Diarra et al. 2002). Elle a une masse moléculaire de 79,5 kDa et est présente dans le lait de chamelle à une teneur de 0,09 $g.L^{-1}$, alors que sa concentration moyenne dans le lait de vache est de 0,14 $g.L^{-1}$ avec une masse moléculaire de 76 kDa (El-Agamy et al. 1996 ; Kappeler et al. 2003).

La lactoperoxydase a une masse moléculaire d'environ 78 et 72,5 kDa respectivement dans le lait de chamelle et celui de vache (El-Agamy et al. 1996). Le pH isoélectrique est de 8,63 et 7,90 respectivement pour la lactoperoxydase du lait de chamelle et de vache (Dull et al. 1990). La lactoperoxydase a été identifiée comme un agent antimicrobien dans le lait (De Wit et Van Hooydon, 1996 ; Touch et al. 2004). En effet, cette protéine possède un effet bactéricide très prononcé contre les souches $Gram^-$ comme *Pseudomonas, E.coli, Salmonelles,* et *Campylobacter* (Purdy et al. 1983 ; Borch et al. 1989 ; El-Agamy, 2009) et un effet bactériostatique contre les bactéries $Gram^+$ comme *Streptococcus, Listeria,* et *Staphylococcus* (Earnshaw et Banks, 1989 ; El-Agamy, 2009).

Les immunoglobulines jouent un rôle important dans le système immunitaire et la croissance du nouveau-né. Des immunoglobulines du lait de chamelle ont été isolées et caractérisées : IgG, IgM, IgA avec une prédominance de la classe G composée de plusieurs sous-classes (El-Agamy

et al. 1996 ; El-Agamy, 2000a). Une étude portant sur les activités antivirale et antibactérienne du lait de chamelle a révélé que les immunoglobulines ont une faible activité antibactérienne et une activité antivirale élevée notamment contre les rotavirus (El-Agamy et al. 1992). El-Hatmi et al. (2006) et Konuspayeva (2007) ont rapporté que, 90 jours après la parturition, la quantité d'IgG dans le lait de chamelle était de 0,65 $g.L^{-1}$, alors que la concentration moyenne dans le lait de vache n'est que de 0,32 $g.L^{-1}$ avec une variation pouvant aller de 0,03 à 0,61 $g.L^{-1}$ (Liu et al. 2009). Cette variation est significativement corrélée avec le nombre et le stade de lactation, la production de lait par jour et le nombre de cellules somatiques (Liu et al. 2009).

Outre ces protéines solubles, des travaux de recherche ont rapporté la présence d'autres fractions protéiques dans le lait de chamelle. On peut citer, par exemple :

- la présence d'une protéine analogue à l'insuline appelée « Insulin-like protein » (Agrawal et al. 2011 ; Haroun et al. 2012) ; d'ailleurs le lait de chamelle est utilisé par les nomades pour traiter le diabète. Sa teneur est 5000 et 1000 fois supérieure à celle du lait de vache et du lait humain, respectivement (Konuspayeva, 2007). Agrawal et al. (2003) ont observé que la consommation régulière du lait de chamelle (0,5 L par jour pendant trois mois) améliorait la glycémie chez les patients insulinodépendants, en accord avec les travaux de Shori (2015) qui a indiqué que la consommation du lait de chamelle réduit significativement les taux de glucose dans le sang et la dose d'insuline nécessaire chez les patients insulinodépendants.
- une protéine de 14 kDa, dont l'extrémité N-terminale présente une structure similaire à celle des caséines α et β du lait de chamelle, est riche en résidus cystéines (Beg et al. 1984).
- une protéine de 117 résidus d'acides aminés comportant 16 résidus de cystéine ; cette protéine est homologue à la phosphoprotéine du lactosérum de ratte et à la neurophysine de souris (Beg et al. 1986).
- une protéine à caractère acide nommée Whey Acidic Protein (WAP) dont la concentration dans le lait de chamelle est de 0,16 $g.L^{-1}$ (Kappeler et al. 2003). Cette protéine a aussi été identifiée dans les laits de ratte, de souris et de truie (Hennighausen et Sippel, 1982 ; Campbell et al. 1984 ; Rival et al. 2001 ; Nukumi et al. 2004). Elle a une masse moléculaire d'environ 12,6 kDa avec un pH isoélectrique de 4,70, et est composée de 117 acides aminés avec 17 groupements thiols (Kappeler, 1998).
- une protéine nommée Novel Whey Protein (NWP), ayant une masse moléculaire de 15 kDa et est constituée de 112 résidus d'acides aminés. Cette protéine est pauvre en cystéine et il n'existe pas de similitude structurelle évidente entre cette protéine et d'autres protéines de lait (Beg et al. 1987).

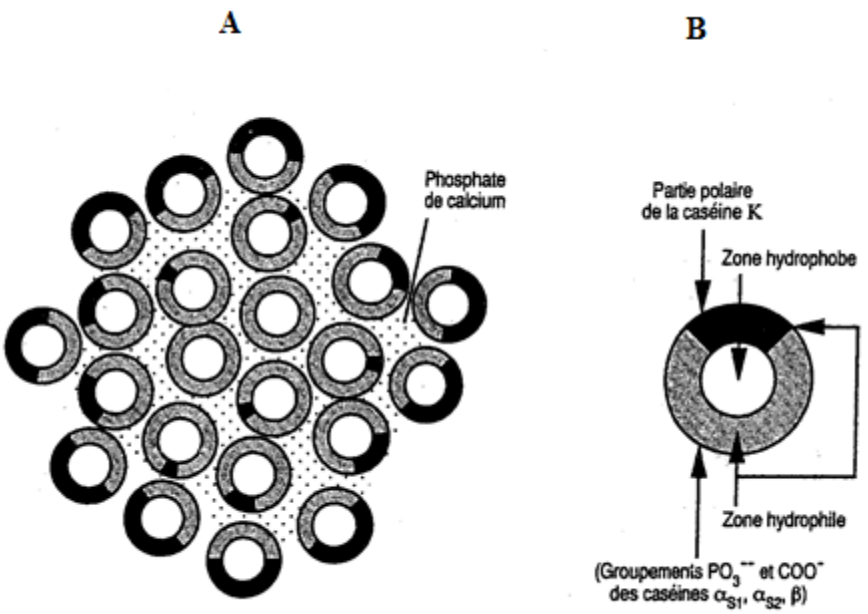

Figure 1 : Structure de la micelle (A) et de la submicelle (B) de caséines (Schmidt, 1980).

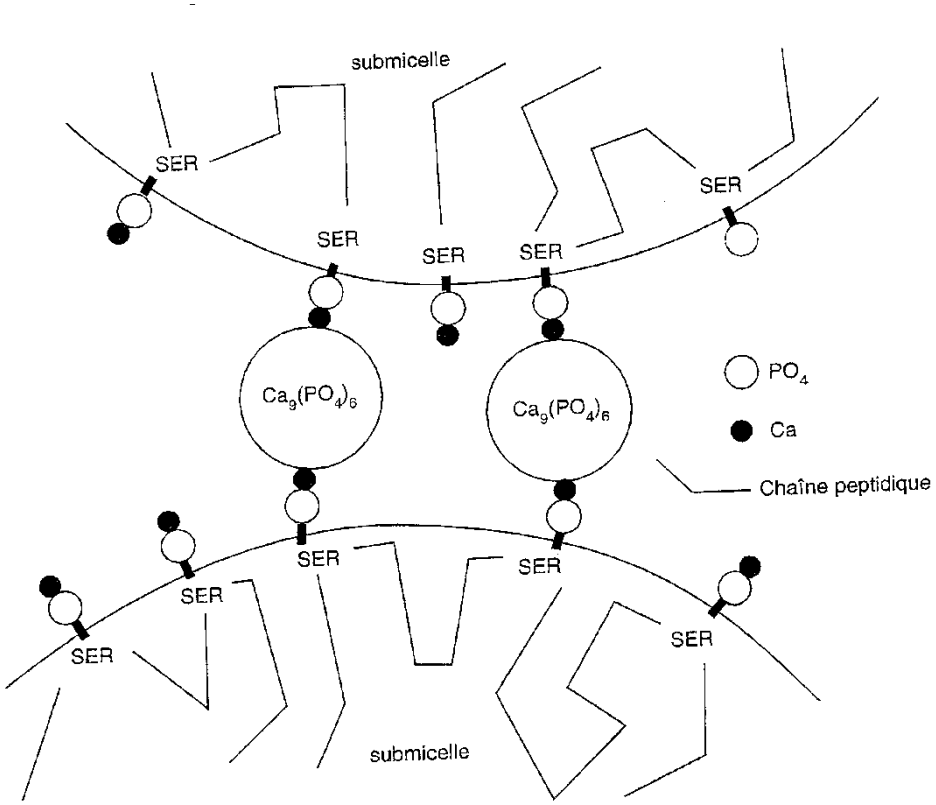

Figure 2 : Schéma de pontage de deux submicelles par le phosphate de calcium (Schmidt, 1982).

- une protéine de reconnaissance du peptidoglycane (PGRP), qui a une masse moléculaire de 19,1 kDa avec un pH isoélectrique de 9,02. Elle est constituée de 172 résidus d'acides aminés et est homologue à celle du lait humain. Sa concentration dans le lait de chamelle est de 0,12 $g.L^{-1}$; elle est riche en arginine mais pauvre en lysine (Kappeler, 1998 ; Kappeler et al. 2004).
- une protéine nommée Camel Whey Basic Protein (CWBP) ayant un pH isoélectrique basique de 9,3 avec une masse moléculaire de 20 kDa (Ochirkhuyag et al. 1998 ; Halima et al. 2014).

La présence de ces protéines dans le lait de chamelle lui confère des vertus nutritionnelles supplémentaires par rapport aux autres types de lait.

2.1.2.2. Caséines

Les caséines sont les protéines les plus abondantes dans le lait ; il en existe 4 types : α_{s1}, α_{s2}, β et κ. Ces différentes caséines s'associent entre elles à l'aide de composés salins dont les principaux sont le calcium et le phosphore pour former les micelles de caséines qui sont des particules sphériques dont le diamètre varie de 90 à 210 nm et de 280 à 550 nm pour le lait de vache et le lait de chamelle, respectivement (Bornaz et al. 2009).

Les travaux d'Attia et al. (2000) et ceux de Kherouatou et al. (2003) ont indiqué que l'organisation de la micelle de caséine du lait de chamelle est compatible avec le modèle moléculaire proposé par Schmidt (1980). Ce modèle suppose que la micelle (**Figure 1 A**) résulte d'une association non uniforme de sous-unités sphériques nommées submicelles (**Figure 1 B**). La submicelle serait constituée d'un cœur hydrophobe de caséines α_{s1} et β, et d'une partie externe, davantage hydrophile, formée de caséines α_{s1}, α_{s2} et κ (Ono et Obata, 1989). Les submicelles seraient reliées entre-elles par des ponts phosphocalciques (**Figure 2**). La caséine-κ est principalement localisée à la surface de la micelle, accessible à la chymosine. Les proportions relatives des caséines varient selon les submicelles : celles ayant une faible teneur en caséine-κ se trouvent à l'intérieur de la micelle, alors que celles qui en sont riches se localisent à l'extérieur de la micelle.

Les ressemblances et les dissemblances entre les caséines (α_{s1}, α_{s2}, β et κ) du lait de chamelle et du lait de vache sont illustrées dans le **Tableau 7**. La caséine-κ étant la protéine laitière jouant un rôle essentiel dans la stabilisation des micelles (Pauciullo et al. 2013), elle sera décrite de façon plus détaillée ci-après.

Caséine-κ

La caséine-κ représente 3,5 % et 13 % des caséines totales respectivement dans le lait de chamelle et de vache (El-Agamy, 2006 ; Al-Haj et Al-Kanhal, 2010 ; Shuiep et al. 2013). Les

Tableau 7 : Composition moyenne des caséines du lait de chamelle et du lait de vache.

Paramètres	Caséine-α_{s1}		Caséine-α_{s2}		Caséine-β		Caséine-κ		Références
	Chamelle	Vache	Chamelle	Vache	Chamelle	Vache	Chamelle	Vache	
Concentration ($g.L^{-1}$)	5	12	2,2	3	15	10	0,8	3,5	Kappeler et al. (1998, 2003) ; Eigel et al. (1984)
Proportion dans les caséines totales (%)	22	40	9,5	10	65	45	3,5	13	Farrell et al. (2004) ; El-Agamy, (2006) ; Al-Haj et Al-Kanhal, (2010) ; Shuiep et al. (2013)
Résidus acides aminés	207	199	178	207	217	209	162	169	Kappeler et al. (1998) ; Tauzin et al. (2002) ; Farrell et al. (2004)
Masse moléculaire (kDa)	24,8	23	22	25	24,9	24	22,6	19	Kappeler et al. (1998) ; Tauzin et al. (2002) ; Farrell et al. (2004) ; Miciński et al. (2013)
Point isoélectrique	4,41	4,26	4,58	4,78	4,76	4,49	4,11	3,97	Eigel et al. (1984) ; Kappeler et al. (1998)
Acides aminés acides	38	31	26	19	22	22	14	17	Eigel et al. (1984) ; Kappeler et al. (1998)
Acides aminés basiques	30	25	27	33	21	20	17	17	Eigel et al. (1984) ; Kappeler et al. (1998)
Résidu cystéine (s)	0	0	2	2	0	0	2	2	Kappeler et al. (1998) ; Farrell et al. (2004)
Groupements phosphoséryls	6	8	9	10	4	5	1	1	Kappeler et al. (1998) ; Farrell et al. (2004)
Résidu proline	19	17	8	10	35	35	22	20	Eigel et al. (1984) ; Kappeler et al. (1998)
Similitude de structure (%)	39		56		64		56		Kappeler et al. (1998)

séquences primaires de la caséine-κ des laits de chamelle et de vache sont présentées dans la **Figure 3**.

La caséine-κ du lait de chamelle est composée d'une séquence de 162 acides aminés avec une masse moléculaire de 22,4 kDa et un point isoélectrique de 4,10 (Kappeler et al. 1998 ; Salmen et al. 2012). En revanche, la séquence de caséine-κ du lait de vache est constituée de 169 acides aminés avec une masse moléculaire de 20,5 kDa (Farrell et al. 2004 ; Salmen et al. 2012). La différence de concentrations en acides aminés de la caséine-κ du lait de chamelle et celle du lait de vache a fait l'objet de quelques travaux de recherche (**Tableau 8**). Salmen et al. (2012) ont montré que la caséine-κ du lait de chamelle était plus riche en lysine, méthionine, thréonine, leucine, phénylalanine et histidine, alors que les teneurs en thréonine, valine et isoleucine étaient plus élevées dans le lait de vache. Ces résultats sont en désaccord avec les travaux de Larsson-Raznikiewicz et Mohamed (1986) qui ont attribué ces variations aux types d'alimentation et aux races de chamelle utilisés pour chaque étude.

Les travaux de Kappeler et al. (1998) ont montré que le site de clivage de la caséine-κ du lait de vache se situe au niveau de la liaison Phe^{105} - Met^{106}, alors que celui du lait de chamelle se trouve au niveau de la liaison Phe^{97} - Ile^{98}. En outre, la caséine-κ du lait de chamelle contient un résidu de proline supplémentaire dans sa séquence (Pro^{95}) qui joue un rôle majeur dans la stabilité de la séquence de la caséine-κ (Kappeler et al. 1998). La séquence de la caséine-κ du lait de chamelle compte cinq sites de résidus glycosylés contre six pour celle du lait de vache. Par contre, les séquences de caséine-κ du lait de chamelle et du lait de vache ont le même nombre de sérine phosphorylé (1 site) (**Figures 3 A et B)**. La glycosylation se trouve au niveau des résidus de thréonine dans la partie C-terminale de la protéine. Cette partie glycosylée forme une chevelure hydrophile à la surface de la micelle contribuant à l'augmentation de la stabilité stérique des micelles grâce à leurs charges négatives (Walstra, 1990).

2.2. Lipides

La plus grande proportion de la matière grasse est constituée de triglycérides qui représentent 98 % et 96 % de la fraction lipidique totale du lait de vache et celui de chamelle, respectivement ; les diglycérides (2 %), le cholestérol (< 0,5 %), les phospholipides (~1 %) et les acides gras libres (0,1 %) sont également présents (Gorban et Izzeldin, 2001 ; Pereira, 2014). La structure d'un globule gras est hétérogène et est formée d'un cœur composé de triglycérides à bas point de fusion et d'une enveloppe constituée de phospholipides, lipoprotéines, enzymes et agglutinines (Amiot et al. 2002) (**Figure 4)**.

Tableau 8 : Concentration moyenne en acides aminés de la caséines-κ du lait de chamelle et du lait de vache (Salmen et al. 2012).

Acides aminés (%)		Lait de chamelle	Lait de vache
Acides aminés essentiels	Lysine	8,15	7,55
	Thréonine	3,09	4,64
	Valine	5,30	5,83
	Méthionine	2,39	1,98
	Isoleucine	4,68	5,09
	Leucine	8,15	6,81
	Phénylalanine	5,08	4,95
	Histidine	3,02	2,90
Acides aminés non essentiels	Acide aspartique	5,72	6,34
	Acide glutamique	18,51	18,01
	Glycine	1,22	1,40
	Sérine	4,90	5,41
	Tyrosine	4,70	5,16
	Arginine	3,81	3,43
	Alanine	2,14	3,70

(A) Caséine-κ du lait de chamelle

EVQNQEQPTC FEKVERLLNE KTVKYFPIQF VQSRYPSYGI NYYQHRLAVP[50]

INNQFIPYPN YAKPVAIRLH AQIPQCQALP NIDPPTVERR PRPRPS**F**[97]↓**I**[98]AI[100]

PPKKT*QDKT*V NPAINTVATV EPPVIPT*AEP AVNTVVIAEA S**SEFITTST*P[150]

ETT*TVQITST EI[162]

(B) Caséine-κ du lait de vache

QEQNQEQPIR CEKDERFFSD KIAKYIPIQY VLSRYPSYGL NYYQQKPVAL[50]

INNQFLPYPY YAKPAAVRSP AQILQWQVLS NTVPAKSCQA QPTTMARHPH[100]

PHLS**F**[105]↓**M**[106]AIPP KKNQDKTEIP TINT*IASGEP T*ST*PTT*EAVE ST*VATLEDS**P[150]

EVIESPPEIN TVQVT*STAV[169]

Figure 3 : Comparaison des séquences primaires de la caséine-κ des laits de chamelle (A) et de vache (B) selon Kappeler et al. (1998).

↓ **:** site de l'hydrolyse par la chymosine ; * les résidus glycosylés ; **la sérine phosphorylée.

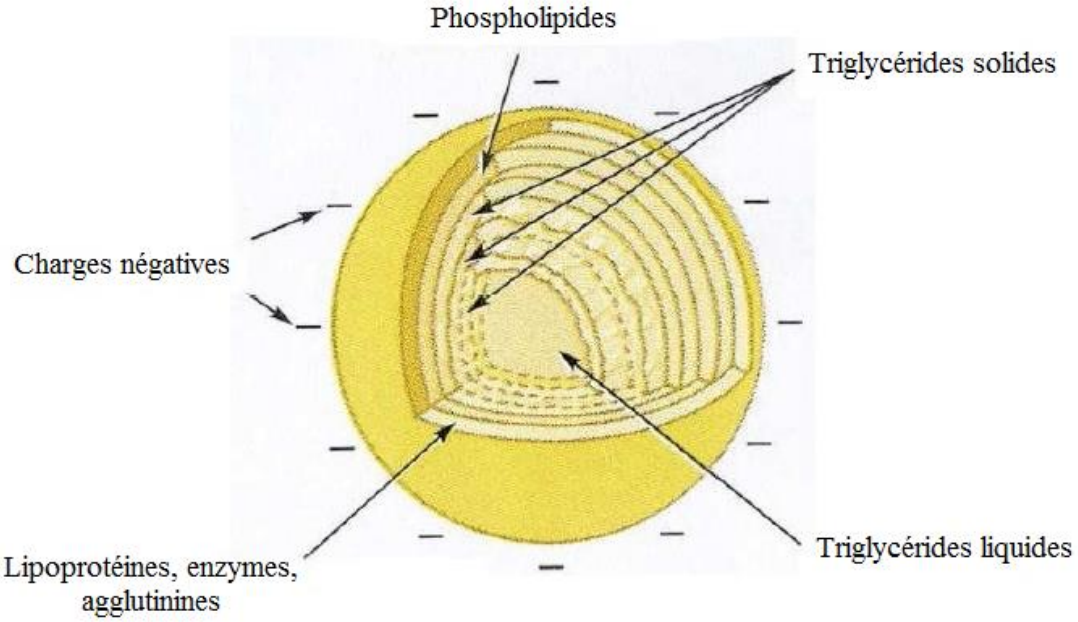

Figure 4 : Structure d'un globule de matière grasse (Amiot et al. 2002).

Tableau 9 : Concentration moyenne en acides gras du lait de chamelle et du lait de vache.

Acides gras	**Point de fusion (°C)**	**Teneur en acides gras totaux (%)**	
		Lait de chamelle (Konuspayeva et al. 2008)	**Lait de vache** (Ceballos et al. 2009)
C4:0	-8	0,37	3,84
C6:0	-3,5	0,38	2,28
C8:0	+16,5	0,40	1,69
C10:0	+31,5	0,35	3,36
C12:0	+43,5	1,01	3,83
C14:0	+54	12,83	11,24
C14:1	- 4,5	0,73	0,49
C16:0	+63	31,30	32,24
C16:1	+1,5	6,93	1,53
C17:0	+60	0,68	0,18
C17:1	+61,3	0,35	0,08
C18:0	+70	16,33	11,06
C18:1	+13,5	21,40	21,72
C18:2	-5	1,37	2,41
C18:3	-11	0,60	0,25
C20:0	+75	0,02	0,11
C20:1	+23,5-	0,01	0,03

Comme dans le cas des protéines, les lipides du lait de chamelle et ceux du lait de vache présentent des caractères communs, tels que la présence des acides gras saturés, mono-insaturés et polyinsaturés (Gorban et Izzeldin, 2001). Cependant, les lipides se distinguent par le diamètre de leurs globules et leurs teneurs en acides gras et en cholestérol. En effet, le diamètre moyen des globules gras du lait de chamelle est de 2,99 µm, plus petit que celui du lait de vache qui est de 3,78 µm (El-Zeini, 2006). Le **Tableau 9** montre que, comparativement au lait de vache, le lait de chamelle contient une faible proportion d'acides gras à chaîne courte (C4-C12) et une teneur plus élevée en acides gras à chaîne longue (C14: 0, C14: 1, Cl6: l, Cl7: 0, Cl7: l et C18: 0). En effet, El-Agamy (2009) a rapporté que la proportion d'acides gras à chaîne courte et à chaîne longue dans le lait de chamelle sont respectivement de 14,80 et 85,20 % (contre 27,72 et 72,81 % dans le lait de vache, respectivement). En outre, le ratio acides gras insaturés / acides gras saturés est plus élevé dans le lait de chamelle (0,43) que dans le lait de vache (0,30), ce qui donne un avantage nutritionnel au lait de chamelle (Konuspayeva et al. 2008). La teneur moyenne en cholestérol du lait de chamelle est plus élevée (34,21 mg.100 g^{-1}) que celle du lait de vache (25,63 mg.100 g^{-1}) comme le rapportent les travaux de Gorban et Izzeldin (1999), Konuspayeva et al. (2008) et Al-Haj et Al-Kanhal (2010).

Les différences de teneur et de composition en lipides induisent des changements aux niveaux de la température de fusion et de solidification de la matière grasse comme le rapportent les travaux d'Abu-Lehia (1989), Rüegg et Farah (1991) et Al-Haj et Al-Kanhal (2010). Ces auteurs ont trouvé des températures de fusion et de solidification de la matière grasse du lait de chamelle de 41,9 ± 0,9 °C et 30,5 ± 2,2 °C, respectivement ; alors qu'elles sont respectivement de 32,6 ± 1,5 °C et 22,8 ± 1,6 °C pour le lait de vache.

2.3. Glucides

Le lactose est le principal glucide présent dans le lait. C'est un diholoside composé d'une unité de glucose et d'une unité de galactose. Il peut se trouver dans le lait sous deux formes anomériques : α-lactose et β-lactose qui sont en équilibre en solution aqueuse. L'assimilation du lactose nécessite au préalable son hydrolyse par la β-galactosidase (lactase) qui a une préférence particulière pour la forme β (Schaafsma, 2008). L'hydrolyse du lactose est relativement lente comparée aux autres glucides, ce qui fait du lactose un glucide assurant aux consommateurs une énergie régulière et prolongée. Le pouvoir sucrant du lactose est relativement faible puisqu'en solution modèle, il n'est que le quart et le tiers de celui d'une solution de fructose et de saccharose, respectivement. La teneur en lactose dans le lait humain

Tableau 10 : Concentration moyenne en sels minéraux du lait de chamelle et du lait de vache.

Type de lait	Concentration (mg.L^{-1})									Références
	Macro-éléments					Oligo-éléments				
	Na	K	Ca	Mg	P	Fe	Cu	Zn	Mn	
Lait de chamelle	702	1586	1078	122	641	2,64	-	-	-	Sawaya et al. (1984)
	270	450	1310	140	510	0,40	-	-	-	Gnan et Shereha, (1986)
	431	725	300	45	-	2,80	-	-	-	Elamin et Wilcox, (1992)
	902	2110	1462	108	784	3,40	-	-	-	Bengoumi et al. (1994)
	688	1464	1180	125	889	2,37	1,44	6,08	0,82	Mehaia et al. (1995)
	581	1704	1182	74	769	1,30	-	-	-	Gorban et Izzeldin, (1997)
	660	1720	1230	90	1020	-	-	-	-	Attia et al. (2000)
	581	1628	1117	66	814	2,30	0,63	5,27	0,13	Soliman, 2005
	590	1560	1140	105	-	2,90	-	5,47	0,51	Al-Haj et Al-Kanhal, (2010)
Lait de vache	501	1402	1127	117,73	961	-	-	-	-	Gaucheron, (2005)
	498	1475	1203	135	953	0,71	0,18	3,92	0,038	Soliman, (2005)
	444	1362	1166	103	867	0,31	0,31	4,12	0,041	Medhammar et al. (2012)

(-) : valeurs non déterminées.

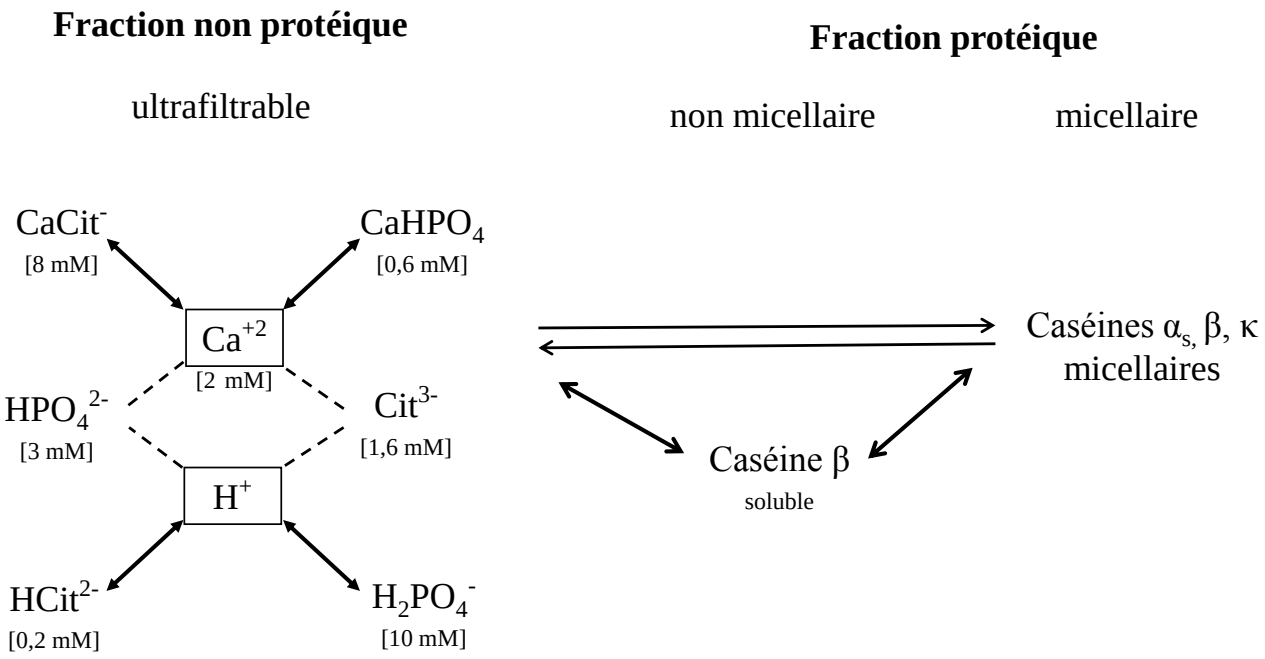

Figure 5 : Les équilibres salins du lait (Brulé, 1981).

est plus élevée (70 $g.L^{-1}$) que celle dans les laits de chamelle et de vache qui sont respectivement de 45,4 et 46 $g.L^{-1}$ (Schaafsma, 2008 ; Konuspayeva et al. 2009).

Comme pour les autres types de lait, l'alimentation influence significativement la teneur en lactose dans le lait de chamelle. En effet, la grande variation de la teneur en lactose du lait de chamelle peut être attribuée aux types de plantes consommées par les chameaux dans le désert (Khaskheli et al. 2005). En revanche, dans le lait de vache, cette teneur semble être dépendante de la saison comme le rapportent les travaux de Lujerdean et al. (2007).

2.4. Minéraux

Bien qu'ils représentent une fraction mineure, les minéraux du lait possèdent un rôle important tant sur le plan physico-chimique que technologique. Les teneurs en macro et oligo-éléments sont présentées dans le **Tableau 10**. La teneur totale de minéraux dans les laits de chamelle et de vache est quasiment similaire 8,15 et 8,50 $g.L^{-1}$, respectivement (Gaucheron, 2005 ; Konuspayeva et al. 2009).

La variation de la composition minérale du lait de chamelle est principalement d'ordre physiologique, bien que d'autres paramètres semblent influencer sa composition comme la saison, l'état sanitaire de la mamelle, le stade de lactation, la race, l'alimentation, etc. (Mehaia et al. 1995 ; Farah, 1996). Même si la composition en macroéléments est similaire dans les deux types de lait, le lait de chamelle se caractérise par sa richesse en oligo-éléments. En effet, les travaux d'Al-Awadi et Strikumar (2001) ont rapporté que le lait de chamelle contenait des teneurs en manganèse et en fer de 0,080 et 3,16 $mg.L^{-1}$, respectivement contre 0,028 et 0,29 $mg.L^{-1}$, respectivement dans le lait de vache.

Les éléments minéraux majeurs sont répartis entre les phases soluble et colloïdale (Holt et Jenness, 1984). En effet, l'équilibre et les répartitions des minéraux dans le lait sont présentés dans la **Figure 5** et ils dépendent du milieu : pH, température, acidification, ajout de minéraux, etc. (Le Graet et Brulé, 1993).

Dans la phase soluble, certains minéraux (sodium, potassium et chlorure) se trouvent essentiellement à l'état dissous sous forme d'ions libres. Les autres minéraux (calcium, magnésium et citrate) sont préférentiellement liés sous forme de sels ($CaCit^-$ et $MgCit^-$). Dans le cas du phosphate, la majeure partie de cet élément est sous forme libre (~ 10 mM) et son état d'ionisation dépend du pH (formes $H_2PO_4^-$ ou HPO_4^{2-}). Il peut également être lié avec le calcium pour former du $CaHPO_4$.

Tableau 11 : Concentration moyenne en vitamines du lait de chamelle et du lait de vache.

Vitamines	Lait de chamelle ($mg.L^{-1}$)			Lait de vache ($mg.L^{-1}$)	
	Sawaya et al. (1984)	Kappeler, (1998)	Zhang et al. (2005) ; Zhao et al. (2015)	Farah, (1993)	USDA, (2009) ; Medhammar et al. (2012)
A (Rétinol)	0,15	0,15	0,97	0,27	0,47
B_1 (Thiamine)	0,32	0,58	0,13	0,57	0,51
B_2 (Riboflavine)	0,40	0,78	1,24	1,56	1,75
B_3 (Niacine)	4,47	4,46	-	0,63	0,93
B_5 (Acide pantothénique)	0,85	0,85	-	3,63	3,81
B_6 (Pyridoxine)	0,51	0,50	0,54	0,50	0,41
B_9 (Acide folique)	0,0040	0,004	-	0,053	-
B_{12} (Cobalamine)	0,0015	0,002	-	0,004	0,0046
E (Tocophérol)	-	0,51	1,50	0,15	-
C (Acide ascorbique)	23,26	29,07	30,07	12,60	-

(-) : valeurs non déterminées.

Dans la phase colloïdale, les minéraux (phosphate, calcium et minoritairement, le citrate et le magnésium) sont associés à la caséine au sein des micelles.

2.5. Vitamines

Les vitamines représentent 0,1 % de la composition globale du lait. Elles existent sous 2 formes : les vitamines liposolubles (A, D, E et K) et les vitamines hydrosolubles (B et C) (Sawaya et al. 1984 ; Stahl et al. 2006 ; Haddadin et al. 2008) (**Tableau 11**). Malgré les variations de la teneur en vitamine C dans le lait de chamelle, sa teneur est trois (Farah et al. 1992) à cinq (Stahl et al. 2006) fois plus élevée que celle du lait de vache. El-Agamy (2009) a rapporté que la quantité de la vitamine C variait de 23,26 à 50,39 $mg.L^{-1}$ dans le lait de chamelle contre 2,91 à 22,29 $mg.L^{-1}$ dans le lait de vache. En comparaison avec le lait de vache, la teneur en vitamine B_3 (niacine) est plus élevée dans le lait de chamelle (Sawaya et al. 1984), alors que les teneurs en vitamines A et B_2 sont les plus faibles (Sawaya et al. 1984 ; Farah et al. 1992 ; Stahl et al. 2006). Selon l'USDA (2009), la consommation quotidienne d'un quart de litre de lait de chamelle assure 15,5 % des apports recommandés en cobalamine (vitamine B_{12}), 8,25 % en riboflavine (vitamine B_2), 5,25 % en vitamine A, 10,5 % en acide ascorbique (vitamine C), 10,5 % en thiamine (vitamine B_1) et 10,5 % en pyridoxine (vitamine B_6). Alors que la consommation de la même quantité de lait de vache permet de répondre aux besoins recommandés journaliers de 43,5 % en vitamine B_{12}, 36 % en vitamine B_2, 11,5 % en vitamine B_6, 3,5 % en vitamine C, 9 % en vitamine A et 9 % en vitamine B_1.

3. Coagulation du lait

La coagulation du lait est la première étape dans la production de la plupart des produits laitiers, pendant laquelle le lait est transformé, par des enzymes spécifiques et / ou des bactéries lactiques, en un coagulum semi-solide viscoélastique. La coagulation du lait correspond à une déstabilisation de l'état micellaire des caséines, et selon le mode de coagulation employé, les mécanismes de formation du coagulum et ses caractéristiques diffèrent.

L'analyse des différentes étapes de formation du gel est d'une importance majeure. Par exemple, la détermination du temps de coagulation est une étape clé puisqu'elle affecte de manière significative le rendement fromager. Si le découpage est réalisé avant la fin de la coagulation, des pertes de graisse et de particules de caillé fines se produisent induisant par conséquent une diminution du rendement fromager. Par contre, si le caillé est trop ferme, la

Tableau 12 : Caractéristiques des coagulums en fonction du mode de coagulation (Lenoir et al. 1985).

Caractéristiques	Gel « ferment lactique »	Gel « présure »
Temps de prise	Long (de 6 à 15 heures)	Court de (10 à 30 minutes)
pH	<4,6	6,7-6,5
Minéralisation	Faible	Forte
Structure micellaire	Détruite	Modifiée
Fermeté	Forte	Faible
Friabilité	Forte	Faible
Plasticité	Forte	Faible
Elasticité	Faible	Forte
Perméabilité	Forte	Faible
Contractibilité	Faible	Forte
Tension	Faible	Forte
Aptitude aux traitements mécaniques	Faible	Forte
Egouttage	Spontané, lent, faible	Rapide et important si actions mécaniques et thermiques fort
Aptitude à l'évaporation	Forte	Faible
Humidité du caillé égoutté	Forte	Faible
Cohésion du caillé égoutté	Faible	Forte

synérèse est retardée entraînant une augmentation de la teneur en eau du fromage, ce qui prolonge la durée d'affinage des fromages (Benedito et al. 2002).

3.1. Coagulation par voie acide

La coagulation par voie acide est de nature électrochimique. Une diminution du pH est induite soit par : i) l'action de bactéries lactiques naturellement présentes dans le lait et / ou ajoutées sous forme de ferments lactiques transformant le lactose en acide lactique, ou ii) acidification chimique (injection de CO_2 et / ou de glucono-δ-lactone (GDL)). L'abaissement du pH de 6,7 à 4,4 conduit à la formation du gel lactique. En effet, les micelles de caséines sont présentes dans le lait sous forme d'agrégats de quatre protéines (caséines α_{s1}, α_{s2}, β et κ), et leur stabilisation est réalisée par des interactions hydrophobes et ioniques. Ces agrégats sont renforcés par la présence de phosphate de calcium à l'intérieur des micelles, tandis que l'extérieur de ces agrégats est stabilisé par la caséine-κ chargée négativement et qui assure des répulsions électrostatiques. McMahon et al. (2009) ont rapporté que la coagulation acide du lait de vache peut être divisée en trois phases en fonction de la diminution de pH. La première phase (de pH 6,7 à 5,3) est caractérisée par la dissociation des micelles des caséines. La deuxième phase de l'acidification se situe entre pH 5,3 et 4,9, et est caractérisée par une réassociation des protéines qui forment des particules colloïdales plus compactes. La dernière phase commence à un pH inférieur à 4,8 impliquant une agrégation rapide des caséines colloïdales conduisant à la formation d'un réseau protéique qui apparait à un pH d'environ 4,8. Au cours de l'acidification, la neutralisation des charges négatives en surface des micelles de caséines conduit à une augmentation du diamètre moyen des micelles. Le gel formé par acidification est friable, de faible contractibilité et sans cohésion (**Tableau 12**).

3.2. Coagulation par voie enzymatique

Un grand nombre d'enzymes protéolytiques ont la propriété de coaguler le lait. Les plus utilisées sont des enzymes d'origine animale. Les préparations coagulantes d'origine végétales sont rarement utilisées du fait de leur pouvoir coagulant très variable. La présure est considérée comme le coagulant d'origine animale le plus utilisé. Elle est constituée de deux enzymes protéolytiques : chymosine (80 %) et pepsine (20 %). La présure est principalement obtenue à partir de la caillette de veau.

L'ajout de la présure au lait entraîne sa coagulation par hydrolyse de la caséine-κ située à la surface de la micelle en coupant la liaison peptidique Phe^{97} - Ile^{98} et Phe^{105} - Met^{106} du lait

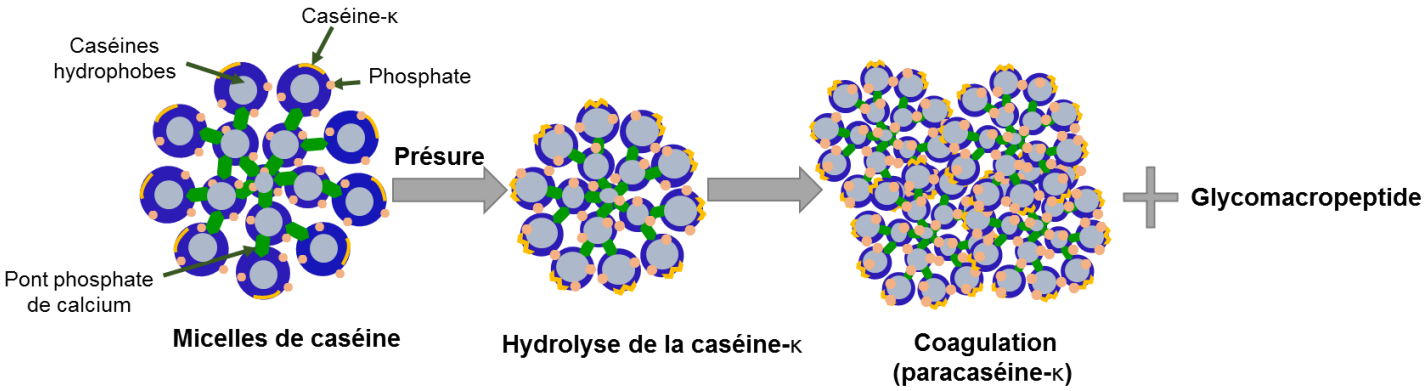

Figure 6 : Étapes de formation d'un gel par action de la présure sur les caséines du lait (d'après le modèle de Schmidt, 1980).

de chamelle et de vache, respectivement. Cette hydrolyse libère le glycomacropeptide (GMP) qui est la partie hydrophile de la caséine-κ chargée négativement et responsable des répulsions électrostatiques (fragments 106–169 et 98–162 dans le cas du lait de vache et de chamelle, respectivement). Le GMP est soluble et est expulsé hors de la micelle. Le fragment 1–105 et 1–97 respectivement pour les laits de vache et de chamelle, correspondant à la paracaséine-κ, qui est hydrophobe et reste à l'intérieur de la micelle, et interagit avec d'autres composants de la micelle par des liaisons hydrophobes entraînant la formation d'un gel (Amiot et al. 2002).

La coagulation du lait par voie enzymatique correspond à trois phases (**Figure 6**) :

- La phase primaire qui correspond à l'hydrolyse de la caséine-κ. Cette action entraîne la formation de deux fragments peptidiques : la paracaséine-κ qui reste associé à la micelle et le GMP qui est exsudé dans la phase du sérum. Il en résulte une réduction des charges négatives et des répulsions stériques de telle sorte que les micelles de caséines deviennent susceptibles à l'agrégation (Anema, 1997).
- La phase secondaire qui correspond à la formation d'un gel par agrégation des micelles. L'agrégation démarre lorsque 85 à 90 % de la caséine-κ est hydrolysée, ce qui correspond à environ 60 % du temps nécessaire pour observer visuellement la coagulation.
- Au cours de la phase tertiaire, des modifications majeures ont lieu au niveau de l'organisation des micelles agrégées *via* la mise en place de liaisons phosphocalciques et des ponts disulfures. Le gel formé est structuré et possède entre autres une bonne élasticité, une faible friabilité et un fort pouvoir de contraction permettant sa bonne aptitude à l'égouttage par synérèse (**Tableau 12**).

• Propriétés du lait de chamelle à la coagulation

Le lait de chamelle est une matière première agricole qui reste peu valorisée dans l'industrie agro-alimentaire. Cela s'explique par sa faible aptitude à se transformer en produits dérivés. Afin de lever les verrous technologiques au développement de nouveaux produits, quelques études ont été menées dans le but d'améliorer les propriétés de coagulation du lait de chamelle. Les faibles teneurs en matière sèche et en caséine-κ, le diamètre élevé des micelles de caséines et l'absence de β-lactoglobuline sont parmi les principales difficultés pour sa transformation en produits laitiers dérivés (Ramet, 2003 ; Kappeler et al. 2003 ; El-Agamy, 2006 ; El-Zubeir et Jabreel, 2008 ; Al-Haj et Al-Kanhal, 2010 ; Zhao et al. 2015).

En ajoutant du chlorure de calcium ($CaCl_2$) à une concentration de 15 g.100 L^{-1} ou en mélangeant du lait de chamelle avec du lait de vache et / ou de brebis (10 à 30 %), les caractéristiques des gels obtenus ont été améliorées puisqu'une diminution du temps de

gélification de 20 à 30 % et une augmentation de la fermeté de gels (doublement) ont été observées par rapport au lait de chamelle témoin (Farah et Bachmann, 1987 ; Ramet, 1990, 1994a, b, 2001, 2003). Ces travaux ont été récemment confirmés par Hailu et al. (2016) qui ont indiqué que l'enrichissement du lait de chamelle avec le $CaCl_2$ à une concentration de 20 g.100 L^{-1} contribuait à diminuer le temps de gélification de 703 à 494 secondes et à augmenter la fermeté des gels de 14 à 55 Pa, en comparaison au témoin (lait sans ajout de calcium).

La majorité des études susmentionnées ont été réalisées avec la présure extraite de la caillette de veau comme agent coagulant. D'autres travaux de recherche ont porté sur l'utilisation de la pepsine de veau (Wangoh et al. 1993 ; Ramet, 1994a) ou de la chymosine de chamelon (Wangoh et al. 1993 ; El-Agamy, 2000b ; Kappeler et al. 2006 ; Saliha et al. 2011 ; Haroun et al. 2012 ; Konuspayeva et al. 2014). Ces travaux ont souligné que le meilleur coagulum est obtenu en utilisant de la chymosine tirée de l'estomac de chamelon ou de la pepsine de veau. Il est à indiquer que l'utilisation de la pepsine de veau, ayant une activité protéolytique assez prononcée, peut induire l'apparition de défauts d'amertume provoquée par l'accumulation de peptides amers dans le produit fini (Ramet, 1993).

4. Techniques d'évaluation de la structure et de la texture des produits laitiers

4.1. Méthodes rhéologiques

La rhéologie est la science qui caractérise le comportement physique de la matière à savoir la déformation, l'écoulement et la désintégration sous l'effet d'une contrainte appliquée. Cette science est omniprésente dans le domaine de la recherche en agro-alimentaire et fréquemment interchangeable avec la texture (Tunick, 2000). Elle permet de déterminer le comportement mécanique des aliments lors des différentes étapes de production et elle conditionne principalement les propriétés texturales et la perception sensorielle du produit fini.

Les propriétés rhéologiques des produits laitiers sont des paramètres importants pour l'évaluation de leur structure / texture (Karoui et De Baerdemaeker, 2007). D'un point de vue rhéologique, le lait et la crème sont assimilés à un produit liquide, alors que le lait concentré, le yaourt, le beurre, la crème glacée et le fromage affiné, etc. sont dotés d'un comportement intermédiaire, appelé en rhéologie un comportement viscoélastique (Shoemaker et al. 1992).

Les méthodes rhéologiques et texturales les plus utilisées pour évaluer la qualité des produits laitiers sont les tests de compression uniaxiale et du cisaillement dynamique ainsi que le profil d'analyse de la texture (TPA).

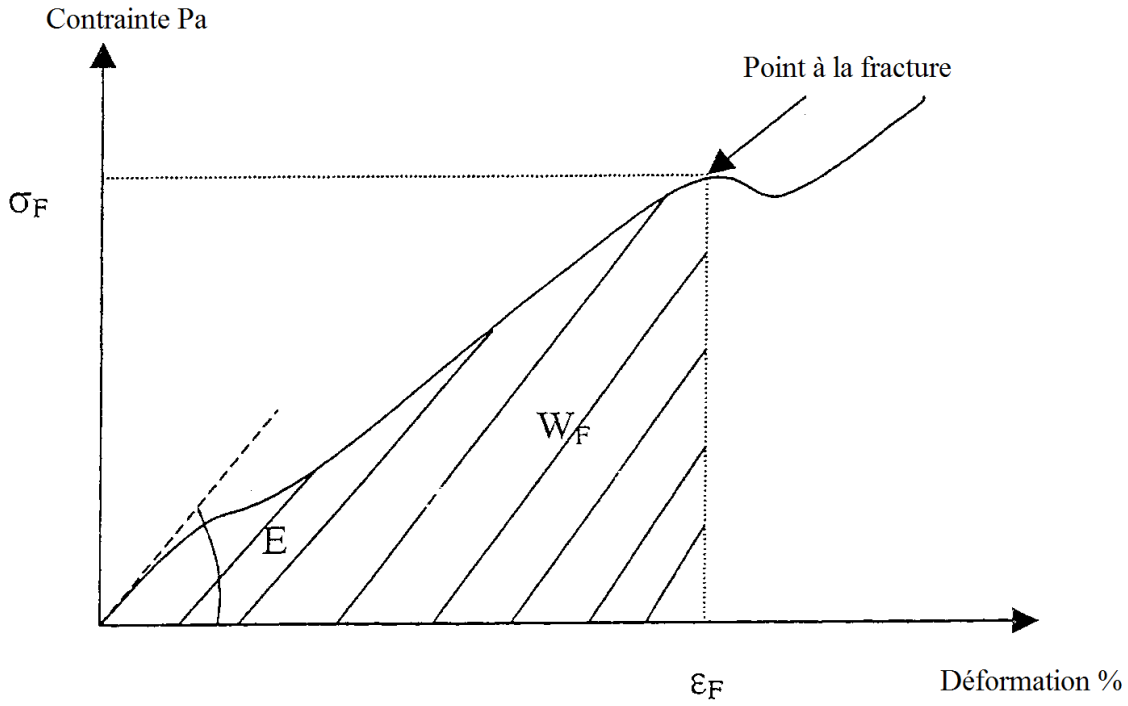

Figure 7 : Allure générale d'une courbe de compression, contrainte en fonction de la déformation, obtenue à partir d'un test de compression uniaxiale (Herbert, 1999).

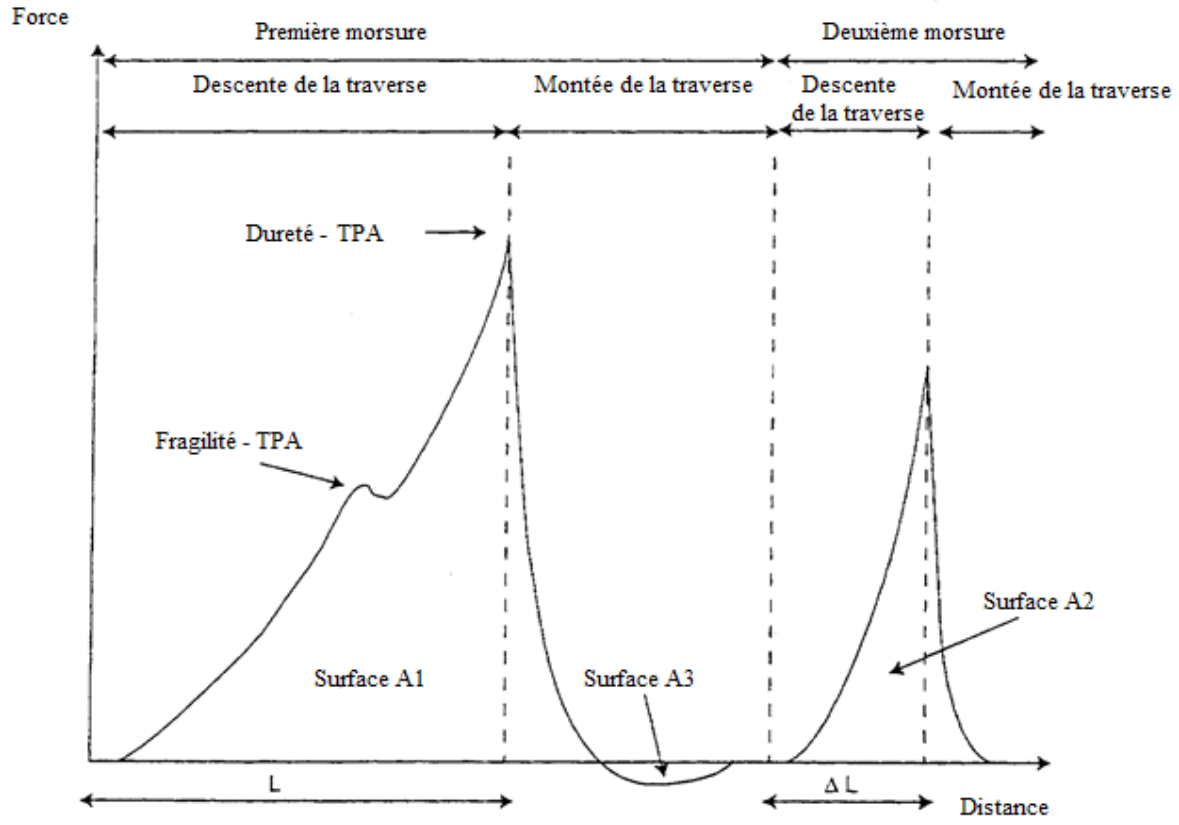

Figure 8 : Allure générale d'un profil d'analyse de la texture (TPA) (Hardy et Scher, 1997). A3 : Intensité de l'adhérence TPA ; A2/A1 : Intensité de la cohésion TPA ; AL/L : Elasticité TPA

4.1.1. Test de compression uniaxiale

Le test de compression uniaxiale consiste à appliquer une contrainte (force par unité de surface) sur l'échantillon, et de mesurer la déformation qui en découle (Tunick, 2000 ; Pappa et al. 2007). L'échantillon de forme cylindrique ou cubique avec des surfaces planes subi une compression uniaxiale entre deux plateaux parallèles, dont l'un est fixe et l'autre avance avec une vitesse constante prédéterminée (Van, 1991). Lors du contact du mobile avec la surface de l'échantillon, un capteur enregistre la force déployée par l'échantillon durant la déformation.

Les données enregistrées sont illustrées sous forme d'une courbe qui représente la contrainte en fonction de la déformation (**Figure 7**), et à partir de laquelle les paramètres suivants sont calculés : le module d'élasticité (E), la contrainte (σ_F), la déformation au point de fracture (ε_F) et l'énergie de la fracture (W_F).

Outre son application aux différentes variétés de fromages (Ak et Gunasekaran, 1992 ; Antoniou et al. 2000 ; Lebecque et al. 2001 ; Famelart te al, 2002 ; Del Nobile et al. 2007 ; Magenis et al. 2014), ce test a été utilisé, récemment, par Munialo et al. (2016) pour étudier les propriétés mécaniques d'un gel fabriqué à partir de lactosérum avec ajout de polysaccharides à différentes concentrations (0, 0,05, 0,1, 0,15, 0,2 et 0,25 %). Les résultats obtenus ont montré que la contrainte à la rupture augmentait avec l'accroissement de la concentration de polysaccharides.

4.1.2. Profil d'Analyse de la Texture

Le Profil d'Analyse de la Texture (TPA) est un test instrumental imitatif ayant pour objectif la reproduction du processus de mastication de manière partielle (Bertola et al. 1991 ; Hardy et Scher, 1997 ; Madureira et al. 2011). Les échantillons, de forme cylindrique, subissent une double compression (test à deux morsures) entre deux plaques d'acier. La force est enregistrée en fonction du temps et à partir de laquelle quatre paramètres texturaux sont mesurés : la dureté, l'élasticité, l'intensité de la cohésion et l'intensité de l'adhérence (**Figure 8**).

La potentialité de la méthode à déterminer la texture de fromages à pâtes pressées non-cuites et cuites a été examinée (Bryant et al. 1995 ; Van Hekken et al. 2013 ; Murtaza et al. 2014 ; Kaminarides, et al. 2015 ; Soodam et al. 2015).

La même technique a été également utilisée pour étudier les propriétés texturales du yaourt (Sandoval-Castilla et al. 2004) et de gels acide et présure (Vasbinder et al. 2003 ; Yuksel, 2013 ; Zhao et al. 2014 ; Pang et al. 2015). Ces auteurs ont indiqué que le TPA permettait de suivre

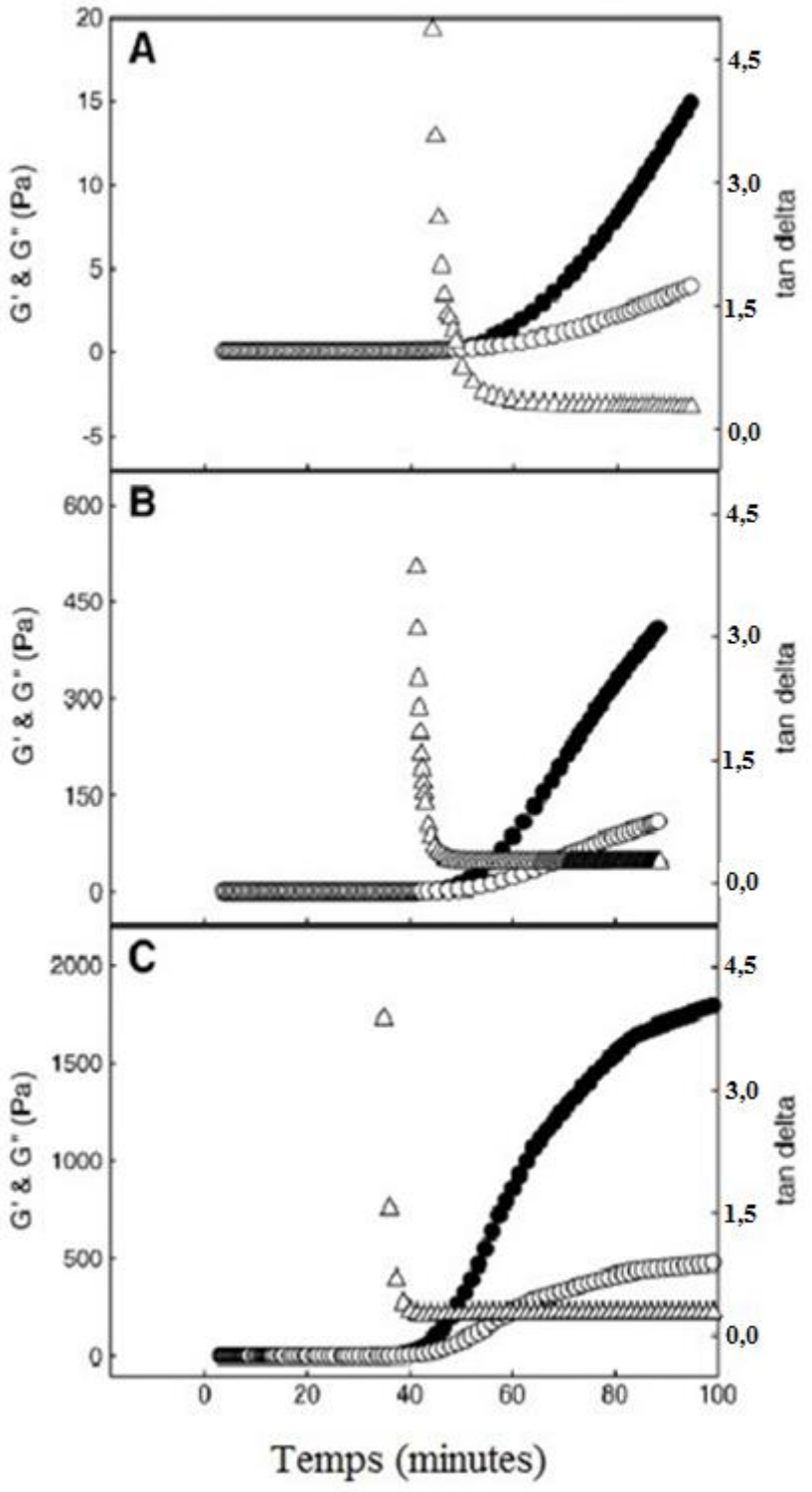

Figure 9 : Évolutions du module élastique G' (●), du module visqueux G" (○) et de la tan delta (tan δ) (Δ) au cours de la gélification du lait de vache écrémé ayant différents facteurs de réduction volumiques (FRV) issus de l'ultrafiltration ; (A : FRV= 1 ; B : FRV=3 ; C : FRV = 5) (Sandra et al. 2011).

les changements de la texture des gels obtenus suite à l'addition, par exemple de calcium et de gélatine.

4.1.3. Test de cisaillement dynamique

Le test de cisaillement dynamique est une technique instrumentale inscrite dans l'approche oscillatoire en rhéologie permettant d'appréhender les modifications structurales susceptibles d'intervenir dans un échantillon (Ma et al. 1996 ; Famelart et al. 2002). Cette méthode est non destructive, ce qui permet de réaliser les mesures sans encourir des dommages structurels à l'échantillon (Gunasekaran et Ak, 2000). Le test repose sur l'application d'une contrainte sinusoïdale dans la région viscoélastique linéaire. Comme le montre la **Figure 9**, différents paramètres peuvent être déterminés comme le module visqueux (G"), le module élastique (G') et tan delta (tan δ) qui correspond au rapport G" / G'. Divers modes opératoires peuvent être mis en œuvre pour l'étude du comportement viscoélastique des produits laitiers : balayage en fréquence, en température, en temps, etc.

Comme l'a montré une recherche bibliographique, le test de cisaillement dynamique a été utilisé pour étudier les caractéristiques rhéologiques des gels obtenus par coagulation enzymatique et acide (Lucey et al. 1998a ; Hemar et al. 2004 ; Zhong et Daubert, 2004 ; Karlsson et al. 2007 ; Blecker et al. 2012 ; Ramasubramanian et al. 2014). Famelart et al. (2002) ainsi que Karoui et Dufour (2003) ont employé cette technique pour déterminer les températures de fusion de la matière grasse de fromages à pâtes pressées non-cuites et cuites.

Le test de cisaillement dynamique présente l'avantage d'être rapide et de permettre d'effectuer un balayage en température qui peut varier de 10 à 100 °C pour les appareils équipés d'un système à effet Peltier. De plus, la connaissance des propriétés dynamiques comme G' et G" permet de calculer d'autres propriétés viscoélastiques comme par exemple tan δ (Gunasekaran et Ak, 2000). Cependant, les analyses rhéologiques en régime transitoire et / ou permanent doivent être effectuées à température constante. Malgré la multitude d'avantages que présentent les méthodes rhéologiques citées ci-dessus, elles souffrent d'un inconvénient majeur consistant à réaliser les mesures sur une échelle de temps relativement assez longue, ce qui par conséquent, pourrait induire une déshydratation de l'échantillon et des modifications au niveau des paramètres rhéologiques.

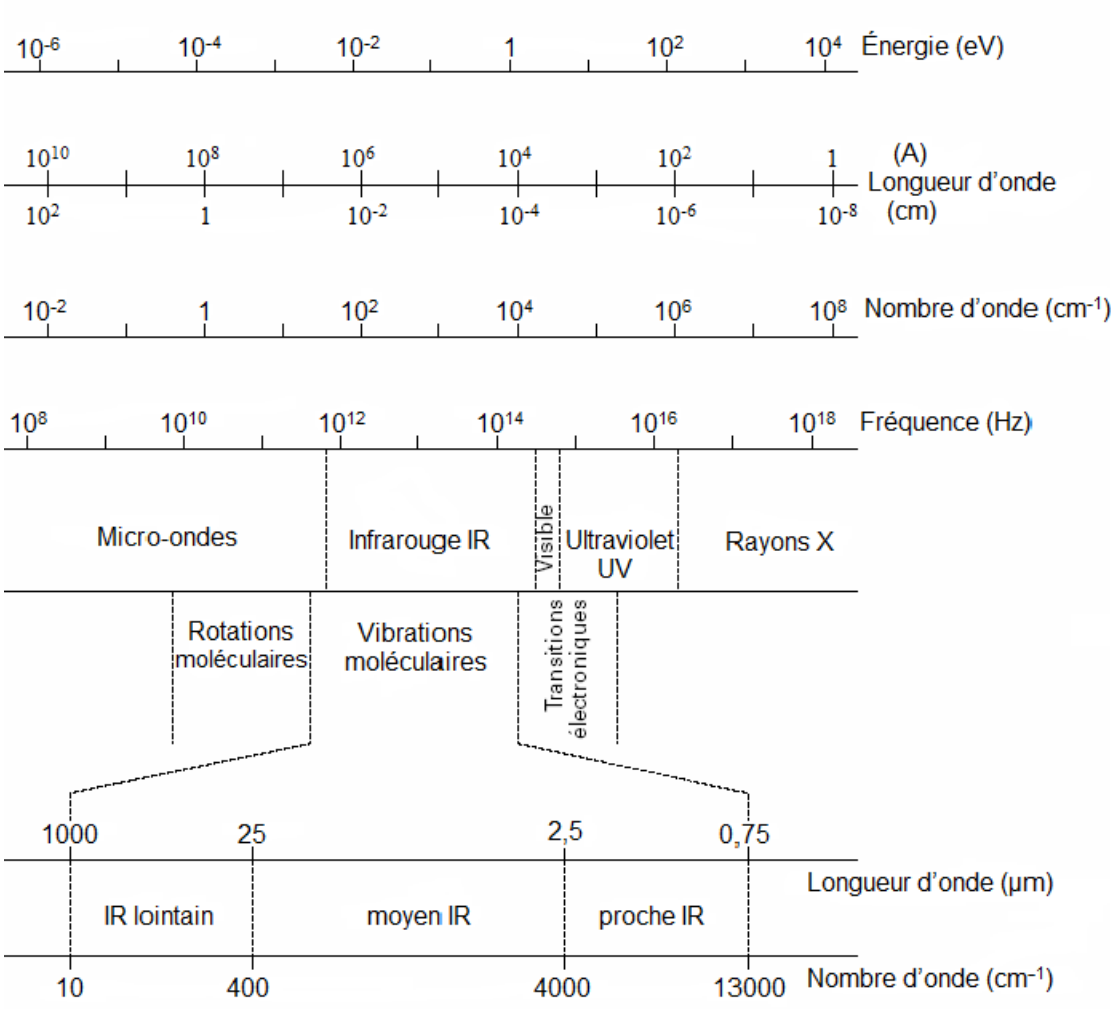

Figure 10 : Domaines spectraux du rayonnement électromagnétique (Dalibart et Servant, 2000).

4.2. Méthodes spectroscopiques

A l'heure actuelle, les méthodes utilisées pour évaluer la qualité des produits laitiers sont majoritairement basées sur des approches rhéologique, physico-chimique, microbiologique, etc. Ces techniques sont, pour la plupart, lourdes à mettre en place, coûteuses, destructives et longues. Ces dernières années, plusieurs travaux ont été menés à la recherche de méthodes rapides, fiables et non-destructives d'évaluation de la qualité des produits laitiers ; il s'agit notamment des techniques spectroscopiques comme, par exemple, les spectroscopies infrarouge, de fluorescence, de résonance magnétique nucléaire (RMN) et Raman.

4.2.1. Spectroscopie infrarouge

La spectroscopie infrarouge est définie comme l'étude de l'interaction de la lumière avec la matière et est basée sur les vibrations des atomes d'une molécule (Bertrand, 2002). Le rayonnement infrarouge (IR) se réfère à des longueurs d'onde plus longues que celles de la lumière visible, mais plus courtes que celles des ondes radio (**Figure 10**) (Dalibart et Servant, 2000).

Un spectre est obtenu en enregistrant l'intensité de la lumière infrarouge absorbée par un échantillon en fonction de la fréquence ou de la longueur d'onde de la lumière incidente (Luykx et van Ruth, 2008). La partie infrarouge du spectre électromagnétique est divisée en trois régions :

- la région du proche infrarouge (PIR) qui est comprise entre 750 et 2500 nm
- la région du moyen infrarouge (MIR) qui se situe entre 2500 et 25000 nm
- la région lointaine infrarouge qui correspond à des longueurs d'onde supérieures à 25000 nm.

Le PIR et le MIR sont connus, en tant qu'outils d'analyses quantitative et qualitative ; ces techniques ont été utilisées avec succès pour contrôler la qualité et pour déterminer l'authenticité des produits laitiers. Les résultats publiés ces dix dernières années ont démontré le potentiel de ces deux techniques, couplées à la chimiométrie à : i) suivre la coagulation du lait (Klandar et al. 2007 ; De Marchi et al. 2009 ; Cipolat-Gotet et al. 2012 ; Lyndgaard et al. 2012; Penasa et al. 2014) ; ii) prédire quelques paramètres physico-chimiques de différentes variétés de fromages (Adamopoulos et al. 2001 ; Karoui et al. 2006a ; Lucas et al. 2008 ; González-Martín et al. 2011) et de beurres (Hermida et al. 2001 ; Heussen et al. 2007) ; iii) déterminer l'authenticité de fromages type Emmental fabriqués dans différents pays européens (Pillonel et al. 2003) ; iv) identifier des fromages Saint-Nectaire en fonction des conditions de fabrication et de la composition du lait de départ (Boubellouta et al. 2010 ; Karoui et al. 2011 ; Valenti et

Tableau 13 : Attribution des bandes spectrales en moyen infrarouge aux principaux composants du lait (Grappin et al. 2000).

Nombre d'onde (cm^{-1})	Liaison impliquée	Fonction du groupement	Mode de vibration	Composant du lait
2955	C-H	$-CH_3$	Élongation asymétrique	Matière grasse
2924	C-H	$-CH_2$	Élongation asymétrique	Matière grasse
2872	C-H	$-CH_3$	Élongation symétrique	Matière grasse
2854	C-H	$-CH_2$	Élongation symétrique	Matière grasse
1746	C=O	Ester carbonyle	Élongation	Matière grasse Acides organiques
1651	C=O	Amide I	Élongation	Protéine
1548	N-H C-N	Amide II	Élongation Déformation Élongation	Protéine
1470-1446	-CH -CH	$-CH_2$ $-CH_3$	Déformation Déformation	Matière grasse + protéine Matière grasse + protéine
1243-1100	-C(O)-O- C-O	Ester	Élongation Élongation	Matière grasse Matière grasse
1112-1050	C-O O-H	Alcool I	Élongation Déformation	Lactose

IRTF: échantillon dilué ou limpide

RTA-IRTF: échantillon turbide, concentré ou solide

Figure 11 : Dispositifs d'infrarouge IRTF et RTA-IRTF.

al. 2013) ; et v) identifier le lait de vache falsifié avec différents types de protéines comme la mélamine mais aussi l'urée et le lactosérum (Santos et al. 2013 ; Zhang et al. 2014).

Le spectre PIR d'un produit laitier correspond principalement aux bandes harmoniques et de combinaisons des liaisons chimiques C-H, N-H et O-H. Cette technique est largement utilisée pour déterminer des constituants organiques présents dans les produits alimentaires et pharmaceutiques. La méthode présente l'avantage d'être rapide (analyse des échantillons en transmittance ou en réflectance sans avoir la nécessité de diluer ou de dissoudre le produit) (Karoui et al. 2003a ; Bassbasi et al. 2014). En revanche, la faible sensibilité de la méthode ne permet pas de mesurer avec précision les composants à faibles concentrations. De plus, la superposition de plusieurs bandes harmoniques et de combinaisons dans la région PIR rend difficile l'attribution des bandes spectrales même pour des composants purs (Grappin et al. 2006).

La partie la plus riche en information et la plus accessible expérimentalement est celle du MIR et qui correspond aux bandes fondamentales de liaison de valence (ν) de groupes fonctionnels d'une molécule. Les bandes spectrales du MIR ont été décrites par Grappin et al. (2000) et présentées dans le **Tableau 13**. Les bandes liées aux vibrations de valence ν (C-H) des groupements CH_3, CH_2 et CH des chaînes d'acides gras des lipides sont principalement localisées dans la zone spectrale 3000 - 2800 cm^{-1}. La région caractéristique des protéines se situe entre 1700 et 1500 cm^{-1} et est dominée par les bandes amide I (élongation C=O ~ 1650 cm^{-1}) et amide II (combinaison hors phase du balancement dans le plan du groupement N-H et de l'élongation C-N ~1550 cm^{-1}), caractéristiques des liaisons peptidiques des protéines. La bande amide I est essentiellement attribuable à une élongation du groupe C=O.

La région spectrale 1500 - 700 cm^{-1} connue sous le nom d'empreinte digitale et où de nombreuses molécules chimiques absorbent la lumière.

Dans le secteur des produits laitiers, la spectroscopie MIR a été moins utilisée que le PIR ce qui s'explique par le fait que l'eau est un constituant majeur de ces produits et contribue fortement au spectre MIR. Avec le développement ces dernières décennies de la spectroscopie infrarouge à transformée de Fourier (IRTF), des informations intéressantes sur les structures des protéines et des lipides ont été obtenues (Casal et Mantsch, 1984). Par ailleurs, l'utilisation de la technique de réflexion totale atténuée (RTA) s'est révélée très utile pour l'enregistrement des spectres MIR de produits laitiers solides, liquides et pâteux simplement étalés sur une lame cristalline de séléniure de zinc (ZnSe), de silicium (Si), de germanium (Ge), etc. (**Figure 11**).

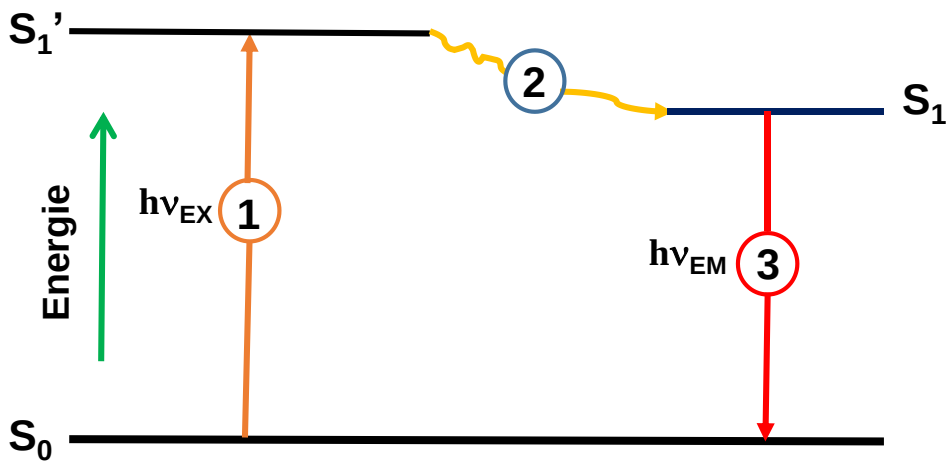

Figure 12 : Diagramme de Jablonski illustrant le principe de base de la spectroscopie de fluorescence (Lakowicz, 1983a).

4.2.2. Spectroscopie de fluorescence frontale

La fluorescence est l'émission de la lumière par une molécule fluorescente ou sous-structure appelée un fluorophore à la suite de l'absorption de la lumière dans l'ultraviolet ou le visible. Ainsi, le fluorophore absorbe de l'énergie sous forme de lumière à une longueur d'onde spécifique et libère de l'énergie sous forme d'émission de lumière à une longueur d'onde plus élevée. Les principes généraux peuvent être illustrés par le diagramme de Jablonski (**Figure 12**).

La fluorescence est le résultat de trois étapes : i) l'excitation, où la lumière est absorbée par la molécule, le fluorophore passe à un état électronique excité S_1' par absorption de photons d'énergie provenant d'une lampe à incandescence ou d'un laser, ce qui signifie qu'un électron absorbe cette énergie et passe du niveau singulet fondamental S_0 à un niveau singulet S_1' ; ii) la molécule reste excitée durant un très court laps de temps (10^{-9} - 10^{-7} secondes) durant lequel des changements conformationnels ont lieu dissipant une faible quantité d'énergie (S_1'- S_1) ; et iii) le fluorophore retourne à son état initial S_0 en émettant un photon d'énergie. Cette émission de photons est appelée fluorescence. L'ensemble des rayonnements émis lors de la désexcitation par fluorescence constitue le spectre d'émission (Karoui et Blecker, 2011).

En raison de sa perte d'énergie, le photon émis présente toujours une énergie moindre que le photon excité : pour une molécule donnée, les longueurs d'onde d'émission seront donc supérieures à celles d'excitation.

La spectroscopie de fluorescence est parmi les techniques analytiques les plus sensibles et qui a été utilisée pour fournir des informations sur la présence de molécules fluorescentes et sur leur environnement moléculaire dans les échantillons. De plus, cette méthode offre une sensibilité 100 - 1000 fois plus élevée que les autres techniques spectroscopiques (Karoui et al. 2007a).

Les sondes de fluorescences peuvent être de deux natures : intrinsèques et extrinsèques.

Les fluorophores intrinsèques sont ceux qui sont naturellement présents dans les aliments. Les produits laitiers contiennent plusieurs fluorophores intrinsèques tels que les acides aminés aromatiques et les acides nucléiques, les tryptophanes des protéines, les vitamines A et B_2 (riboflavine), le NADH (Nicotinamide Adénine Dinucléotide) et tous les composés contenant des doubles liaisons conjuguées (Karoui et al. 2003a, 2011).

L'allure des spectres varie en fonction des traitements appliqués au produit. Karoui et al. (2003b) ont rapporté que le maximum d'intensité de fluorescence du tryptophane à 340 nm des fromages à pâtes pressées non-cuites et cuites (Raclette, Emmental, Comté) diminuait avec

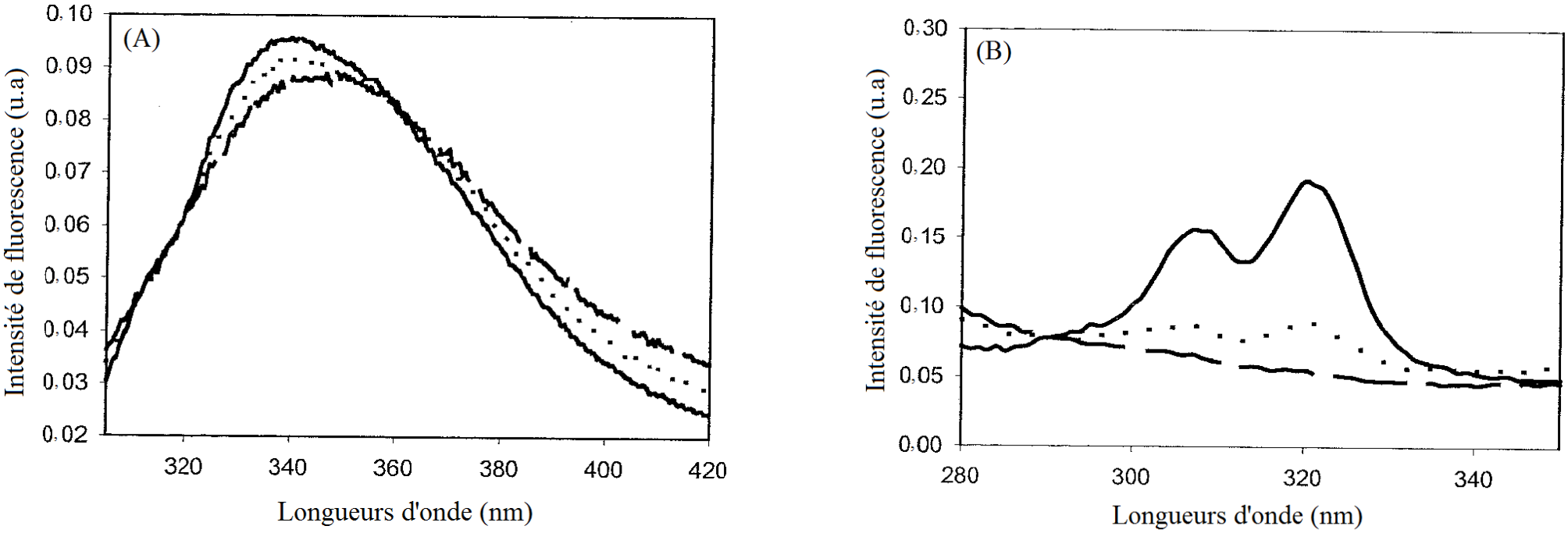

Figure 13 : Changements de l'allure des spectres du tryptophane (A) et de la vitamine A (B) acquis sur des fromages Comté à 5 °C (—), 30 °C (···) et 60 °C (---) (Karoui, 2004).

l'intensité du traitement thermique appliqué aux fromages (**Figure 13 A**), ce qui est en accord avec les résultats obtenus sur le lait de vache (Dufour et Riaublanc, 1997 ; Birlouez-Aragon et al. 2002 ; Kulmyrzaev et Dufour, 2002). Une des explications pourrait résulter de la dénaturation des protéines du lait au cours du traitement thermique ; cette hypothèse a été confortée par la très bonne corrélation ($R^2 = 0,98$) entre l'intensité de fluorescence à 340 nm et la teneur en ß-lactoglobuline native (Birlouez-Aragon et al. 2002 ; Elshereef et al. 2006).

De plus, la forme des spectres de la vitamine A changeait selon l'état physique des triglycérides (**Figure 13 B)**. Karoui et al. (2003b) ont souligné que le rapport des intensités de fluorescence des spectres de la vitamine A à 322 et 295 nm variait selon l'état physique des triglycérides (solide ou liquide). La valeur de ce rapport en fonction de la température a permis de déterminer le point de fusion de la matière grasse émulsionnée (Dufour et al. 1998). Suite à l'application de l'analyse en composantes principales (ACP), une très bonne séparation des laits selon les traitements technologiques qu'ils ont subis (lait natif, chauffé, homogénéisé, chauffé et homogénéisé) a été obtenu (Dufour et Riaublanc, 1997).

Outre les deux sondes intrinsèques (tryptophane et vitamine A), d'autres fluorophores ont été utilisés pour suivre, entre autres, l'état d'oxydation des produits laitiers. Il s'agit, par exemple, de la riboflavine qui conditionne la couleur et la valeur nutritionnelle d'un produit alimentaire (Kristensen et al. 2000 ; Wold et al. 2002 ; Becker et al. 2003 ; Dowell et al. 2008). Les travaux de Becker et al. (2003) et ceux de Karoui et al. (2006b) réalisés respectivement sur le yaourt et des fromages du type Raclette ont montré que la riboflavine est très sensible aux conditions d'entreposage (lumière, oxygène, atmosphère modifiée).

Quant aux fluorophores extrinsèques, ils sont incorporés aux échantillons pour marquer spécifiquement un constituant du produit. Herbert (1999) a utilisé le 1-anilinonaphthalene-8-sulfonicacide (1,8-ANS) comme marqueur des protéines au cours de la coagulation du lait. Cette sonde est bien connue pour ses propriétés hydrophobes et est fréquemment utilisée en spectroscopie de fluorescence (Matarella et Richarson, 1983). Cependant, en plus de son instabilité dans le temps, cette sonde n'est pas spécifique seulement aux protéines puisqu'elle peut marquer des lipides. Les mêmes auteurs ont ensuite utilisé une autre sonde : l'acide fuchsine qui est une sonde spécifique aux protéines et présente une excitation maximale à 540 nm. D'autres sondes apolaires telles que le 9,10-diphénylanthracène et le diphénylhexatriène (DPH) ont été également examinées par Herbert (1999) pour marquer les lipides dans les produits laitiers.

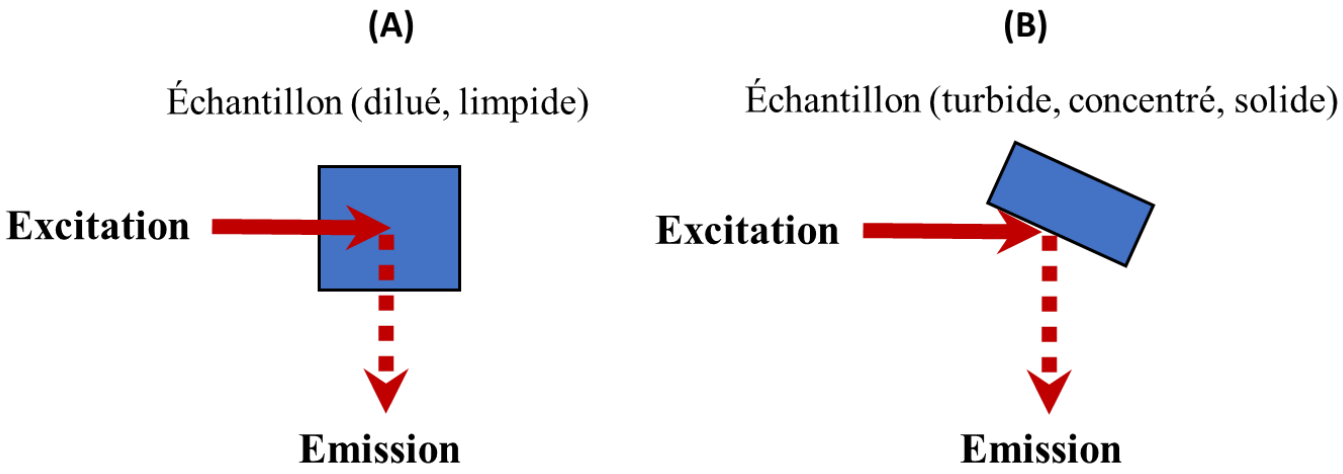

Figure 14 : Dispositifs de fluorescence à angle : droit (A) et frontal (B).

La spectroscopie de fluorescence à angle droit a été appliquée pour des échantillons dilués et limpides (Lakowicz, 1983b). Cette technique ne peut pas être utilisée sur des échantillons turbides comme le lait. En effet, si l'échantillon à analyser présente une absorbance supérieure à 0,1, un effet d'écran se produit induisant une diminution de l'intensité de fluorescence par le phénomène d'extinction (*quenching*) de fluorescence et une distorsion des spectres d'excitation (Genot et al. 1992). De plus, en raison de leur nature physique (solide ou viscoélastique), certains produits laitiers ne peuvent pas être analysés par la spectroscopie à angle droit. Une solution consiste, dans ce cas, à diluer l'échantillon jusqu'à atteindre des absorbances inférieures à 0,1. Par contre, les résultats obtenus sur des solutions diluées ne permettent pas de fournir les mêmes informations contenues dans les échantillons de départ, en particulier, lorsque les informations recherchées concernent les changements de conformation (Genot et al. 1984). Pour pallier cette difficulté, la spectroscopie de fluorescence à angle frontal a été développée pour étudier des échantillons en poudres, turbides et concentrés (Genot et al. 1992). Dans ce cas, seule la surface de l'échantillon est examinée (**Figure 14**). Les photons émis sont collectés sous un angle variant de 30 à 60 ° par rapport à l'échantillon afin de minimiser la collection de photons réfléchis.

5. Analyse multidimensionnelle des données

La chimiométrie est définie comme la science, qui repose sur l'application des méthodes mathématiques et statistiques pour traiter les données acquises sur un produit de façon optimale, et extraire le maximum d'information contenue dans le tableau de données. Le développement des méthodes analytiques, en particulier, les techniques spectroscopiques, est intimement lié au progrès des outils chimiométriques. En effet, les spectres enregistrés sur un produit alimentaire présentent généralement de faibles différences à l'œil nu. Il est donc nécessaire d'appliquer des méthodes chimiométriques pour traiter ces tableaux de données. Cette démarche a été utilisée avec succès dans le domaine de la spectroscopie PIR depuis maintenant plusieurs années.

Les méthodes statistiques (**Figure 15**) sont divisées en deux groupes : méthodes descriptives telles que l'ACP, l'ACC (Analyse Canonique des Corrélations), l'ACCPS (Analyse en Composantes Communes et Poids Spécifiques) et le PARAFAC (PARAllel FACtor analysis) et des méthodes prédictives comme l'AFD (Analyse Factorielle Discriminante) et la méthode des moindres carrés partiels (PLS).

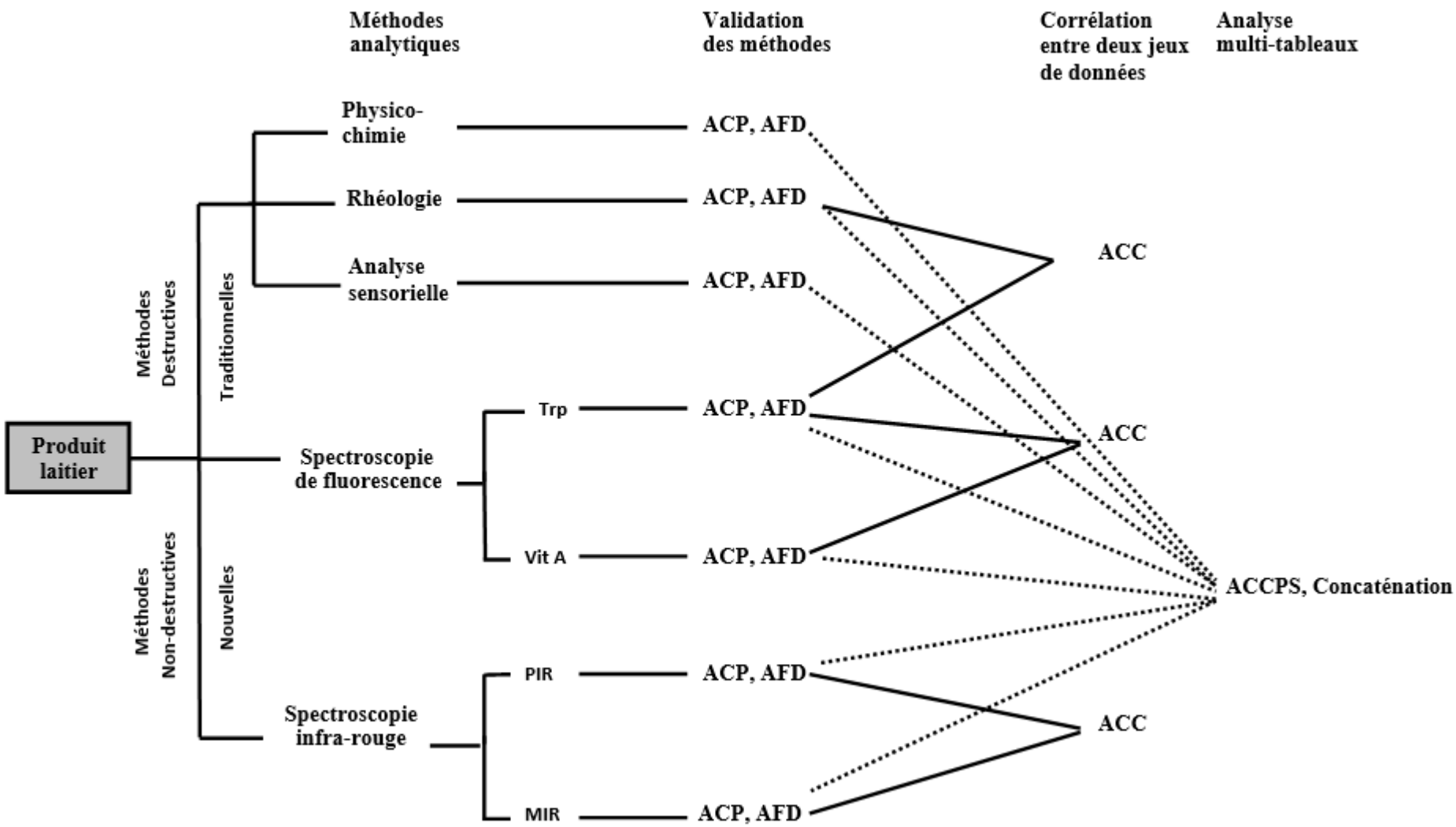

Figure 15 : Évaluation des tableaux de données enregistrés sur un produit laitier à l'aide des outils chimiométriques.

5.1. Méthodes descriptives

5.1.1. Analyse en Composantes Principales

Quand le nombre de données à analyser devient important, un examen direct est difficile. Avant de commencer une étude quantitative, il faut observer les données spectrales pour apprécier leur structure et détecter, par exemple, la présence d'un spectre aberrant (Roggo, 2003).

L'ACP est particulièrement bien adaptée à l'étude exploratoire des données spectrales. Elle calcule de nouvelles variables, appelées composantes principales (CPs) qui sont des combinaisons linéaires des variables de départ. Ces CPs sont mutuellement orthogonales. Puisque l'objectif de l'ACP est la simplification, il faut choisir la dimension de l'espace de représentation en effectuant un compromis entre deux objectifs : prendre un espace de faibles dimensions et conserver une variance expliquée maximale. La première composante choisie doit donc être la combinaison linéaire des variables d'origine décrivant la droite d'allongement maximum du nuage. Les autres composantes doivent respecter les règles suivantes :

- être orthogonales aux composantes précédentes.
- respecter la règle de description de l'allongement maximum.

L'ACP projette les données spectrales dans un espace de représentation de faibles dimensions (Danzart, 1990). Lorsque les données de départ sont des spectres de fluorescence ou d'infrarouge, les vecteurs propres associés aux CPs apparaissent comme analogues aux spectres de produits purs, et les coordonnées factorielles sont analogues à des concentrations (Bertrand et al. 2006).

5.1.2. Analyse Canonique des Corrélations

L'ACC est une méthode statistique descriptive multidimensionnelle qui présente des analogies à la fois avec l'ACP, pour la construction et l'interprétation de graphiques, et avec la régression linéaire, pour la nature des données.

L'ACC permet d'identifier et de quantifier les relations linéaires pouvant exister entre deux groupes de variables quantitatives enregistrées sur les mêmes échantillons. De façon précise, on cherche la corrélation maximale entre une combinaison linéaire choisie du premier ensemble des variables et une combinaison linéaire sélectionnée du deuxième ensemble des variables (Bouroche et Saporta, 1998). Les couples des variables suffisamment corrélés peuvent alors être identifiés avec des combinaisons linéaires et sont appelés les variables canoniques

(Devaux et al. 1993). Si le 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). L'objectif de l'ACC est de déterminer des combinaisons linéaires des variables pour chacun des groupes, de façon à ce que les variables canoniques du premier groupe soient les plus corrélées possibles 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 de données doivent être orthogonales entre elles. Des cartes de ressemblance des individus peuvent être également tracées pour l'ensemble de données. Ces cartes sont sensiblement identiques lorsque les coefficients de corrélation canonique sont élevés.

En pratique, l'application directe de l'analyse canonique aux spectres infrarouge et / ou de fluorescence engendre des artéfacts du fait des redondances importantes existant entre les variables spectrales. Dans ce cas, une solution consiste à appliquer l'ACP sur chaque tableau de données 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).

En utilisant l'ACC, Karoui et Dufour (2003) ont trouvé une très bonne corrélation (R^2 = 0,98) entre les données rhéologiques et celles issues de la vitamine A et du tryptophane. En outre, l'ACC a été également utilisée pour étudier les modifications des protéines au cours de l'affinage de fromages à pâte pressée en analysant conjointement les spectres du MIR et de fluorescence (Dufour et al. 2000 ; Mazerolles et al. 2001).

5.1.3. Analyse en Composantes Communes et Poids Spécifiques

L'ACCPS est une méthode qui permet de traiter simultanément des tableaux multiples appariés par lignes. Elle stipule l'existence de composantes communes pour tous les tableaux mais les poids de ceux-ci pour chacune des composantes peuvent être différents. Cette méthode a été introduite pour analyser des tableaux dans le cadre de l'évaluation sensorielle (Qannari et al. 2000). L'ACCPS consiste à trouver des variables latentes qui décrivent au mieux l'ensemble des tableaux (Qannari et al. 2000). Pour chacune des dimensions extraites, la méthode attribue un poids spécifique à chacun des tableaux. L'ACCPS présente plusieurs intérêts : elle peut

s'appliquer à des tableaux dont le nombre de variables n'est pas identique. La méthode d'attribution du poids spécifique n'impose pas de «consensus» : les tableaux qui contiennent une information différente de celle des autres restent représentés. Cette caractéristique est intéressante lorsqu'on souhaite étudier, par différentes techniques analytiques, des matrices alimentaires, comme les produits laitiers.

Pour chaque composante commune, des profils spectraux (infrarouge et fluorescence) et / ou des tableaux de corrélation (physico-chimie, rhéologie, etc.) sont obtenus. Cette méthode a été appliquée avec succès pour suivre les modifications des protéines au cours de l'affinage de fromages expérimentaux de type pâte pressée en utilisant simultanément les données de spectroscopie infrarouge et de fluorescence (Mazerolles et al. 2002). Karoui et al. (2006b) ont appliqué la méthode ACCPS sur les données de fluorescence et physico-chimiques pour suivre au cours de l'affinage les modifications structurales d'un fromage à pâte pressée non cuite type Raclette. Les mêmes auteurs ont appliqué avec succès l'ACCPS sur des fromages à pâte molle (Karoui et al. 2007b) et des fromages Emmental de différentes origines géographiques (Botosoa et Karoui, 2013), puisqu'une bonne discrimination des échantillons en fonction du procédé de fabrication, de l'origine géographique et de la zone d'échantillonnage (surface et centre pour les fromages à pâte molle) a été obtenue.

5.2. Méthodes prédictives

Les méthodes prédictives (discrimination et régression) utilisées pour construire une équation d'étalonnage à partir de données spectrales sont nombreuses. Elles peuvent être classées en méthodes de discrimination et méthodes de régression.

5.2.1. Analyse Factorielle Discriminante

L'AFD est l'une des méthodes de discrimination qui a pour but de mettre en relation une variable qualitative indiquant l'appartenance d'un individu ou un groupe qualitatif (Vigneau et al. 2006). Les deux objectifs de l'AFD sont de séparer au mieux les groupes d'individus identifiés au préalable à l'aide des variables quantitatives et d'affecter de nouvelles observations aux groupes dont elles sont les plus proches. L'approche de cette méthode consiste à choisir les axes factoriels qui séparent au mieux les centres de gravité des groupes et de projeter les points correspondant aux observations d'un groupe donné de telle sorte que ces projections soient les plus concentrées possible autour de leur centre de gravité afin d'éviter le recouvrement des groupes après projection.

Les facteurs discriminants, qui sont des combinaisons linéaires des variables d'origine, doivent restituer au mieux l'inertie du nuage de points formé par les centres de gravité des groupes (Lebart et al. 1977). Les facteurs discriminants sont calculés de telle façon que :

- la variance intragroupe soit minimale ;
- la variance intergroupe soit maximale.

Typiquement, on fait appel en analyse discriminante à un jeu de données d'apprentissage (étalonnage) qui sert de référence pour construire la règle d'affectation, auquel est adjoint un jeu de validation qui sert à vérifier la pertinence du modèle de discrimination (Vigneau et al. 2006).

Dans le cas de données spectrales, les variables étant très redondantes, l'inversion de la matrice de variance-covariance, nécessaire pour définir les fonctions linéaires discriminantes, est généralement impossible. Une solution consiste à remplacer, dans l'AFD, les données spectrales par les CPs, résultantes de l'application de l'ACP sur les données spectroscopiques, associées à des valeurs propres non nulles (Devaux et al. 1988). Mais l'utilisation de CPs comme variables de départ pour l'AFD pose le problème de choix de leur nombre à introduire dans le modèle. En choisissant trop peu de CPs, il y a un risque de ne pas prendre en compte des informations spectrales utiles à la discrimination. A l'inverse, en considérant trop de CPs, les dernières seront source d'instabilité du modèle préétabli. Une solution consiste à choisir les composantes par une procédure pas à pas (Devaux et al. 1988).

Karoui et al. (2004, 2005, 2011) ont utilisé l'AFD pour discriminer : i) des fromages Emmental fabriqués pendant les périodes estivale et hivernale en provenance de divers pays européens (Allemagne, Autriche, Danemark, Finlande, France, Suisse) ; et ii) du lait de brebis en fonction du système d'alimentation (soja, féverole).

5.2.2. Régression des moindres carrées partiels

La régression PLS est actuellement la méthode la plus connue et la plus utilisée dans de nombreux domaines comme la bio-informatique, l'agroalimentaire, la médecine, etc. (Rosipal et Krämer, 2006 ; Vigneau et al. 2006). Elle a été introduite par Wold (1966) et a fait l'objet de nombreuses adaptations et développement.

Comme pour l'AFD, 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 dans l'ancienne base. La PLS construit les 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 selon les directions les plus pertinentes en termes de prédiction des variables Y (Geladi et Kowalski, 1986). La régression sur la matrice des variables latentes est utilisée pour construire l'équation de prédiction.

Il existe de nombreuses versions d'algorithmes de régression PLS. Elles diffèrent au niveau des normalisations et des calculs intermédiaires, mais elles aboutissent toutes à la même régression. Parmi ces versions, on peut citer, par exemple, l'algorithme NIPALS initialement proposé par Sjöström et al. (1983) et l'algorithme SIMPLS initialisé par De Jong (1993). S'il s'agit d'une seule variable Y à prédire, on parle de régression PLS univariée (PLS1), alors que la régression PLS multivariée (PLS2) est appliquée lorsque plusieurs variables Y sont à prédire (Vigneau et al. 2006).

Dans le domaine des produits laitiers, la régression PLS a été appliquée pour prédire le temps d'affinage de 63 fromages commerciaux du type Mondego, et ce à partir de trois paramètres physico-chimiques (pH, activité de l'eau et teneur en matière sèche). Les résultats obtenus étaient encourageants puisque l'erreur moyenne de prédiction était approximativement de 12 jours (Poveda et al. 2004). Dans une autre étude, Frister et al. (2000) ont employé la même approche statistique pour corréler les paramètres chimiques (peptides, caséines et produits de dégradation de la caséine) avec le goût amer de fromages Cheddar âgés de 6 mois. Les résultats obtenus ont montré que les peptides et les produits de dégradation des caséines issus de la protéolyse des caséines α et β sont corrélés positivement avec l'amertume.

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Review

Analytical methods coupled with chemometric tools for determining the authenticity and detecting the adulteration of dairy products: A review

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ABSTRACT

Authenticity of dairy products has become an urgent issue for producers, researchers, governments, consumers and so on due to the increase of falsification procedures inducing lost large of money as well as the confidence of consumers. The determination of the authenticity and the detection of adulteration of milk and dairy products have been determined by several analytical techniques (e.g., physico-chemical, sensory, chromatography, and so on). Although these methods are considered as the reference ones, they required sophisticated analytical equipment's and skilled operators; they are also time consuming and need both the purchase and disposal of chemical reagents. Therefore, there is a need to find cheap and fast methods for the determination of the authenticity and the detection of adulteration of these products. Thus, spectroscopic techniques such as fluorescence spectroscopy, near infrared (NIR), mid infrared (MIR), nuclear magnetic resonance (NMR), among others, in combination with multivariate data analysis methods could be considered helpful tools in this domain. The advantages and disadvantages of each technique will be also discussed in this review.

1. Introduction

Food adulteration has been practiced since a long time ago and becomes increasingly in the last years more sophisticated. Foods and ingredients presenting high-value are the most vulnerable for adulteration (Karoui & De Baerdemaeker, 2007). Determination of food authenticity and detection of adulteration have become an important question in quality control and safety of food. Indeed, consumer awareness has increased about food quality and safety, geographical origin and agricultural practices, mainly after the spread of foodborne diseases around the world (Gonzálvez & de la Guardia, 2013). Non-authentic food products arising from the adulteration and fraud. The replacement of original substance partially or completely with more easily available and cheap substance is the most common procedure performed by defrauders such as the addition of: i) flavors/aromas to improve the value of cheap products; ii) and/or cheap substances to the food products (Hrbek, Vaclavik, Elich, & Hajslova, 2014; Van Leeuwen, Prenzler, Ryan, & Camin, 2014). Indeed, milks could be adulterated by several ways such as mix different types of milk, whey, neutralizing to mask acidity, melamine, salt or sugar to mask extra water or high solid contents, among others (Kartheek, Smith, Muthu, & Manavalan, 2011). For example, Souza et al. (2011) reported that commercial ultra-high temperature milks (UHT) available in the Brazilian market presented at least one adulterant, such as starch, chlorine, formalin, hydrogen peroxide, urine, etc. Other fraudulent means can be used such as the excessive addition of water into milk resulting in the decrease of nutritional quality of the dairy product, or addition of non-milk fat/oil into dairy products (Santos & Pereia-Filho, 2013). The authenticity of dairy products can be also related to their geographical origin and processing technology (Hrbek et al., 2014). Another aspect linked to the authenticity of milk and dairy products concern the need to avoid labeling of conventional milk as a product from organic farming (Molkentin & Giesemann, 2007).

Milk and dairy products are essential foods for human food, where they are considered very important for some consumer groups including children, pregnant women and elderly due to their high nutritional values (Souza et al., 2011). Indeed, milk is one of the seven (7) top foods that could be adulterated (Moore, Spink, & Lipp, 2012). The percentage of adulteration of milk in world varied according to the country. For example, in India, more than

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60% of milk is unsafe and adulterated with paint, detergent (Food Safety and Standards Authority of India, 2012), where as in Brasil, it is around 10% (Brazilian Institute of Geography and Statistics, 2012). This fact has been widely registered as the Global 'melamine scandal', broken out in 2008 in China, and was related with serious health risks for consumers (Chan, Griffiths, & Chan, 2008; European Food Safety Authority, 2010) due to the use of chemical products to adulterate protein levels in diluted milk. In China, these adulterated products resulted in illness of 294,000 individuals, hospitalization of 50,000, and death of 6 children's (Domingo, Tirelli, Nunes, Guerreiro, & Pinto, 2014).

Nowadays, the determination of the authenticity and the detection of adulteration of dairy products is a major concern in order to: i) assure the traceability system from milk to fork; and ii) ensure that dairy products are correctly labeled in terms of which animals are actually processed for consumption. In order to protect consumers from toxic and harmful compounds in milk and dairy products such as antimicrobials, mycotoxins, residues of cleaners and sanitizers, pesticides residues, heavy metals and so on, requirement for the dairy products traceability has been imposed in developed countries (Jooste, Anelich, & Motarjemi, 2014). Indeed, the traceability system represent a helpful tool in the domain of food safety, where it could effectively trace quality and decrease information asymmetry problems of adverse selection and moral risk in the food system (Peres, Barlet, Loiseau, & Montet, 2007; Zhang, Zhang, Dediu, & Victorn, 2011). Although relevant authenticity issues of dairy products include species, feeding, and farming system, other factors such as processing conditions, packaging, and so on could be considered.

Determination of the authenticity and adulteration detection of dairy products has been performed by using several analytical methods. The physico-chemical methods are the most used ones for authenticating dairy products (Karoui & De Baerdemaeker, 2007; Souza et al., 2011). Although heavy, these techniques are considered among the most interesting ones for the authentication and adulteration purposes. With regard to sensory methods, they have been utilized to determine some attributes (e.g., leakage of whey for appearance, butter or milk flavor, butter taste and rubbery texture in Coalho cheeses) as pointed out by Cavalcante et al. (2007). Other techniques namely liquid and gas chromatography, isotope ratio analysis, and DNA based method, have also been found extremely helpful in dealing with the problems related to adulteration, and authentication of dairy products (Karoui & De Baerdemaeker, 2007; Sharma, Rajput, Poonam, Dogra, & Tomar, 2009; Pajor, Galló1, Steiber, Tasi, & Póti, 2009). As for the physico-chemical methods, liquid and gas chromatographic techniques, which are considered as official ones, for detecting harmful substances are time consuming, high cost, labor intensive and need complex sample pretreatment procedures (Lin, 2009). The isotope ratio analysis and DNA based methods presented also the same drawbacks. For all these reasons, there is a need to develop rapid, inexpensive and efficient analytical methods for the detection of frauds and authentication of milk and dairy products. Recently, more attention has been paid to the development of non-invasive and non-destructive techniques such as infrared, fluorescence, nuclear magnetic resonance (NMR), and so on. These techniques are fast, of relatively low cost, environmentally friendly, and provide a great deal of information with only one test, making them suitable for on-line and/or at-line process control. In addition, spectroscopic techniques often require little or no sample preparation and are relatively easy to operate. Although the advantages of these new analytical techniques, it is sometimes difficult to determine the authenticity and to detect the adulteration of milk and dairy products with high accuracy. This could be explained by the fact that the composition of milk may be related with other factors such as: i) influence of the environment as climate and season; ii) individual differences between the animals; iii) lactation stage, and so on. These factors would have significant impacts on the authenticity of milk and dairy products, and thus might contribute to a difficult authentication of these products.

This review paper will provide a comprehensive overview of the applications of different analytical techniques, in combination with multivariate data analysis, to determine the authenticity and to detect the adulteration of dairy products during the last 7 years. Actual examples illustrating the utilization of these techniques in both laboratory and industrial environments will be discussed as well as their advantages and disadvantages.

2. Chemometric tools

Chemometric can be defined as the science, which based on the application of mathematical and statistical methods in order to process data acquired on a food product in an optimal way. These techniques have proven their abilities as useful tools for the determination of the authenticity and/or the detection of adulteration; indeed, these chemometric tools could be applied when it's not possible to attain the results through the analysis of one single food property but it needs the generation of multivariate data sets (i.e., analysis of more than one sample property) as pointed out by Capuano, Rademaker, van den Bijgaart, & van Ruth, (2014). The multivariate analysis can be applied aims to detect specific hallmarks or fingerprints in a multivariate dataset that might be used to infer about the authentic nature of a food product. . The chemometric tools allow optimal application of the analytical methods, in particular, spectroscopic ones, through the extraction and interpretation of valuable information from large and complex data sets, identification of patterns in the data and development of calibration models in many analytical fields (Souza et al., 2011). The multivariate approach has proven beneficial in analysis of nonselective signals and specifically with respect to handling of interferents and as a diagnostic tool for the detection of deviating samples or outliers (Izenman, 2009). These powerful methods and the computer technology necessary to use them have only become readily available in recent years; their application has become a significant feature for the analytical techniques used to evaluate the quality of dairy products. A large number of scientific studies have been published over the last years wherein chemometrics has been applied to data generated from a variety of analytical methodologies (chromatography, spectroscopy, mass spectrometry, calorimetry, wet chemistry) for the authentication of dairy products (Capuano et al., 2014). A broad range of chemometric tools is now available including data reduction tools, regression techniques, and classification methods (Roggo et al., 2007).

The best-known and most widely used variable-reduction method is the principal component analysis (PCA). PCA is a mathematical procedure, which decomposes the data matrix with n rows (samples) and p columns (variables) into the product of a scores matrix. The scores are the position of the samples in the space of the principal components (PCs) while the loadings are the contributions of the original variables to the PCs. All the PCs are mutually orthogonal, and each successive PC contains less of the total variability of the initial data set.

Another variable-reduction method used to present the data to emphasize the natural groupings in the data set is hierarchical cluster analysis (HCA). The HCA approach involves the assessment of similarity between the samples based on their measured properties (variables). The samples are grouped in clusters in terms of their nearness in the multidimensional space, and the results are presented in the form of dendrograms to facilitate the visualization of the sample relationships (Almeida, Barbosa, Pais, & Formosinho,

2007). Similar data reduction approaches including canonical correlation analysis (CCA) and common components and specific weights analysis (CCSWA) could be used.

Exploratory techniques for data analysis, such as PCA, are unsupervised methods, meaning that they just show the data as they are. Conversely, supervised chemometric methods such as discriminant analysis look to determine features within data, explicitly oriented to address particular issues (Roggo et al., 2007). A wide variety of discriminant analysis has been investigated for this purpose including linear discriminant analysis (LDA), factorial discriminate analysis (FDA), partial least squares discriminant analysis (PLS-DA), quadratic discriminant analysis (QDA), and so on.

The regression techniques such as partial least squares regression (PLSR) has been used in order to find the fundamental relations that explain a significant proportion of the information content between two matrices (X and Y), which allows to overcome the problems of over-fitting, multicollinearity and outliers. This method is considered as a helpful tool to build predictive models when there are many extremely collinear factors (Abdi, 2007). Classification methods are used to separate and classify new data points by constructing predictive models allowing them to be used as a useful tool for non-targeted analysis of food adulterations, and answer the question of whether a new sample can be accepted or rejected by a predefined class (Forina, Oliveri, Lanteri, & Casale, 2008; Brereton, 2011; Xu, Shi, Cai, Zhong, & Tu, 2015).

In the case of complex data represented in various formats (databases, texts, images, sounds, video, and so on) chemometric methods such as PCA, FDA, PLSR and so on failed to produce robust and efficient predictive models with these multiform data. To remedy with this, several methods based on the concepts of statistical learning theory, including support vector machine (SVMs), artificial neural network (ANN), probabilistic neural networks (PNN), bayesian belief networks (BBN), etc. have been proposed.

3. Traditional methods used for the determination of the authenticity and detection of adulteration of dairy products

3.1. Physico-chemical analysis

The physico-chemical properties of dairy products are considered as an important tool to determine the authenticity and the detection of adulteration of dairy products (Hettinga, van Valenberg, & van Hooijdonk, 2008). Indeed, there are several factors, which might modify of the detection of adulteration of dairy products such as the milk zone production, and production system characteristics including methods of feeding, milking and cooling, microbial contamination (Tsioulpas, Grandison, & Lewis, 2007; Gabbi et al., 2013).

In this context, the effect of different feeding systems (i.e., soybean meal, scotch bean) on physico-chemical characteristics of ewe's milk samples during 10 weeks lactation period have been studied by Rouissi, Dridi, Kammoun, De Baerdemaeker, and Karoui (2008). The authors showed that the diets given to the herd affected only the lactose and fat contents. Indeed, the replacement of soybean meal by scotch bean induced a significant decrease and increase of fat and lactose levels, respectively. The obtained results were in agreement with those of Maâmouri et al. (2008) who stated that the inclusion of scotch bean in the diet of ewes resulted in a significant decrease ($P \leq 0.05$) of fat content, and a significant increase of lactose level. These results were confirmed, later, by Hammami et al. (2010) who pointed out no significant difference ($P \geq 0.5$) between all the compositional parameters (pH, protein, ash and freezing point) except fat and lactose values of ewes milk under the three feeding systems used (ewes fed ad libitum with three iso-energetic diets: corn (control), soybean rich and scotch bean rich-diets). Hilali et al. (2011) stated that milk fat content of ewes was higher in diets containing urea-treated straw in comparison with control diet containing barley, wheat bran, and barley straw. A summary overview of physico-chemical methods used for determining the authenticity and the detection of adulteration of dairy products is given in Table 1.

In a different approach, Souza et al. (2011) pointed out that physico-chemical parameters coupled with chemometric tools such as PCA and HCA succeeded to determine the authenticity and adulteration of Brazilian UHT milk, in agreement with the findings of Awan et al. (2014) who achieved to detect the presence of various chemical adulterants (i.e., formalin, cane sugar, starch, glucose, alkalinity and benzoic acid) in different types of milk based on their chemical composition. Gomes et al. (2013) have determined the physicochemical characteristics of fermented dairy beverages made with mixtures of milk and whey from goats, cows, and guava jelly. One of the main conclusions reached by the authors was that the level of fat beverages made with goat's milk was higher ($P < 0.05$) than those produced with cow's milk and the goat's and cow's milk mixture. However, total solid and protein values of cow's milk beverages were higher ($P < 0.05$) than those observed with mixture of the two milks and goat's milk beverages.

Regarding studies on cheeses, Kędzierska-Matysek, Florek, Skałecki, Litwińczuk, and Chruścicki (2014) have compared physicochemical characteristics of two traditional cheeses produced in two different polish regions: Protected Designations of Origin (PDO) Oscypek cheeses produced with a mixture of sheep's and cow's milk, and native traditional Gazdowski cheeses made with cow's milk. The authors pointed out that PDO Oscypek cheeses contained significant higher total solids ($P < 0.01$), protein in dry matter ($P < 0.05$) and lower salt content ($P < 0.05$) than the native traditional Gazdowski cheeses. The authors concluded that the authenticity of PDO Oscypek cheeses from other types of cheeses could be determined based on physico-chemical parameters. The above-mentioned findings were in agreement with those of Dervisoglu and Aydemir (2007) who succeeded by using the physico-chemical properties to distinguish between Kulek cheeses made from raw and heated milk. Indeed, titratable acidity was found to be higher in cheeses made from heated milk while total solid, salt, and fat contents were higher in cheeses made from raw milk.

In another study, Okpala, Piggott, and Schaschke (2010) have determined the impact of high-pressure on the physico-chemical properties of fresh soft cheeses. The authors stated that protein contents in treated cheeses were lower than the non-treated one ($p < 0.001$); the pH value of cheeses increased with high pressure, in agreement with the findings of Rynne et al. (2008) who pointed out that high-pressure-treated to cheeses increased pH values in comparison with control ones.

Although the physico-chemical analyses are considered as reference methods providing accurate and reliable results on the authenticity of dairy products, they are time consuming and need a lot of pollutant reagents, relatively expensive, cannot be utilized at/on-line, and involve the use of chemicals and trained labor. Additionally, the physico-chemical characteristics of milk can be changed according to animal breeds, feed and lactation period, where these factors play an important role in influencing on the composition of milk.

3.2. Liquid chromatographic techniques

High-performance liquid chromatography (HPLC) is one of the analytical techniques used to separate, identify and quantify the components in a mixture. Components may be separated by sending the sample mixture into the flow of mobile phase, which

Table 1
A summary overview of physico-chemical methods used for determining the authenticity and detecting the adulteration of dairy products.

Dairy product	Objective	Principle parameter	Main result	Reference
Milk	Effects of different feeding systems on physico-chemical properties of ewes' milk.	pH, density, non-fat in dry matter, fat, protein, lactose, and ash.	- No significant difference of the feeding systems on the pH, density, and protein. - Replacement of soybean meal with scotch bean caused a significant decrease of fat content (7.85 vs. 6.75 g/100 g) and a significant increase in lactose levels (3.49 vs. 3.61 g/100 g). - Milk fat content was higher in diets containing urea-treated straw than that containing barley, wheat bran, and barley straw.	Rouissi et al., 2008; Maàmouri et al., 2008; Hammami et al., 2010; Hilali et al., 2011.
	Detection of adulteration of milk.	Physico-chemical compositions.	- PCA and HCA applied to the physico-chemical parameters allowed to authenticate and detect adulteration of UHT milk.	Souza et al., 2011; Awan et al., 2014.
Fermented dairy beverages	Comparison of physico-chemical parameters of goat's and cow's milk.	Fat, protein, and total solids.	- Fat content of beverages made of goat's milk was higher than that produced with cow's milk. - Total solids and protein values of cow's milk were significantly higher than those observed in goat's milk.	Gomes et al., 2013.
Cheese	Identification of cheeses.	Total solids, protein in dry matter, salt, titratable acidity.	- PDO Oscypek cheeses contained more significant total solids, protein in dry matter and less salt than the native traditional Gazdowski cheeses. - Titratable acidity was higher in Kulek cheeses made from heated milk, while total solids, salt, and fat were higher in cheeses produced with raw milk.	Kędzierska-Matysek et al., 2014; Dervisoglu & Aydemir, 2007.
	Effect of high-pressure.	pH, protein.	- Protein content in treated cheeses was lower than that in the non-treated ones. - pH values of cheese increased with high-pressure.	Okpala et al., 2010; Rynne et al., 2008.

HCA; hierarchical cluster analysis, PCA; principal component analysis, PDO; protected designation of origin.

pass through the column. The various components travel through the column at different velocities and interact with the sorbent (called stationary phase). Dependent of the stationary phase type, components might be separated based on its chemical nature such as their molecular mass, charge, and so on. Several detectors could be used in HPLC such as ultraviolet–visible (UV–vis), fluorescence, electrochemical, diffractometer, among others (Luykx & van Ruth, 2008).

HPLC method is used in food analysis for quantitative analyses of compounds present in food but also to verify the quality of product with the increased productivity (Nollet & Toldra, 2012). For milk and dairy products, HPLC methods could be considered as useful tools to perform several measurements such as: i) dairy product components; ii) levels of additive used; and iii) environmental pollutants and xenobiotics (Table 2). Indeed, HPLC coupled to mass spectrometry (MS) has been used to: i) identify and quantify some milk compounds such as vitamins, lactose, protein and so on (Plozza, Trenerry, & Caridi, 2012; Fusch, Choi, Rochow, & Fusch, 2011; Feligini, Bonizzi, Buffoni, Cosenza, & Ramunno, 2009; Cunsolo, Muccilli, Saletti, & Foti, 2011); ii) determine the authenticity and traceability of milk (Picariello, Mamone, Addeo, & Ferranti, 2012; Losito, Carbonara, Monaci, & Palmisano, 2007); and detect adulterated dairy products (Müller et al., 2008).

In this context, Sharma et al. (2009) have used HPLC technique for the detection of stachyose peak (sugar from soymilk) in milk samples for up to 5%. These findings were confirmed by Cordewener et al. (2009) and Russo et al. (2012) who succeeded respectively to: i) discriminate between non adulterated skim milk powder from those adulterated with soy and pea protein by using LC–MS method; and ii) detect buffalo mozzarella adulteration with bovine milk by using ultra (U)HPLC–MS. The authors stated that the high sensitivity of this technique provide a useful tool for monitoring complex matrices such as dairy products. Recently, Jablonski, Moore, and Harnly (2014) succeeded by using UHPLC–UV to detect skim milk powder adulterated with soy, pea, brown rice, and hydrolyzed wheat protein at higher adulteration levels varying in the 0.5–10% range.

In a similar approach, Filazi, Sireli, Ekici, Can, and Karagoz (2012) have developed RP–HPLC method for the detection of melamine in different dairy products (pasteurized milk, UHT milk, milk powder, powdered infant formula, fruit yogurt, soft cheese). Their results showed that melamine was absent in infant formulas and pasteurized and UHT milk, whereas it exists in the other types of dairy products at different percentages. The authors stated that the detection and quantification limits were sufficient to determine with high accuracy melamine in milk and dairy products under the safety limits recommended by the Codex Alimentarius Commission (i.e., 1 mg/kg for infant formula, 2.5 mg/kg for dairy products). Regarding the absence of melamine in milks, the above-mentioned results were in disagreement with those of He, Liu, Huang, Yang, and Liao (2008) and Salman et al. (2012) who succeeded by using respectively HPLC and RP–HPLC ion pairing chromatography with UV detection to detect melamine in milk and milk powder with detection limits of 1 mg/kg and 0.01 μg/mL, respectively. Recently, Finete, Gouvêa, Marques, and Netto (2015) have developed a new HPLC method coupled with fluorescence detection to determine the melamine content in bovine UHT whole milk with melamine detection and quantification limits of 0.0081 and 0.027 mg/mL, respectively. The obtained results were in agreement with those of Ge, Wu, Wang, Liang, and Sun (2015) who developed a molecularly imprinted solid-phase extraction–ultra-performance liquid chromatography (MISPE–UPLC) to separate and determine cyromazine, ammelide, melamine, and ammeline in milk samples. One of the main conclusions reached by the authors was that MISPE–UPLC method could be considered as a useful tool due to its highly selective and sensitive for monitoring and quality assurance of milk, where the detection limits were of 1.25, 1.25, 2.59, and 6.42 μg/kg, respectively.

In another study, Motta et al. (2014) have employed LC–MC–MC with electrospray ionization to detect the adulteration of milk with whey using casein-macropeptide as a biomarker (a peptide that results from the remained chymosin in whey during the manufacture of cheese). The authors found that LC could be applied to detect the adulteration of milk with whey since it showed satisfactory precision (<11%) with a detection and quantification limit of 1.0 μg/mL and 5.0 μg/mL, respectively.

Regarding studies on cheeses, Rodríguez, Ortiz, Sarabia, and Gredilla (2010) used HPLC with diode-array detection to verify that the type of milks used for cheese making correspond to those appearing in their label. To conduct the research study, the authors used the protein chromatographic profiles of cheese and milk extracts, which correspond to the pure samples of bovine, ovine and caprine milk, and also to binary and ternary mixtures. By applying a series of chemometric tools such as PCA and PLS, the authors succeeded to detect 3.92, 2.81 and 1.47% of ovine, caprine and bovine milk, respectively in cheese manufactured from mixtures of milk. The obtained results were in agreement with those of: i) Czerwenka, Muller, and Lindner (2010) who succeeded by using LC–MS to detect adulteration of buffalo's milk with cow's milk utilizing the β-lactoglobulin as a marker; and ii) Sforza et al. (2008) who reached to differentiate with high accuracy between cheeses produced with cow's and sheep's milk samples. Similar results were obtained on cheese samples during their ripening stage. Indeed, Masotti, Hogenboom, Rosi, De Noni, and Pellegrino (2010) and Sforza, Cavatorta, Galaverna, Dossena, and Marchelli (2009) pointed out that pyroglutamyl-γ3-casein peptide, and *N*-γ-glutamyl- as well as *N*-pyroglutamyl- and *N*-lactoyl-amino acids could be used as indicators for monitoring ripening stage of Grana Padano and Parmigiano–Reggiano cheeses, respectively.

Thanks to HPLC–MS, it is possible to authenticate milk and dairy products on the basis of some specific components. Various molecules (Desirable and undesirable) in milk and dairy products may be identified and quantified. In addition, HPLC is a helpful technique, where it allows simultaneously to measure several compounds in one measurement. Moreover, this technique is an essential tool for reliable certification of authenticity of milk and dairy products. However, LC techniques applied to determine the authenticity and the detection of adulteration of dairy products generally include sample extraction procedures, which are often regarded as bottlenecks in analytical methods. A drawback of the approach certainly is the requirement of a rather expensive and complex LC–MS system.

3.3. Gas chromatographic techniques

Gas chromatographic (GC) is one of the most commonly separation technique used in food research to analyze volatile substances, aromas, and pesticides (Karoui & De Baerdemaeker, 2007). A sample containing different components can be analyzed by GC, where the mixture of components is vaporized without decomposition in a heated chamber. Subsequently, components become separated after the passage of gas mixture through the column as they interact with the coating of the column. The column contains a liquid stationary phase, which is adsorbed onto the surface of a thin fused-silica capillary tube. The transport of sample through the column carried out by a chemically inert gas as helium. Several detectors can be employed in GC such as mass spectrometry (MS), flame ionization detector (FID), electron capture detector (ECD), among others, but MS detector is the most used in food research (Luykx & van Ruth, 2008). GC with different detectors has been

Table 2
A summary overview of chromatographic methods used for determining the authenticity and detecting the adulteration of dairy products.

Dairy product	Objective	Analytical technique	Main result	Reference
Milk	Detection of milk adulteration.	HPLC LC–MS UHPLC–MS UHPLC–UV LC–ESI MS–MS	- Presence of stachyose peak (sugar from soymilk) in milk can be used as effective parameter for milk adulteration with soymilk. - Higher sensibility for the detection of buffalo Mozzarella adulteration with bovine milk. - Good discrimination between non-adulterated skim milk powders from those adulterated with soy, brown rice, pea protein and hydrolyzed wheat protein. - Good detection of milk adulteration with whey since the detection and quantification limit was of 1.0 μg/mL and 5.0 μg/mL, respectively.	Sharma et al., 2009, Russo et al., 2012; Cordewener et al., 2009; Jablonski et al., 2014; Motta et al., 2014.
	Detection of melamine in different types of milk.	RP–HPLC HPLC HPLC coupled with fluorescence detection MISPE–UPLC	- The limits of detection and quantification were sufficient to detect melamine in milk powder, and UHT bovine milk.	Filazi et al., 2012; He et al., 2008; Salman et al., 2012; Finete et al., 2015; Ge et al., 2015.
Cheese	Authentication and detection of adulteration of cheese.	LC–MC HPLC HPLC–DAD with PCA, and PLS	- Discrimination between cheeses produced from cow's and sheep's milk. - Pyroglutamyl-γ3-casein peptide and *N*-γ-glutamyl-, *N*-pyroglutamyl- and *N*-lactoyl-amino acids can be used as markers for monitoring cheese ripening time. - HPLC-DAD allows detecting 3.92, 2.81 and 1.47% of ovine, caprine and bovine milk in cheeses elaborated with mixture of milk.	Sforza et al., 2008; Masotti et al., 2010; Sforza et al., 2009; Rodríguez et al., 2010.
Milk	Authentication of organic milk from conventional milk.	GC–FID GC–MC	- Good discrimination between organic and conventional milks based on fatty acid contents. - Organic milk had significantly higher contents of polyunsaturated fatty acids, conjugated linonenic, branched fatty acids, and α-linolenic acid. - Fatty acid profile of organic milk was significantly different from that of conventional milk. - Phytanic acid content and diastereomer ratio could be used for differentiating organic milk from other types of milk.	Collomb et al., 2008; Molkentin & Giesemann, 2007; Molkentin, 2009; Slots et al., 2008; Butler et al., 2011; Schröder et al., 2012; Capuano et al. (2014)
Milk	Authentication of milk according to the feed, breed, geographical origin, and type of milk.	GC–MS GC–FID	- Good discrimination between milk samples according to the diet, breed, geographical origin based on fatty acids profile. - Unsaturated fatty acids and long-chain fatty acids ≥18 were efficient markers to discriminate milk according to their origin country. - Milks collected from grazing and pasture hay have a richer profile in mono and sesquiterpenes compounds than those collected from the mixed hay group. - Triacylglycerol profile can be used to discriminate of milk according to the feeding system.	Soják et al., 2013; Fedele et al., 2007; Pajor,Galló1, Steiber, Tasi, & Póti, 2009; Capuano et al., 2014; Gaspardo et al., 2010; Poulsen et al., 2012; Palladino et al.2010; Capuano et al., 2014.
Cheese	Determination the authenticity of cheeses.	GC–MC with PCA GC–FID GC–MC	- A clear differentiation between natural Mozzarella cheeses from the imitated ones. - Level of the most common CLA isomer, *cis-9, trans-11*, ranged from 0.74% to 0.83% in cheeses produced in winter and autumn. - Volatile organic compound profile of cheeses was affected by dairy farming system and lactation stage. - Cheeses from cows grazed on Trifolium alpinum-dominated pastures was richer in long-chain fatty acid, unsaturated, monounsaturated, and odd-chain saturated fatty acid, while those from Festuca nigrescens pastures	Kim et al., 2014; Romano et al., 2011; Bergamaschi et al., 2015; Falchero et al., 2010.

			contained more short- and medium-chain, saturated fatty acid, and α-linolenic acid.	
Butter	Detection of the adulteration of butter with non-milk fat.	GC–FID GC–MC	- A large capacity of the detection of foreign fat in bovine milk fat. - The LDA applied to the data sets allowed correct classification of 94.4% of samples adulterated with non-fat milk less than 10%. - The application of MLR to the profiles of triacylglycerols allowed to detect 75.3 and 98.11% of mixtures of milk fat with non-milk fat of vegetable origin and of animal origin with levels 5 and 10% of adulteration. - Analysis of fatty acid composition by GC–MC was not an efficient tool to detect the adulteration of butter with non-milk fat.	Molkentin, 2007; Gutiérrez et al., 2009 Gutiérrez-Tolentino et al., 2007; Derewiaka et al., 2011.

CLA; conjugated linoleic acid isomers, GC–FID; gas chromatography – flame photometric detector, GC–MS; gas chromatography–mass spectrometry, HPLC, high-performance liquid chromatography, HPLC-DAD; high-performance liquid chromatography with diode-array detection, LC–MS; liquid chromatography/mass spectrometry, LC–ESI MS–MS; liquid chromatography coupled to mass spectrometry with electrospray ionization, LDA; linear discriminant analysis, MISPE–UPLC; molecularly imprinted solid-phase extraction–ultra-performance liquid chromatography, MLR; multiple linear regression, PCA; principal component analysis, PLS; partial least squares, RP–HPLC; reversed-phase high-performance liquid chromatography, UHPLC–MS; ultra-high-performance liquid chromatography-mass spectrometry, UHPLC–UV; ultra-high-performance liquid chromatography-ultraviolet.

used to perform qualitative and quantitative analyses (Luykx & van Ruth, 2008) and ii) exploited to determine the authenticity of food by obtaining a fingerprinting chromatography profile (Cajka, 2013).

Authentication of organic milk is consider as a reliable indicator due to its high price and the restriction of natural resources. The selling of conventional milk as organic one is a type of fraud and thus it is necessary to adopt all necessary procedures for protecting consumers. In this context, Collomb et al. (2008) have attempted to quantify fatty acid compositions of organic and conventional milks (from integrated farming), by using high-resolution GC–FID. Their research showed that organic and conventional milks did not significantly differ with respect to saturated fatty acid; however, organic milk samples had significantly higher contents of poly-unsaturated fatty acids, conjugated linonenic acid and branched fatty acids. These results were in agreement with those of Molkentin and Giesemann (2007) and Molkentin (2009) who explored the α-linolenic acid content of milk fat as marker for the authentication of organic retail milk. Their results showed that organic milk contains high levels of α-linolenic acid confirming those of Slots, Sorensen, and Nielsen (2008) and Butler, Stergiadis, Seal, Eyre, and Leifert (2011) who succeeded to discriminate between organic and conventional farm milk based on α-linolenic acid. Recently, Schröder, Lutz, Tangwan, Hajazimi, and Vetter (2012) indicated that phytanic acid content and diastereomer ratio could be used for differentiating organic milks from others since a high phytanic acid level and a low diasteromer ratio were observed in organic milks. However, Capuano, Elgersma, Tres, and van Ruth (2014) pointed out that phytanic acid content could not be considered as a reliable tool to differentiate organic bovine milk from other milks, while diastereomers ratio might be used as indicator of organic farming.

Fatty acid profile obtained by GC has been used to determine the geographical origin of milk (Table 2). In this context, Gaspardo, Lavrenčič, Levart, Del Zotto, and Stefanon (2010) found that unsaturated fatty acids and long-chain fatty acids $\geq$18 can be used as efficient markers for the discrimination of milk samples based on country of origin, in agreement, with the findings of Poulsen et al. (2012) and those of Palladino et al. (2010) who pointed out that the variation of fatty acid compositions in milk can be related with the source of origin and breed. Recently, Capuano, Boerrigter-Eenling, Elgersma, and van Ruth, (2014) indicated that the triacylglycerol profile of milk was different according to the feeding system (fresh grass feeding, pasture grazing and organic/biodynamic farming).

In a similar approach, Soják et al. (2013) succeeded by using GC–MS to determine fatty acid milk contents, in particular, the content of conjugated linoleic acid, oleic acid and 12:0–16:0 saturated fatty acids with regard to variation among individual ewe, breed, parity and milk yield of ewes grazed on natural pasture. The obtained results confirmed the previous findings of Fedele, Pizzillo, Claps, and Cifuni (2007) who stated that the highest mono and sesquiterpenes contents were found in milks gathered from grazing goats, while the lowest one was observed for those collected from mixed hay treatment. Milks collected from grazing and pasture hay showed a richer profile in mono and sesquiterpene compounds than those collected from the mixed hay group, in agreement, with the findings of Pajor et al. (2009) who pointed out that grazing increased the total conjugated linoleic acid content of both milks and cheeses. These differences were attributed mainly to the botanical composition in the two feeding systems regardless of the species (Galina, Osnaya, Cuchillo, & Haenlein, 2007; Samková, Pešek, Špička, Pelikánová, & Hanuš, 2009). This investigation was recently confirmed by Bergamaschi et al. (2015) who found that the volatile organic compound profile of model cheeses was affected by dairy farming system and lactation stage, in agreement with previous findings of Falchero et al. (2010) where variation in cheese fatty acid

composition according to the grazing was observed. Indeed, cheeses from cows fed Trifolium alpinum-dominated pastures were richer in long-chain fatty acid, unsaturated, monounsaturated, and odd-chain saturated fatty acid, while those obtained from Festuca nigrescens pastures were richer in short and medium-chain, saturated fatty acid, and α-linolenic acid.

With regard to Mozzarella cheeses, Kim et al. (2014) applied PCA to the fatty acid profile and phytosterol content data sets and observed a clear differentiation between natural Mozzarella cheeses from the imitated ones. However, the authors failed to discriminate between natural Mozzarella cheeses from the processed ones showing some limit of GC for the differentiation between these two cheese varieties. These findings supported those of Romano, Giordano, Chianese, Addeo, and Musso (2011) who indicated that the level of the most common CLA isomer, *cis*-9, *trans*-11, ranged from 0.74% in winter cheeses to 0.83% in autumn cheeses.

In a different approach, Molkentin (2007) has used GC to detect the adulteration of butter with foreign fat and satisfactory results were obtained. However, some limitations needed to be considered with technologically processed milk fat, fat from low-fat milk products such as skim milk or buttermilk, as well as with milk fat from other species than cows. Gutiérrez et al. (2009) succeeded by applying linear discriminant analysis to detect adulteration of milk fat with non-milk fat (fish, peanut, corn, olive, and soy oils) since correct classification of 94.4% of the samples with levels <10% of adulteration was obtained. These results confirmed previous findings of the same research group (Gutiérrez-Tolentino et al., 2007) who found that application of multiple linear regression to the triacylglycerols profile allowed to detect 75.3 and 98.11% milk mixed with vegetable fat (canola and sunflower oils) from those not mixed with non-milk fat and of animal origin (fish oil, lard and tallow). Whereas, Derewiaka, Sosinska, Obiedzinski, Krogulec, and Czaplicki (2011) pointed out that the analysis of fatty acid composition was not an efficient tool to detect the adulteration of milk fat in butter due to variability of fatty acid composition.

Thanks to its very high sensitivity, versatility, and reproducibility, GC has become one of the most used techniques for the analysis of volatile compounds in dairy products. As LC, a drawback of GC is the requirement of expensive and complex system. In addition, skilled operators are needed to perform and interpret results.

3.4. Isotope ratio mass spectrometer (IRMS)

IRMS is one of the techniques, which used to obtain information about the geographic origins of food. This technique may differentiate between the samples containing identical chemical components based on their isotope content. The isotope ratios of elements most commonly used for the evaluation of food included $^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$ and $^{34}S/^{32}S$ (Luykx & van Ruth, 2008). IRMS method has been used the first time by the European wine industry to authenticate the origin of wine and to detect the adulteration (Crittenden et al., 2007). This technique is more and more used to determine the authenticity of food, particularly when traditional methods cannot give accurate results (Guo, Wei, Pan, & Li, 2010). Indeed, information on the geographical and environmental origin of biological products such as milk and dairy products may be obtained based on stable isotope composition of different elements.

IRMS technique combined with chemometric methods has been used in several studies to determine the authenticity and geographical origin of milk and dairy products (Table 3). In this context, Crittenden et al. (2007) have evaluated the multi-element isotope ratio analysis to determine the origin of milk cows' from

Table 3
A summary overview of isotope ratio mass spectrometer method used for determining the authenticity of dairy products.

Dairy product	Objective	Isotope ratio analysis	Technique	Main results	Reference
Milk	Authentication of milk.	$^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{34}S/^{32}S$, $^{87}Sr/^{86}Sr$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{34}S/^{32}S$, and $^{2}H/^{1}H$.	IRMS	- Good discrimination of milk from different regions using the $\delta^{18}O$ and $\delta^{13}C$. - $\delta^{18}O$ allowed to discriminate milk samples according to the altitude, while it failed to determine the production zone at the scale of the year. - A clear separation of milk samples originating from two alpine sites and from different pasture types. - $\delta^{2}H$ of bulk milk solids would help to authenticate dietary regime and geographical origin. - Good discrimination between organic milks and conventional milks based on $\delta^{13}C$ and $\delta^{15}N$.	Crittenden et al., 2007; Bontempo et al., 2012; Chesson et al., 2010; Scampicchio et al., 2012; Engel et al., 2007; Ehtesham et al., 2015; Chung et al., 2014; Erich et al., 2015.
Cheese	Authentication of cheese.	$\delta^{13}C$, $\delta^{2}H$, $\delta^{15}N$, and $\delta^{34}S$, $\delta^{18}O$.	IRMS	- Discrimination of European hard cheeses according to their geographical origin, diet and cheese making processes. - Good discrimination between different cheese varieties produced in alpine and pre-alpine Italian areas. - Isotopic fingerprint C, N, O, and H, allowed the discrimination of buffalo's cheese originating from the Amazon basin and Brazil. - C and N isotope allowed to trace milk samples along cheese manufacturing.	Camin et al., 2012; Bontempo et al., 2011; Silva et al., 2014; Capici et al., 2015.

IRMS; isotope ratio mass spectrometer.

seven dairying regions in Australia and New Zealand. The authors succeeded to distinguish cows' milk from different regions using the $\delta^{18}O$ isotope ratios of milk, and the $\delta^{13}C$ of skim milk and casein, confirming the isotope fractionation patterns on the basis of latitude and climate. These results were in agreement with those of Bontempo, Lombardi, Paoletti, Ziller, and Camin (2012), Chesson, Valenzuela, O'Grady, Cerling, and Ehleringer (2010) and Scampicchio et al. (2012) who found respectively that: i) the stable isotope ratios of H, C, N and O of milk samples are linked to the terroir since a clear separation of milk samples originating from two alpine sites and from different pasture types within each site was observed; ii) the $\delta^{2}H$ and $\delta^{18}O$ values of milk and cow drinking water were correlated suggesting the potential use of this technique for the determination of the geographical origin of such products; and iii) the isotope ratio of $\delta^{13}C$ and $\delta^{15}N$ can differentiate between milk samples according to both their geographical origin and type of processing, confirming previous findings of Engel et al. (2007) who showed that the $\delta^{18}O$ allowed to distinguish between milk samples according to their altitude, since milk water $\delta^{18}O$ enrichment was higher in lowland (<500 m altitude) than in upland (>700 m altitude). However, the $\delta^{18}O$ values failed to discriminate the production area throughout the year due to the variability of the sampling period.

Recently, Ehtesham, Hayman, Van Hale, and Frew (2015) found significant relationships between the isotopic composition of milk and feed fatty acids. For example, $\delta^{2}H$ values of milk samples were related with values of corresponding fatty acids in feed and $\delta^{2}H$ of farm water. Thus, the measurements of $\delta^{2}H$ of milk could help to authenticate the type of feed and geographical origin, confirming previous findings of the same research group (Ehtesham et al., 2013) who showed that the isotope ratio $\delta^{2}H$ value of milk fatty acids could be considered as a promising tool for the determination of the geographical origin. In a similar approach, Chung, Park, Yoon, Yang, and Kim (2014) have used stable isotope ratio of carbon and nitrogen ($\delta^{13}C$ $\delta^{15}N$) for determining organic milk authenticity. Their results showed that the mean $\delta^{13}C$ and $\delta^{15}N$ values of organic milk were respectively significantly higher and lower than that of conventional milk, in agreement with the findings of Erich et al. (2015).

Regarding studies on cheeses, Camin et al. (2012) pointed out that H, C, N and S stable isotopes of seven types of European hard cheeses were not only related with geographical origin, but also to cow diet and cheese making processes. The use of these stable isotopes allowed to discriminate between the most famous PDO Parmigiano Reggiano cheese originating from 9 European and 2 extra-European imitators. The obtained results confirmed those of Bontempo et al. (2011) who found that casein $\delta^{13}C$ and $\delta^{15}N$ allowed to distinguish cheese samples collected from different Italian regions. These results were, recently, confirmed by: i) Silva et al. (2014) who by using multi-stable isotope analysis succeeded to discriminate buffalo's cheese samples originating from the Amazon basin, Brazil; and ii) Capici, Mimmo, Kerschbaumer, Cesco, and Scampicchio (2015) who pointed out that isotopic ratio is able to trace milk samples along the cheese manufacturing.

IRMS is a helpful tool for determining the authenticity and geographical origin of milk and dairy products. However, this technique has some limits such as: i) the necessity to take into account the impact of environmental factors such as climate, animal breed, and altitude; ii) the isotope values obtained from animals raised in different zones with the same feed can be deceptive and thus do not reflect the reality of product region; and iii) identical isotopic signature could be observed when it is demanded for tracing the milk products obtained from the animals raised by similar feeds and in the zones with similar climate and geological site (Zhao et *al.*, 2014). However, this technique could be considered an alternative tool for the traceability and identification of milk and dairy products.

3.5. DNA based method

DNA based method is a biological technique that has been used to determine the authenticity and the detection of adulteration of food products based on the detection of nucleic acid probes (DNA or RNA) and determination of their sequence (Luykx & van Ruth, 2008; Cawthorn, Steinman, & Witthuhn, 2012). Currently, this technique is widely employed for the verification and confirmation of food authenticity, where it helps government, food processors, consumers to correctly identify food species (Teletchea, 2009; Maralit et al., 2013; Melo Palmeira et al., 2013). Due to their highly sensitivity and reproducibility, this technique become more commonly and the most used in last decades to replace protein based methods (De et al., 2011). In dairy products, milk can be considered as a source of DNA for further DNA based analysis, where it contains a large quantity but highly variable number of somatic cells depending on the physiological status of animal. This technique can represent an ideal alternative to detect the adulteration in dairy products due to the stability of DNA under the different chemical treatments and high temperature (Ganopoulos, Sakaridis, Argiriou, Madesis, & Tsaftaris, 2013). Among the DNA-based techniques, Polymerase chain reaction (PCR) is one of the molecular techniques most commonly used for identifying the species exist in food products, where little quantities of DNA can be detected using species-specific primers (Mafra, Ferreira, & Oliveira, 2008).

This technique has been used in several reports for the identification and authentication of milk origin species and dairy products. In this context, Cottenet, Blancpain, and Golay (2011) have developed a multiplex real-time (RT)-PCR to detect DNA of cow's and buffalo milk in 119 freeze-dried Asian milk samples. The obtained results showed that this technique was able to identify cow's milk in buffalo milk with a detection limit of 1%. One of the main conclusions reached by the authors was that PCR could be used as a reliable tool to differentiate Asian milk samples, in agreement, with the findings of Dalmasso, Civera, La Neve, and Bottero (2011), Dalmasso, Sacchi, and Bottero (2012) and Drummond et al. (2013) who pointed out that RT-PCR method enabling the detection at a level of 2% the presence of cow's milk in buffalo milk and cow's milk in donkey milk. These results confirmed previous findings of López-Calleja et al. (2007a, 2007b) and Zhang, Fowler, Scott, Lawson, and Slater (2007) who succeeded to detect goats' milk in sheep's milk and cow's milk in ewes' milk mixtures. Rodrigues et al. (2012) were able to detect cow's milk in goats' milk at 0.5%, in agreement with Kotowicz, Adamczyk, and Bania (2007) and Abdel-Rahman and Ahmed (2007) who pointed out the ability of PCR restriction fragment length polymorphism to discriminate buffalo, cattle and sheep milk.

Regarding studies on cheese, De et al. (2011) showed that PCR technique allowed to differentiate cheeses made from cow's and buffalo milk even at a level of 0.1%. The obtained results were in agreement with those of Mafra, Roxo, Ferreira, and Oliveira (2007), Feligini, Alim, Bonizzi, Enne, and Aleandri (2007) and Lopparelli, Cardazzo, Balzan, Giaccone, and Novelli (2007) who, respectively, pointed out that: i) the application of a duplex PCR permitted to detect 0.1% of cows' milk in cheese made from goats' milk allowing to quantify cheese adulteration with cows' milk in the 1–60% range; ii) RT-PCR amplifications method was able to detect the fraudulent addition of cow's milk in water buffalo Mozzarella cheese; and iii) RT Taqman-PCR method can be used for the detection of bovine milk in Mozzarella cheese. The obtained results were, recently, confirmed by: i) Gonçalves, Pereira, Amorim, and

van Asch (2012) who found that the use multiplex PCR could be used to simultaneous detection of cow's, sheep's, goat's, and buffalo milk in different types of dairy products (like standard cheese, PDO cheese, cottage cheese, powdered milk, UHT milk, fresh milk, yogurt, cream, and butter); and ii) Ganopoulos et al. (2013) who have used RT-PCR coupled with high resolution melting (HRM) to detect bovine, ovine and caprine species and authenticate Greek PDO Feta cheese. Their results showed that HRM method could be considered as effective and accurate method for detecting bovine milk present in putative Feta at 0.1% and quantifying the ratio of goat's to sheep's milk mixture in different Feta cheese commercial products.

The main advantages of DNA based method are rapid, sensitive, robust, and relatively simple to amplify DNA. However, it is important to know the exact sequences, which flank both ends of the target DNA region (Luykx & van Ruth, 2008). Additionally, reactions crossed may be bringing when DNA fragments of similar species within a sample were used, and lower amplification is obtained from DNA more fragmented. Therefore, special care is necessary during the sample treatment and before to DNA amplification since it may change the final results.

3.6. Sensory analysis

The sensory analysis of dairy products is a scientific discipline, which measure, explain and comprehend human reactions regarding the characteristics of product by discerning the five senses: smell, sight, taste, touch and sound. These analyses often carried out by trained panelists (Bodyfelt, Drake, & Rankin, 2008). In sensory evaluation, the analyses included the detection and characterization of both the qualitative and quantitative sensory components of products considered among the most tools developed in the arsenal of the sensory scientist (Varela & Ares, 2012). The properties of a product such as aroma, appearance, flavor, texture, aftertaste and sound are the qualitative aspects, which distinguish it from others. Several of the published research papers about milk and dairy products discussed the potential detection of adulteration of dairy products on the basis of their sensory properties. In this context, Gomes et al. (2013) evaluated the sensory properties of fermented dairy beverages made with goat's milk, cow's milk and a mixture of the two milks. The authors stated that by using quantitative descriptive analysis technique, no difference was detected by the panel between the three fermented beverages.

The sensory characteristics of dairy products can be described based on its appearance, aroma, texture, flavor, and color. In this context, Brighenti, Govindasamy-Lucey, Lim, Nelson, and Lucey (2008) have assessed the sensory properties of cream cheeses, which contain different fat levels. The results obtained from a trained panel showed significant differences ($P < 0.001$) between cheese fat levels; indeed, full-fat cream cheeses were more firm, cohesive, difficult to dissolve, and to spread and less sticky than Neufchatel and fat-free cream cheeses. Lteif, Olabi, Baghdadi, and Toufeili (2009) have also characterized the sensory properties of bovine and ovine Halloumi cheese of different fat levels. One of the main results achieved by the trained panelists revealed that ovine cheese was found to be more yellow and harder than bovine cheese. In addition, cheeses at low-fat and reduced-fat were more yellow and harder than full-fat cheese. The authors concluded that bovine cheese was more acceptable than ovine cheese where received significantly higher scores, in agreement with the findings of Queiroga et al. (2013) who found that the sensory acceptability of caprine milk was improved when bovine milk was added to caprine milk due to the reduction odor and flavor of caprine milk. In a similar approach, Golinelli et al. (2014) have used sensory analysis to detect the adulteration of *frescal* (fresh) goat cheese with bovine milk. Their results showed that around half of the consumers were capable to identify adulteration at 10% cow milk, in agreement with the findings of Santos et al. (2011) who pointed out the possibility to discriminate curd cheeses made with goat's milk from those made with goat's and cow's milk mixtures. Cheeses manufactured from cow's milk with 60% of goat's milk received the lowest scores for flavor. In a different approach, Felfoul, Bornaz, Baccouche, Sahli, and Attia (2015) have studied the impact of milk-fat replacement by emulsified olive oil on sensory characteristics of Gouda cheeses in comparison with the low-fat without fat replacers and full-fat cheese. The principal results showed that the overall impression score of all products at low-fat was significantly lower than full-fat cheese.

Krause, Miracle, Sanders, Dean, and Drake (2008) attempted to determine the effect of refrigerated (5 °C) and frozen (−20 °C) butter storage on their sensory characteristics (color, flavor, and texture). The authors stated that butter flavor changes occurring over time depended mainly of storage temperatures ($P < 0.05$), while color and firmness did not change, regardless of storage time and conditions.

Sensory analysis methods are useful techniques, where it can provide the answers to several questions that contribute in the success of product such as the product stability and variability, comparison to competitor product(s). However, these techniques have some limits such as variability in the information provided about the product, which result of individual variations, leading to an ambiguous answer of the test. Moreover, sensory methods are costly and not always practical for large-scale commercial purposes.

4. Spectroscopic techniques used for the determination of the authenticity and the detection of adulteration of dairy products

In the last years, spectroscopic techniques in the different electromagnetic radiation spectrum (i.e., ultraviolet (UV), visible (VIS) and infrared (IR)) have become increasingly the most commonly used methods in food research for determining the authenticity and detecting adulteration. The main advantages of these techniques are fast, low-costs, nondestructive, the ability of detecting contaminants in food with good accuracy as well as they are considered ideal alternatives to replace the reference ones (Domingo et al., 2014; Karoui & De Baerdemaeker, 2007).

4.1. Fluorescence spectroscopy

Fluorescence spectroscopy is one of the fast and sensitive analytical techniques that has been used for characterizing molecular environments in a wide variety of biological samples such as dairy products (Karoui, Dufour, Schoonheydt, & De Baerdemaeker, 2007). Fluorescence is the emission of light by a fluorescent molecule or substructure (called a fluorophore) following the absorption of ultraviolet or visible light (Karoui & Blecker, 2011). Indeed, the absorption of the light energy by fluorophore is at a specific wavelength and the liberation of energy in the form of light emission is at a longer wavelength (Karoui & Blecker, 2011). This technique has recently becomes the most widely used as a tool in biological science associated to food technology (Oto et al., 2013).

The measurements with classical right-angle fluorescence spectroscopy should perform on dilute solutions with absorbance of the sample below 1. At a higher absorbance (than 1), the inner filter effect induce a decrease of fluorescence intensity and a distortion of emission spectra.

To avoid these problems, front-face fluorescence spectroscopy (FFFS) was developed, where only the surface of the material is

illuminated and examined (Karoui & Blecker, 2011). The emitted photons are collected at an angle comprised between 30 and 60° to the surface of the sample inducing the minimum of artifacts induced by the photons reflected from the sample; this technique allows a quantitative investigation of fluorophores in powders as well as in concentrated and turbid samples.

In the last decade, the potential of using fluorescence in milk and dairy products has been increased with the propagated application of chemometrics due to relative simplicity of the instrument for acquiring spectra (Luykx & van Ruth, 2008). Moreover, this technique has a sensitivity which is 100–1000 times higher than other spectrophotometric techniques, and it can provide information about fluorescent molecules and their environment in all sorts of biological samples (Karoui, Dufour, & De Baerdemaeker, 2007). Fluorescence spectroscopy combined with chemometric tools has been used for monitoring the authenticity and the detection of adulteration of different dairy products (Table 4). Indeed, dairy products contain several intrinsic fluorophores including the aromatic amino acids and nucleic acids (AAA + NA), tryptophan, tyrosine, and phenylalanine in proteins; vitamins A and B_2; nicotinamide adenine dinucleotide (NADH) and chlorophyll, all compounds containing in their structure conjugated double bonds such as conjugated linoleic acid, and numerous other compounds found at a low or very low concentration in food products have the properties to fluoresce (Karoui & Blecker, 2011).

Hammami et al. (2010) assessed the potential of FFFS and synchronous fluorescence spectroscopy (SFS) to discriminate between milk samples belonging to Sicilo-Sarde ewes fed with three different feeding groups (control, soy bean meal, and scotch bean meal) during 11 weeks lactation period. By applying PCA, separately, to the fluorescence spectral data, only a small discrimination between the 3 milk groups was observed. The same authors have then applied the concatenation technique to all the intrinsic fluorophores (i.e., AAA + NA, tryptophan, vitamin A and riboflavin) and a clear discrimination of ewe's milk according to their feeding systems was obtained, confirming previous findings of Rouissi et al. (2008) and those of Zaïdi et al. (2008) and Hammami et al. (2013) who succeeded to discriminate between ewe's milks according to their genotype (Comisana and Sicilo–Sarde-ewe's).

In a different approach, 9 nonfat dry milks collected from 3 manufacturers and stored at 4 temperatures (4, 22, 35, and 50 °C) for 8 weeks were assessed by scanning Maillard products, tryptophan, and riboflavin spectra (Liu & Metzger, 2007). The application of PCA to these spectra allowed a clear discrimination between milks according to their variation in nonfat dry milk and technological processes. These results were, later, confirmed by Ntakatsane, Yang, Lin, Liu, and Zhou (2011) reporting that FFFS could be used to differentiate milk samples according to their composition (whole, reduced fat, skimmed, low lactose, and high protein) and geographical origin.

Blecker, Habib-Jiwan, and Karoui (2012) assessed to determine the effect of heat treatments (60 and 80 °C during 20 min) and rennet-induced coagulation temperatures (30 and 40 °C) on the kinetic of milk coagulation by using SFS. By applying a series of chemometric tools, a clear discrimination was observed between: i) raw milks and heated ones; and ii) milks renneted at 30 °C from those renneted at 40 °C. These results were, recently, confirmed by Hougaard, Lawaetz, and Ipsen (2013) reporting the ability of FFFS combined with parallel factor analysis (PARAFAC) to discriminate milk samples according to heating intensity (72–120 °C).

In another approach, the potential of fluorescence spectroscopy in detecting adulteration of milk fat with vegetable oil was evaluated by Ntakatsane, Liu, and Zhou (2013). One of the main results reached by the authors is the potential use of FFFS to discriminate commercial butter from milk according to the source of the fat. These results were, recently, confirmed by Dankowska, Małecka, and Kowalewski (2015) who pointed out the ability of FFFS to quantify the adulteration of hard cheese with cheese-like products, with lowest detection limits of 3.0%.

FFFS has also been used to differentiate between cheeses according to their geographical origin, technological process and sampling zone. Karoui et al. (2007) succeeded to differentiate between the external and central zones and the manufacturing process of 3 varieties of soft cheeses. The same research group (Karoui, Dufour, and De Baerdemaeker, 2007) assessed the potential use of the 400–640 nm emission spectra after excitation set at 380 nm to monitor the oxidation changes of semi-hard cheeses throughout ripening. One of the main conclusions reached by the authors was that the 400–640 nm emission fluorescence spectra were found primarily affected by light and oxygen then by the physicochemical changes that occurred throughout ripening. The obtained results were, after, confirmed by Sinelli, Limbo, Casiraghi, and Torri (2011) and Veberg, Olsen, Nilsen, and Wold (2007) who evaluated the application of fluorescence spectroscopy method, combined with chemometric tools, to assess the light induced oxidation of semi-hard cheeses and butter during storage. The authors stated that this method is very sensitive to measure the development of light-induced oxidation in cheese products, and that fluorescence spectroscopy detected riboflavin decay that appears to be the most lightly absorbing constituent of cheese.

In a similar approach, Othman et al. (2011) monitored changes occurring in Cantal cheeses throughout ripening period (30, 120 and 200 days) using SFS. By applying a series of chemometric tools such as PCA and FDA, the authors succeeded to: i) differentiate cheeses according to their ripening stage since 100% of correct classification was obtained; and ii) attribute changes in bands (i.e., 290, 322, and 355 nm) to the modifications occurring in the physicochemical properties of cheese matrix. The same research group (Abbas, Karoui, & Aït-Kaddour, 2012) explored the potentiality of this technique to predict some chemical parameters (pH, fat, dry matter, protein and soluble nitrogen) of French blue-veined cheeses belonging to four brands (Fourme d'Ambert, Fourme de Montbrison, Bleu d'Auvergne and Bleu des Causses). The application of PLSR allowed to predict ash and protein ($R^2 > 0.8$) when two groups (Fourme and Bleu) were used in the data sets, while fluorescence spectroscopy failed to predict the above-mentioned parameters when the 4 groups were utilized (Fourme d'Ambert, Fourme de Montbrison, Bleu d'Auvergne and Bleu des Causses).

The use of 3D fluorescence spectroscopy in dairy products is limited. An example of the 3D fluorescence spectra obtained during heat treatment of milk is illustrated Fig. 1. Heat treatment of milk samples at 60 °C for 30 min induced an increase of the fluorescence intensity with excitation emission set at 350 and 480 nm compared to milk samples heated at 55 °C for 30 s; these compounds could be attributed to fluorescent Maillard reactions.

The excellent potential of this technique combined with multivariate statistical analyses to determine the authenticity and to detect the adulteration of dairy products has also been demonstrated. Although these advantages, the interpretation of fluorescence spectral data is complex since some other fluorescence components could fluoresce due to energy transfer.

4.2. Infrared spectroscopy

Infrared spectroscopy is one of the spectroscopic techniques most widely used, where it measures the wavelength and intensity of infrared light absorbed by a sample (Luykx & van Ruth, 2008). This method is based on the vibrations of the atoms of a molecule. The infrared spectrum of a sample is recorded by passing a beam of infrared light through the sample and then determine what part of

Table 4
A summary overview of use the fluorescence spectroscopy method for determining the authenticity and detecting the adulteration of dairy products.

Dairy product	Objective	Method	Fluorophore	λ ex (nm)	λ em (nm)	Main result	Reference
Milk	Authentication of ewe's raw milk according to their feeding systems, and genotypes.	FFFS	AAA + NA Tryptophan Riboflavin Vitamin A	250 290 380 250–350	280–450 305–400 400–640 410	- Good discrimination of milk samples with regard to feeding systems, genotypes. - The best result was obtained with vitamin A spectra, since 88.6% of correct classification was observed.	Hammami et al., 2010; Hammami et al., 2013; Rouissi et al., 2008; Zaïdi et al., 2008.
	Identification of nonfat dry milk and milk originating from different manufacturers and geographical origins.	FFFS	Maillard products Tryptophan riboflavin	360 290 380	380–480 305–450 400–590	- Good discrimination between milk samples according to their composition and geographical origin.	Liu & Metzger, 2007; Ntakatsane et al., 2011.
	Identification of raw milk from the heated one.	FFFS, SFS	Excitation-emission	250–800 250–350	310–860 260–360	- Good discrimination between milks renneted at 30 °C from those renneted at 40 °C. - Good discrimination between milk samples according to heating (72–120 °C).	Blecker et al., 2012; Hougaard et al., 2013.
Cheese, milk fat	Detection of cheese and milk fat adulteration.	FFFS, SFS	Vitamin A 3D Excitation-emission	250–390 250–550 240–700	410 310–700 320–780	- FFFS method allowed to detect adulteration of hard cheese with cheese-like products. - Ability to detect up to 5% of vegetable oil adulteration in the milk fat.	Ntakatsane et al., 2013; Dankowska et al., 2015.
Cheese	Authentication of cheese.	FFFS, SFS	Tryptophan Riboflavin Vitamin A Excitation-emission	290 380 280–350 250–500	305–450 400–640 410 330–580	- Discrimination of cheeses according to their manufacturing processes, sampling zones (external and central), and ripening stages.	Karoui et al., 2007; Othman et al., 2011
Cheese, butter	Monitoring the oxidation changes of cheeses and butter.	FFFS		380 382	400–640 410–750	- The 400–640 nm emission could be used as a useful probe for monitoring cheese oxidation during ripening. - FFFS can measure both degradation of photosensitizers and formation of lipid oxidation products of light-exposed butter.	Karoui, Dufour, & De Baerdemaeker, 2007; Sinelli et al., 2011; Veberg et al., 2007.

AAA + NA; aromatic amino acids and nucleic acid, FFFS; front face fluorescence spectroscopy, SFS; synchronous fluorescence spectra.

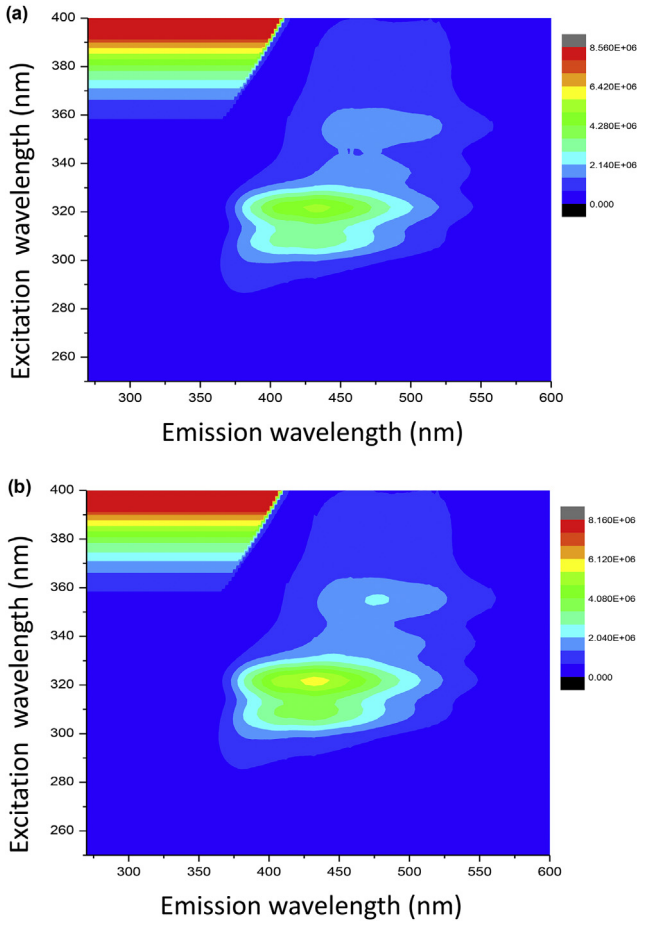

Fig. 1. 3D fluorescence spectra of milk samples heated at 60 °C for 30 s (a) and 30 min (b).

the incident radiation is absorbed in a particular energy. The energy at which any peak in an absorption spectrum appears corresponds to the frequency of a sample molecule vibration. This technique allows to determine functional groups present within a sample since each functional group of a molecule has a unique vibrational frequency. By taking into account the effects all the different functional groups, it results a spectrum representing a unique molecular "fingerprint" that may be used to confirm the detection of adulteration of a sample (Luykx & van Ruth, 2008).

The infrared can be divided into three spectral regions; the near- mid- and far-infrared, named for their relation to the visible spectrum. The higher-energy near-infrared (NIR) (~14,000–4000 cm^{-1}), the mid-infrared (MIR) (~4000–400 cm^{-1}), and the far-infrared (~400–10 cm^{-1}).

4.2.1. Near infrared spectroscopy

NIR spectroscopy is a method that uses the electromagnetic spectrum range varying in the 780–2500 nm, and can provide complex structural informations about the behavior of vibration of bond combinations. The response of the molecular bonds O–H, C–H, C–O and N–H can be characterized by the NIR region of the electromagnetic spectrum (Cen & He, 2007). During the last decades, NIR spectroscopy has become one of the most efficient tools for determining the authenticity of food products such as milk and dairy products (Huang, Yu, Xu, & Ying, 2008). For example, the technique was utilized for monitoring rennet coagulation of milk (Cipolat-Gotet, Cecchinato, De Marchi, Penasa, & Bittante, 2012; Lyndgaard, Engelsen, & van den Berg, 2012; Klandar, Lagaude, & Chevalier-Lucia, 2007), and physico-chemical parameters of cheeses (González-Martín, Hernández-Hierro, Revilla, Vivar-Quintana, & Ortega, 2011; Lucas, Andueza, Rock, & Martin, 2008) and butters (Heussen, Janssen, Samwel, & van Duynhoven, 2007).

Valenti et al. (2013) have evaluated the potential use of NIR spectroscopy to differentiate milk samples according to the feeding system, breed and altitude of the farm (Table 5). The application of PLS-DA to the collection spectral data sets, allowed to distinguish three main feeding systems defined by the dominant forage: maize silage, pasture and hay. However, the NIR failed to discriminate between milk samples collected from the upland and lowland groups, and milks from different cow breeds, in agreement, with previous findings of Mouazen, Dridi, Rouissi, De Baerdemaeker, and Ramon (2007, 2009) who succeeded to differentiate between ewe's milk samples obtained from different feeding systems (pasture vs. box feeding). However, the above-mentioned authors failed to differentiate between milk samples according to their genotypes (Comisana and Sicilo-Sarde). Aulrich and Molkentin (2009) succeeded to discriminate milk samples according to their production systems (organically and conventionally). These results were partially, later, confirmed by Coppa et al. (2012) who underlined that NIR spectroscopy is better suited to the authentication of milk according to their feeding systems although it failed to determine their geographical origin.

In another approach, the NIR technique has demonstrated its ability to determine the nutritional parameters. In this context, infant formula samples, milk powder samples, and liquid milk sample were scanned in the 9000–4500 cm^{-1} spectral range to detect melamine (Balabin & Smirnov, 2011). By applying a series of chemometric tools (PLS, polynomial-PLS, ANN, support vector regression (SVR)), the authors succeeded to detect the presence of melamine even at a level below 1 ppm (i.e., 0.76 ± 0.11 ppm) confirming previous findings of Yuan, He, Ma, Wu, and Nie (2009), Lu et al. (2009) and Dong et al. (2009) who by using NIR achieved to detect adulterated milk with melamine. The obtained results were, recently, confirmed by Zhang et al. (2014), Fu et al. (2014) and Li and Ding (2010) who respectively succeeded to: i) discriminate raw cow's milks from the adulterated ones with different pseudo proteins and thickeners (melamine, urea, or ammonium nitrate); ii) detect melamine in milk powders; and iii) detect milk with plant butter, vegetable protein, and starch. These results were in disagreement with those of Ni, Zhong, Zhang, Zhang, and Huang (2014) who failed to identify the adulteration of milk with different components (i.e., starch, melamine, urea, and ammonium nitrate) when used at concentration below 0.1%.

Aliakbarian et al. (2015) assessed the potential use of NIR to monitor the fermentation of skim milk after addition of aqueous phenolic extracts from olive and grape pomace. By applying chemometric tools to the spectral data sets, it was possible to discriminate between samples of probiotic milk according to the type of extract added and to evaluate the 'stability' of the product confirming NIR spectroscopy as a valid support to classical analysis. These results confirmed previous findings of Cozzi, Ferlito, Pasini, Contiero, and Gottardo (2009) who by applying discriminant analysis to the NIR spectra observed a clear separation between alpine and factory products, in agreement with those of Ottavian et al. (2012) and Andueza, Agabriel, Constant, Lucas, and Martin (2013) who reported respectively that: i) NIR could be considered as a reliable tool for real-time authentication of Asiago d'allevo cheese according to different farm management, ripening age, production period and production height; and ii) NIR could discriminate between cheese samples according to their feeding system (pasture vs. preserved-forage samples). However, other

Table 5
A summary overview of near and mid-infrared spectroscopy method used for determining the authenticity and detecting the adulteration of dairy products.

Dairy product	Objective	Methods	Wavenumber range (cm^{-1})/or Wavelength (nm)	Main results	Reference
Milk	Authentication of milk according to the feeding system, breed, altitude, and production system.	Reflectance-NIR Reflectance-Vis–NIR	1100–2498 nm 300–1710 nm	- Good discrimination of milk samples according to the feeding and production systems (organically and conventionally). - NIR failed to discriminate milk samples from different breeds, and between milks collected from the upland and lowland regions.	Valenti et al., 2013; Mouazen et al., 2007, 2009; Aulrich & Molkentin, 2009.
	Detection of the melamine, different pseudo proteins and thickeners in different types of milk.	Transmittance-NIR Reflectance-NIR Reflectance-NIR NIR-hyperspectral imaging	1110–2500 nm 830–2500 nm 1000–2500 nm 990–1700 nm	- NIR has a high ability for the detection of melamine in dairy products with the limit of detection lower than 1 ppm. - Good discrimination of raw milk from milk adulterated with melamine and other pseudo proteins and thickeners. - NIR hyperspectral imaging with SAM, SCM, and EDM may detect melamine in milk powders at 200 ppm.	Balabin & Smirnov, 2011; Lu et al., 2009; Dong et al., 2009; Zhang et al., 2014; Fu et al., 2014; Ni et al., 2014; Li & Ding, 2010.
Cheese, milk	Authentication	Reflectance-NIR Absorptions NIR Reflectance-Vis–NIR	1100–2500 nm 1100–2500 nm 400–2500 nm	- Good discrimination of cheese samples according to the farm management, ripening time, feeding system, production period, and geographical region. - NIR failed to discriminate between lowland and mountain cheeses, and between fresh and freeze-dried cheeses. - Good discrimination between fermented milk samples according to the type of extract added.	Cozzi et al., 2009; Ottavian et al., 2012; Andueza et al., 2013; Aliakbarian et al., 2015; Lucas, Andueza, Ferlay, & Martin, 2008.
Butter	Detection of butter adulteration with cheaper vegetable fats.	Absorptions-NIR	715–2500 nm	- Good detection of vegetable fats in butter in the 5100–6400 cm^{-1} spectral range.	Heussen et al., 2007.
Milk	Authentication of milk according to the feeding system, breed, altitude of the farm and production system.	Reflectance-MIR Absorption-MIR Absorption-FTIR	3000–900 cm^{-1} 5012–926 cm^{-1} 5008–925 cm^{-1}	- Good discrimination of milk samples according to their feeding and production systems (organically and conventionally). - MIR failed to discriminate milk samples from different breeds, and between the upland and lowland groups.	Karoui et al., 2011; Maâmouri et al., 2008; Valenti et al., 2013; Capuano et al., 2014.
	Detection of milk adulteration.	FTIR ATR-FTIR ATR-FTIR Absorption-FTIR	5000–1000 cm^{-1} 4000–500 cm^{-1} 4000–700 cm^{-1} 4000–650 cm^{-1}	- FTIR can detect adulterated milk with 0.05% and 0.075% of sodium bicarbonate and citrated respectively. - FTIR detect soymilk in milk samples with detection limits low than 2%. - The application of SIMCA allowed to discriminate control milk samples from the adulterated ones. - Detection of the melamine in milk, as well as cheese whey in milk powder. - Adulteration of milk with maltodextrin and water.	Cassoli et al., 2011; Jaiswal et al., 2015; Santos et al., 2013; Jawaid et al., 2013; Nicolaou et al., 2010; Mauer et al., 2009; Aparecida de Carvalho et al., 2015; Liu et al., 2015.
Cheese	Authentication.	ATR-MIR ATR-FTIR	3000–900 cm^{-1} 4000–700 cm^{-1}	- The 3000–2800 cm^{-1} allowed to discriminate cheeses according to their manufacturing processes, sampling zones, and geographical origins. - Discrimination of cheeses according to their ripening conditions, ripening stages, and cheese making procedures.	Karoui, Mouazen, Ramon, Schoonheydt, et al., 2006; Karoui, Mazerolles, Bosset, De Baerdemaeker, & Dufour, 2007; Boubellouta et al., 2010; Lerma-García et al., 2010.
Butter	Authentication.	ATR-FTIR ATR-FTIR ATR-FTIR	4000–650 cm^{-1} 4000–700 cm^{-1} 4000–600 cm^{-1}	- A clear discrimination between butter and margarine samples. - Good discrimination of butter according to their geographical origin, production season, and feeding regime.	Koca et al., 2010; Gori et al., 2012; Bassbasi et al., 2014.

ATR–FTIR; *attenuated total reflectance–Fourier transform infrared spectroscopy, ATR-MIR;* attenuated total reflectance-mid-infrared, EDM; Euclidian distance measure, FTIR; Fourier transform infrared, MIR; mid-infrared, NIR; near-infrared, SAM; spectral angle measure, SCM; spectral correlation measure, SIMCA; soft independent modeling of class analogy.

findings (Cozzi et al., 2009; Andueza et al., 2013) failed to discriminate between: i) lowland and mountain cheeses; and ii) fresh and freeze-dried cheeses, confirming those of Lucas, Andueza, Ferlay, and Martin (2008) who pointed out that NIR failed to differentiate between fresh and freeze-dried cheeses.

Finally, NIR method has also been used by Heussen et al. (2007) to detect the presence of cheaper vegetable fats in butter. The authors stated that the 5100–6400 cm^{-1} could be used to detect adulteration of butter. However, NIR was found to be less efficient for the determination of C4:0 fatty acid levels compared to GC.

The main advantages of NIR spectroscopy are rapid, cheap, non-destructive and very simple, where it does not need any sample preparation. Moreover, this technique allows to measure several components together. By contrast, NIR spectroscopy has some disadvantages such as the low sensibility, where the components at low concentration cannot be measured with high accuracy. In addition, due to the: i) superposition of many different overtone and combination bands in the NIR region, a very low structural selectivity of NIR spectra compared to the MIR; and ii) presence of several signals and noise overlapped which give rise to a spectrum with broad peaks, some difficulty could be encountered during the interpretation of the NIR spectra.

4.2.2. *Mid-infrared spectroscopy*

MIR spectroscopy is a method that measures the absorption of radiation in the range of 4000 to 400 cm^{-1}. This region may be divided into four broad regions: the region of single bonds (4000–2500 cm^{-1}), region of triple bonds (2500–2000 cm^{-1}), region of double bonds (2000–1500 cm^{-1}), and the region of other bond deformations (fingerprint) (1500–400 cm^{-1}) (Karoui, Downey, & Blecker, 2010). A transition between the ground and fundamental states, lead to only one type of vibrational response, which derived in the MIR absorbance. Therefore, the particular type of organic bond is the exclusive representation of peaks existing in a spectrum of MIR absorbance (Cheng et al., 2013). In the past years, MIR spectroscopy technique has been developed for studying the organic compounds structure such as protein secondary structure of milk and dairy products (Carbonaro & Nucara, 2010).

Currently, MIR spectroscopy has become more available for analyzing dairy products, mainly after the introduction of attenuated total reflection (ATR). The main advantages of this technique is that little or no sample preparation is required (Karoui et al., 2010). MIR has been used to monitor technological process, determination of the geographical origin, and detection of adulteration of dairy products (Fagan, O'Donnell, Rudzik, & Wüst, 2009; Karoui, Mouazen, Dufour, Pillonel, et al., 2006; Karoui, Mouazen, Ramon, Schoonheydt, & De Baerdemaeker, 2006; Karoui et al., 2010).

Several researchers have used MIR to determine the authenticity and the detection of adulteration of dairy products (Table 5). Karoui, Hammami, Rouissi, and Blecker (2011) explored the MIR combined with multivariate data analysis to discriminate between ewe's milk samples according to their feeding systems (controls, ewes fed scotch bean and ewes fed soybean) during 11 weeks of lactation period. By applying a series of chemometric tools such as PCA and FDA to the 3000–2800, 1700–1500 and 1500–900 cm^{-1} spectral region, a classification rate of 71.7% according to the feeding system was obtained. The obtained results were in agreement with the findings of Maâmouri et al. (2008) who succeeded by using the 3000–2800 cm^{-1} and 1500–900 cm^{-1} spectral region to discriminate between milk samples of ewes fed soybean meal from those fed scotch bean meal. These results were recently confirmed by: i) Valenti et al. (2013) who demonstrated that MIR has an excellent capability to distinguish milk from hay- and pasture-based systems and those from maize silage- and pasture-based systems; the same research study pointed out that MIR failed to discriminate milk samples collected from the upland and lowland regions; and ii) Capuano et al. (2014) who were able by using FTIR coupled with chemometric tools such as PLS-DA to discriminate between milks collected from cows fed fresh and aged grass and organic milk from conventional milk.

Cassoli, Sartori, Zampar, and Machado (2011) adulterated raw milk with three different compounds: sodium bicarbonate, sodium citrate, and non-acid cheese whey and MIR spectra were scanned. The capability of MIR to detect milk adulteration with 0.05% and 0.075% of sodium bicarbonate and citrate sodium, respectively was demonstrated. However, a low sensitivity of MIR was observed when milk samples were adulterated with nonacid whey, even when added at high concentrations (20%). Santos, Pereira-Filho, and Rodriguez-Saona (2013) succeeded by using MIR to detect and quantify adulterated milk with whey, urea, hydrogen peroxide, synthetic urine and synthetic milk, in agreement with the findings of: i) Jawaid, Talpur, Sherazi, Nizamani, and Khaskheli (2013) who attempted by using FTIR to detect melamine at 2.5 ppm in liquid and powder milk samples; ii) Nicolaou, Xu, and Goodacre (2010) and Mauer, Chernyshova, Hiatt, Deering, and Davis (2009) who accomplished to respectively detect the binary and tertiary milk mixture composed of sheep's, goat's, and cow's milk and to detect the melamine at 1 ppm in infant formula powder; and iii) Aparecida de Carvalho et al. (2015) who detected the presence of cheese whey in milk powder. Recently, Liu, Ren, Liu, and Guo (2015) succeeded by using new comprehensive index, called Q, to detect milk adulteration with maltodextrin and water (as low as 1.0% of adulteration proportions), and with other nine kinds of synthetic adulterants (as low as 0.5% of adulteration proportions). This is in agreement with the findings of Jaiswal et al. (2015) who accomplished to detect the presence of soymilk in milk at a level of 2%.

With regard to soft and hard cheeses, several researchers reported that the 3000–2800 cm^{-1} could be considered as a fingerprint allowing clear discrimination of: i) soft cheeses according to their manufacturing process and sampling zones (Karoui, Mouazen, Ramon, Schoonheydt, et al., 2006); and ii) Emmental cheeses according to their geographic origin (Karoui, Mazerolles, Bosset, De Baerdemaeker, & Dufour, 2007). These results were, later, confirmed by Boubellouta, Karoui, Lebecque, and Dufour (2010) reporting a clear discrimination between saint-Nectaire PDO cheeses and Savaron cheeses produced by using different manufacturing and ripening conditions. A similar approach sampling was used by Lerma-García, Gori, Cerretani, Simó-Alfonso, and Caboni (2010) in Pecorino cheeses and a clear differentiation of cheeses according to their ripening times and manufacturing process was obtained.

In another approach, Koca, Kocaoglu-Vurma, Harper, and Rodriguez-Saona (2010) executed a feasibility study of the use of ATR-FTIR (4000–650 cm^{-1}) in combination with multivariate statistical analysis to detect the presence of margarine adulteration in butter. A distinctive band located around 1741 cm^{-1} was observed allowing a clear discrimination between butter and margarine samples. These results were, recently, confirmed by Gori, Cevoli, Fabbri, Caboni, and Losi (2012) who pointed out the ability of MIR to discriminate between butter samples according to their production seasons, and those of Bassbasi, De Luca, Ioele, Oussama, and Ragno (2014) who succeeded to authenticate Moroccan butters collected from different regions.

MIR spectroscopy has some advantages such as rapidity of measurements, high repeatability, low-cost equipment and easy sampling. However, the strong absorption of water in the MIR region especially in the aqueous samples is the main disadvantage of this technique that could induce some difficulty in the interpretation of the spectra. For example, the useful absorptions from protein can be obscured effectively by O–H bending band (~1650 cm^{-1}).

4.3. Nuclear magnetic resonance (NMR)

NMR is a research technique that is used for studying physical and chemical properties of atoms or the molecules in which they are contained by exploiting the magnetic properties of certain atomic nuclei. By using this technique, a large amount of information can be reached about the molecular structure of an organic sample. This technique is based upon the measurements of radio-frequency radiation absorbed by atomic nuclei in a strong magnetic field. The absorption of the atomic nuclei might be affected by the local environment around atoms, which cause slight modification of the local to the external magnetic field. Several isotopes may be used in NMR, among them ^{15}N, ^{17}O and ^{31}P, but ^{1}H and ^{13}C that are the most commonly employed (Luykx & van Ruth, 2008). NMR methods applied to food analysis can be divided into three main groups: magnetic resonance imaging (MRI), low-field NMR, and high-resolution NMR (Erikson, Standal, Aursand, Veliyulin, & Aursand, 2012).

NMR has been used to determine the authenticity and identify of milk and dairy products (Table 6). In this context, Sacco et al. (2009) used NMR in combination with chemometric tools for determining the geographical origin of 39 milk samples produced in two neighboring Italian regions (Apulia and Basilicata) and in some countries located in the Central-Eastern Europe. Their results showed that peaks of some organic acids, sugars and amino acids could be used to distinguish between the investigated milk samples. In addition, ^{1}H NMR spectra showed higher sugar content in foreign milk than in Southern Italy milk. The authors have then applied PCA to the collected data sets and a clear separation of Italian milk samples from the others was observed, that is confirmed by FDA since correct classification of more than 90% was obtained. Recently, Liu, Cao, Zhang, He, and Xu (2014) have employed low-field NMR method to detect changes in the moisture of fresh milk samples stored at 30 °C for up to 96 h. Their researches showed that total moisture in fresh milk decreased firstly, and then increased during the process of metamorphism; the authors concluded that NMR technique could be used as a rapid method for discriminating fresh milks from the aged ones. In another study, Haque et al. (2015) have utilized high resolution solid state ^{13}C NMR technique to study changes occurring at the molecular level of milk protein concentrate powder, presenting moisture content in the 5.5–16.5% w/w range. The authors indicated that a slight higher rigidity of non-aged milk protein concentrate was observed compared to the long aged one.

NMR technique coupled with multivariate statistical analysis has also been used for authentication goal. Lachenmeier et al. (2009) explored this technique to detect the presence of melamine in infant formula. The ^{1}H NMR at 400 MHz was found to distinguish between melamine-contaminated and melamine-free infant formulas and the authors concluded that this technique could provide quantitative information by integration of individual lines after identification. These results were recently confirmed by Picariello et al. (2013) who by using ^{13}C NMR succeeded to detect synthetic triacylglycerols used for adulterating butter at percentage of 1% w/w, in agreement with the findings of Schripsema (2008) reporting the ability of ^{1}H NMR to discriminate between butter and margarine samples.

In a similar approach, the water-soluble metabolites content of 25 Italian Parmigiano Reggiano cheeses sampled at different ripening stages (14, 24 and 30 months) were compared with 8 samples of “Grana type” cheeses originated from east Europe countries (Consonni & Cagliani, 2008). A clear discrimination of cheeses according to their geographical origin and ripening time was observed. The obtained results were confirmed later by Mazzei and Piccolo (2012) who succeeded by using ^{1}H high resolution magic angle spinning NMR spectroscopy to authenticate 37 Mozzarella cheeses produced from two different sites in Campania, in agreement with those of Piras et al. (2013) and Rodrigues et al. (2011) who pointed out the ability of this technique to discriminate respectively between: i) Fiore Sardo Italian cheeses samples on the basis of their maturation age (during 90 days) and the type of added cultures (i.e., lactic acid bacteria or commercial; and ii) Probiotic cheeses or Synbiotic cheeses according to the ripening time, and type of added probiotic bacteria. In a different approach, Prema et al. (2013) explored the potentiality of ^{1}H NMR to predict CLA content in the lipid fraction of various Canadian cheeses from conventional, organic, and grass-fed dairy sources. Their investigation showed the accuracy of this technique to predict CLA.

The main advantages of NMR spectroscopy are: i) rapid and environmentally friendly; ii) non-destructive, possibility to carry out several analyses by using the same sample; iii) the ability to detect different nuclei, allowing differentiation between molecules or portions of molecules with different mobility. The main disadvantage of this technique is the complex optimization of the instrument compared with other techniques such as infrared and fluorescence spectroscopies, and that measurements are time dependent. Indeed, the nature of pulse sequence used in the experimentation is crucial since the use of wrong one induce a negative outcome of the results.

4.4. Analysis of the data obtained from different analytical techniques

Dairy products are complex foods. The use of only one analytical technique is not enough to obtain all the information about the authenticity and the detection of adulteration of dairy products. Thus, it would be interesting to analyze jointly all the data sets obtained from different analytical techniques. By applying statistical techniques to the whole data sets, it is possible to characterize better the authenticity and identify dairy products as well as to detection adulteration. In this context, Karoui et al. (2011) have applied concatenation technique to discriminate between ewe's milk samples according to their feeding systems (controls, ewes fed scotch bean and ewes fed soybean). The concatenation technique was performed on data sets collected from the two spectroscopic techniques: three MIR spectral regions (3000–2800, 1700–1500 and 1500–900 cm^{-1}) and the four fluorescence spectra (AAA + NA, tryptophan residues, and riboflavin). By applying FDA to the data sets, correct classification amounting to 98% was obtained. One of the main conclusions of this study was that the approach which based on the use of all data registered on milk samples by using infrared and fluorescence methods has proven its ability to distinguish between milk samples according to the feeding system of the ewes. The obtained results confirmed previous findings of the same research group (Rouissi et al., 2008) who applied CCSWA to the physico-chemical and fluorescence spectral data sets. A good discrimination of milks collected from ewes fed soybean meal from those fed scotch bean was obtained. The CCSWA was also applied to infrared and fluorescence data sets collected on skim-milk samples (Boubellouta, Galtier, & Dufour, 2009), and a clear differentiation was observed between milk samples where phosphate was added from those induced by the incorporation of calcium or citrate. One of the main conclusions of this study was that CCSWA could be used a valuable technique for a global characterization of milk samples throughout ripening. This was later confirmed by Botosoa and Karoui (2013) who by applying CCSWA to the fluorescence and infrared data sets succeeded to discriminate Emmental cheeses according to their ripening time and/or their manufacturing process, confirming previous findings of the same research group (Karoui, De Baerdemaeker, & Dufour, 2008). Indeed, by applying

Table 6
A summary overview of nuclear magnetic resonance method used for determining the authenticity and detecting the adulteration of dairy products.

Dairy product	Objective	Type NMR	Frequency (MHz)	Main results	Reference
Milk	Authentication and detection of milk adulteration.	^{1}H NMR Low-field ^{1}H NMR	400	- Good discrimination between milk samples according to their geographical origin. - Good discrimination of fresh milks from the aged ones based on the moisture content. - ^{1}H NMR method at 400 MHz can distinguish between melamine-contaminated and melamine-free infant formulas.	Sacco et al., 2009; Liu et al., 2014; Lachenmeier et al.2009.
Milk protein concentrate powder	Study the changes of molecular structure and dynamics of proteins as a function of moisture content (5.5 −16.5% w/w) and storage period.	^{13}C NMR	75.482	- A slight higher rigidity of molecular domains of protein molecules of non-aged milks compared to the long aged ones at 25 °C. - A decline in milk protein concentrate solubility from 70% (non-aged) to around 29% and 7.5% was observed for samples stored at 25 °C for 3 and 11 weeks, respectively.	Haque et al., 2015.
Cheese	Authentication of cheese.	^{1}H NMR ^{1}H HRMAS NMR ^{1}H NMR	500.13 400 399.95	- Good discrimination of cheeses according to their geographical origin, ripening time, type of added cultures, and type of added probiotic bacteria. - Good discrimination of cheeses (i.e., grass-fed, conventional and organic) according to their CLA content.	Consonni & Cagliani, 2008; Mazzei & Piccolo, 2012; Piras et al.2013; Rodrigues et al., 2011; Prema et al.2013.
Butter	Detection of butter adulteration.	^{13}C NMR ^{1}H NMR	150.90 400	- ^{13}C NMR method allowed to detect synthetic triacylglycerols in butter even at percentage of 1% w/w. - Good discrimination between butter and margarine according to their composition and technology process.	Picariello et al., 2013; Schripsema, 2008.

CLA; conjugated linoleic acid, HRMAS NMR; high resolution magic angle spinning nuclear magnetic resonance, NMR; nuclear magnetic resonance.

FDA to the concatenated data sets: MIR spectra recorded between 3000 and 900 cm^{-1}, fluorescence spectra and physico-chemical data, Karoui, Mouazen, Dufour, Schoonheydt, and De Baerdemaeker (2006) succeeded to discriminate cheeses according to their manufacturing processes (traditional and stabilized cheeses) and sampling zones (surface and centre). Correct classification amounting to 91.7% was obtained. One of the main conclusions of this study was that although concatenation technique did not allow 100% correct classification, the results obtained are promising, confirming recent investigations of the same research group (Karoui et al., 2011) illustrating that the concatenation technique applied to the MIR and fluorescence data sets allowed 98% of correct classification of ewe's milk collected from three different feeding systems.

5. Conclusion

During the past decade, a considerable interest has been paid to dairy products quality and methods of production due to the recent crises and scandals in food industry, which have seriously undermined consumer confidence. The need for rapid analytical techniques to determine the authenticity and to detect adulteration is greater than ever. Demand for high levels for the determination of authenticity of dairy products obviously requires high standards in quality assurance and process control; satisfying this demand in turn requires appropriate analytical tools for milk and dairy products analysis both during production and storage. Sensory, physico-chemical, DNA based method, stable isotope analysis, and chromatographic methods have been evaluated to assess the authenticity and the detection of adulteration of dairy products. However, many of these methods are either time consuming, destructive or require trained personnel, and are therefore not suited for online or large-scale operations.

Recently, a considerable effort has been made by researchers to explore the possibilities of using spectroscopic techniques for authenticity and adulteration of milk and dairy products. These techniques (e.g., NIR, MIR, FFFS, NMR spectroscopy) are mostly relatively low-cost and can be applied in both fundamental research and in the factory as on-line sensors for dairy process monitoring and product quality assessment. Moreover, most of them are non-destructive, rich in information on both molecular structures and physical states, and therefore, provide a fingerprint of product.

The results illustrated in this review suggest that the methodology of coupling different spectroscopic techniques with appropriate chemometric tools could allow to characterize in depth the maximum of information contained in spectral data. Increased research efforts in the field of spectroscopic techniques could address some measurement challenges of dairy products and further explore the physico-chemical changes that are: (i) mostly not fully understood; and (ii) responsible for the modification of the stability, organoleptic, and/or typicality of such products. Even though the present review focused on the milk and dairy products, the principles are broader and generally applicable to other food ingredients and products.

Spectroscopic techniques are very promising tools to determine and/or to detect the authenticity and adulteration of milk and dairy products; they are rapid, non-destructive, effective and reliable. Taking into account the environmental aspect, they are the best solution because there is no chemical involved compared to the reference methods (e.g., physico-chemical analysis, HPLC, and GC–MS, etc.). Finally, they could be considered as excellent alternatives to the standard reference methods for determining the authenticity and/or detecting the adulteration of milk and dairy products.

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RÉSULTATS ET DISCUSSION

Partie 1

Suivi de la cinétique de coagulation du lait de chamelle en comparaison avec le lait de vache : Impact du traitement appliqué au lait et de l'ajout des minéraux

Partie 1 : Suivi de la cinétique de coagulation du lait de chamelle en comparaison avec le lait de vache : Impact du traitement appliqué au lait et de l'ajout des minéraux

Cette partie a fait l'objet de quatre publications :

- Kamal, M., Foukani, M., & Karoui, R. (2016). Fortifying camel and cow milk with calcium and phosphate and their impact on the rheological properties of rennet-induced coagulation. *International Journal of Food Properties* (article 1, soumis).

- Kamal, M., Foukani, M., & Karoui, R. (2016). Effects of heating and mineral supplementation on the physical properties of rennet-induced coagulation of camel and cow milk gels. *Journal of Dairy Research* (article 2, **accepté après modifications**).

- Kamal, M., Foukani, M., & Karoui, R. (2017). Rheological and physical properties of camel and cow's milk gels enriched with phosphate and calcium during acid-induced gelation. *Journal of Food Science and Technology* (article 3, **54, 439–446**).

- Karoui, R., & Kamal, M. (2017). Rennet-induced coagulation of raw and heated camel and cow milk gels determined by instrumental techniques: Effects of added calcium and phosphate. *Journal of the Science of Food and Agriculture* (article 4, **sous presse**).

I. Introduction

Le lait est un aliment de couleur généralement blanchâtre et est l'une des principales ressources alimentaires pour l'humain. Il est consommé à l'état liquide ou après avoir subi des procédés de transformation (laits fermentés, beurres, fromages, etc.).

La texture des produits laitiers demeure un enjeu majeur auprès du consommateur pour l'appréciation de leurs qualités. Elle est liée, d'une part, à la composition physico-chimique du lait de départ, et d'autre part, aux procédés de fabrication et des conditions de conservation (Lobato-Calleros et al. 1998 ; Lucey et al. 2003).

Afin d'améliorer la texture des produits laitiers, il est important de contrôler l'évolution de leur structure au cours de leur transformation. Dans ce cadre, la coagulation du lait est considérée comme l'une des principales étapes conditionnant la texture finale des produits ayant

subi une coagulation acide, enzymatique, ou mixte. Selon le type de coagulation, de nombreuses modifications physico-chimiques ont eu lieu au niveau des micelles de caséines.

Pour investiguer l'impact de la structure moléculaire du coagulum sur sa texture, différents niveaux peuvent être étudiés allant du niveau moléculaire au niveau macroscopique. Bien que quelques méthodes non destructives aient été utilisées pour suivre les changements moléculaires et macroscopiques de la structure du coagulum du lait de vache (Herbert et al. 1999 ; Boubellouta et al. 2011 ; Blecker et al. 2012), à notre connaissance aucune étude n'a été menée sur le suivi de la coagulation acide / enzymatique du lait de chamelle par de telles techniques.

Les objectifs de cette étude étaient de caractériser les changements structuraux au niveau macroscopique et moléculaire au cours de la coagulation du lait de chamelle en comparaison avec le lait de vache dans différentes conditions (lait cru et lait ayant subi un chauffage à 50 et 70 °C pendant 10 minutes) avec et sans ajouts de minéraux (calcium, phosphate) en utilisant le test de cisaillement dynamique et la spectroscopie de fluorescence frontale.

II. Impact du préchauffage et de l'enrichissement du lait de chamelle en minéraux sur les propriétés rhéologiques de gels obtenus par coagulation enzymatique et acide : comparaison avec le lait de vache

Bien que les minéraux représentent une petite fraction dans la composition globale du lait, ils présentent à la fois des intérêts nutritionnels et technologiques (Gaucheron, 2005). Les minéraux sont présents dans le lait dans un état d'équilibre entre la phase soluble et la phase colloïdale. Le calcium et le phosphate représentent les éléments principaux et jouent un rôle important dans cet équilibre avec des concentrations qui varient entre 25 et 35 mM pour les laits de chamelle et de vache (Gaucheron, 2005 ; Bornaz et al. 2009). La concentration de ces deux éléments affecte de manière significative les propriétés du gel. En effet, toute modification dans leur concentration se répercute sur les propriétés rhéologiques du coagulum (Le Graet et Brulé, 1993). Des travaux de recherche ont indiqué que l'augmentation de la concentration du calcium dans le lait de vache améliorait ses propriétés gélifiantes. Récemment, Ramasubramanian et al. (2014) ont souligné que la vitesse de gélification passait de 0,029 à 3,614 $Pa.min^{-1}$ et la fermeté de gels augmentait de 25 à 248,6 Pa respectivement avec l'accroissement de la concentration de calcium ajouté au lait de 10 à 20 mM. De plus, Sandra et al. (2012) ont également indiqué que l'ajout de calcium au lait de vache renforçait la fermeté de gels qui passait de 13 Pa pour le lait témoin (sans ajout de calcium) à 33 Pa pour celui enrichi

en calcium à 1 mM. Au contraire, un enrichissement du lait en phosphate provoque des effets inverses comme le rapportent les travaux de Mittal et al. (1990), Udabage et al. (2001) et Guillaume et al. (2004).

Pour améliorer la qualité du lait de départ pour sa transformation ultérieure, l'industrie laitière procède au chauffage de celui-ci. Cependant, ce processus affecte les aptitudes technologiques. Récemment, Blecker el al, (2012) ont rapporté que le chauffage du lait de vache à des températures au-dessus de 55 °C provoquait la dénaturation des protéines solubles entraînant ainsi un allongement du temps de gélification et une diminution de la fermeté de gel. Cet effet s'expliquerait par les interactions entre les protéines dénaturées de lactosérum, en particulier, la β-lactoglobuline et l'α-lactalbumine avec la caséine-κ, conduisant ainsi à la formation d'un complexe entre protéines sériques dénaturées et caséine-κ *via* la formation de ponts disulfures empêchant l'action de la présure sur la caséine-κ.

Bien que plusieurs études aient été réalisées sur l'impact de l'ajout des minéraux sur les propriétés de gels obtenus à partir du lait de vache (Udabage et al. 2001 ; Choi et al. 2007 ; Sandra et al. 2012 ; Ramasubramanian et al. 2014), à notre connaissance, seules quelques études se sont intéressées à déterminer l'impact de cet enrichissement sur les caractéristiques de gels obtenus à partir du lait de chamelle (Farah et Bachmann, 1987 ; Ramet, 2001). Les objectifs de ce travail étaient de déterminer les propriétés rhéologiques de gels obtenus par coagulation enzymatique et acide du lait de chamelle (cru et chauffé à 50 et 70 °C pendant 10 minutes) suite à l'ajout de minéraux (calcium et phosphate à 10 et 20 mM) en comparaison avec le lait de vache traité dans les mêmes conditions.

II.1. Coagulation par voie enzymatique

Dans cette étude, la coagulation a été réalisée avec du lait de chamelle collecté dans une ferme expérimentale d'El Jem (Tunisie) et du lait de vache obtenu dans une ferme régionale de Lille (France). La coagulation enzymatique a été effectuée en utilisant la chymosine FAR-M®Sticks (CHR Hansen) préparée à partir de 1 g de poudre FAR-M®Sticks dilué dans 20 g d'eau distillée. Les cinétiques de coagulation enzymatique ont été suivies pendant 45 minutes à 36 °C en utilisant le test de cisaillement dynamique (Rhéomètre physica MCR 301). Ce test a été utilisé pour déterminer G', G" et tan δ. A partir du G', les paramètres suivants ont été calculés : i) le temps de coagulation défini comme étant le temps pour lequel G' = 1 Pa comme le rapportent les travaux de Klandar et al. (2007) et Ramasubramanian et al. (2014) ; ii) la vitesse de gélification qui correspond à la pente obtenue par la régression linéaire de l'évolution

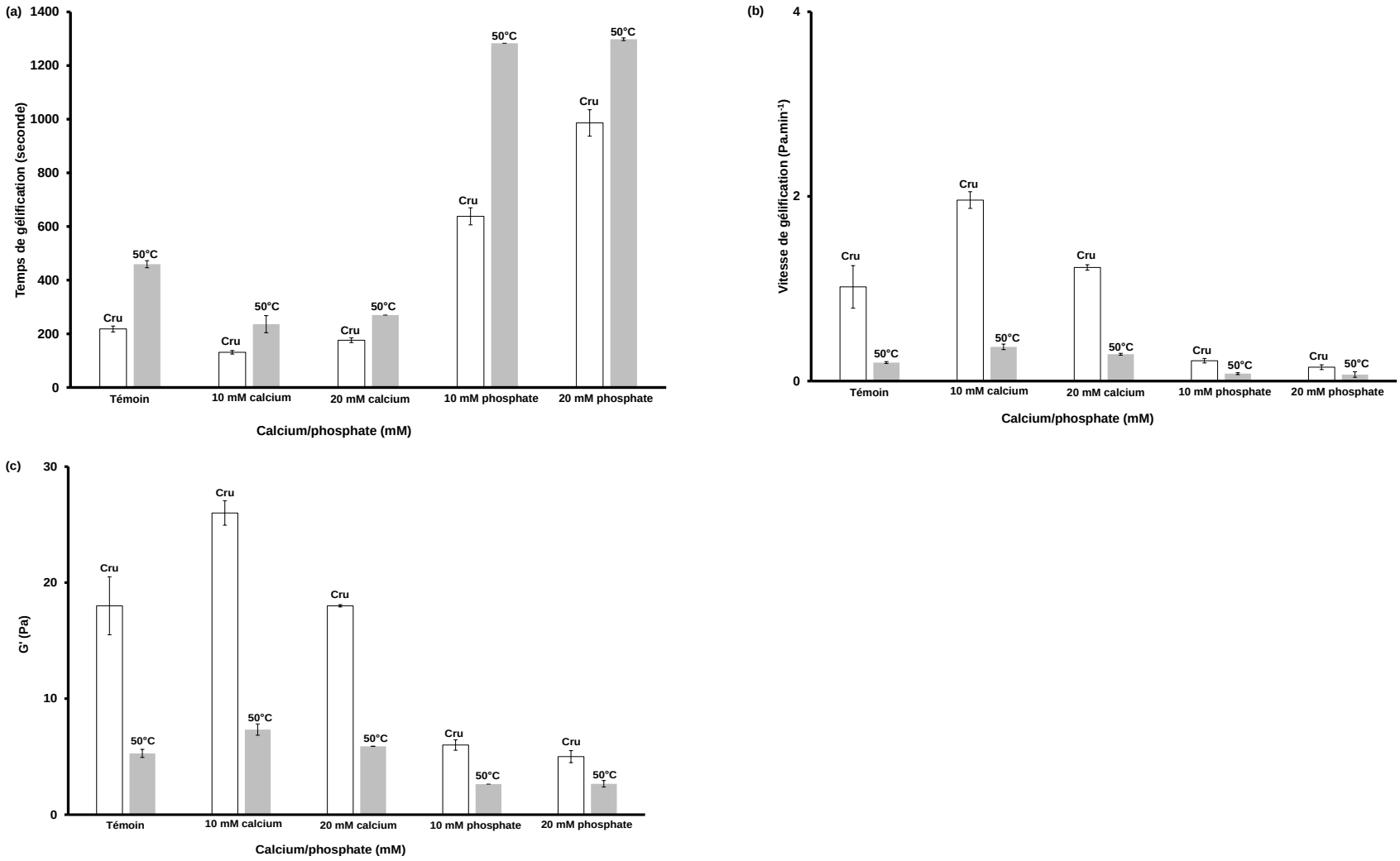

Figure 16 : Impact du préchauffage du lait de chamelle à 50 °C avec et sans ajout de minéraux sur les propriétés rhéologique de gels obtenus par coagulation enzymatique en comparaison au lait cru : (a) temps de gélification ; (b) vitesse de gélification ; et (c) module élastique (G').

de G' en fonction du temps et ce à partir du temps de gélification et pendant une durée de 4 minutes ; et iii) la valeur de G' après 45 minutes de gélification.

Les principaux résultats obtenus montraient que l'ajout de calcium à 10 et 20 mM aux laits de chamelle et de vache crus améliorait les propriétés de gélification par rapport aux laits témoins (sans ajout de minéraux). En effet, le temps de coagulation diminuait de 218 secondes pour le lait de chamelle témoin à 131 et 176 secondes pour ceux enrichis avec 10 et 20 mM de calcium, respectivement, et de 143 secondes pour le lait de vache témoin à 84 et 34 secondes respectivement pour le lait enrichi en calcium à 10 et 20 mM. L'ajout de calcium induisait une augmentation de la vitesse de coagulation qui passait de 1,02 $Pa.min^{-1}$ pour le lait de chamelle témoin à 1,96 et 1,23 $Pa.min^{-1}$ au lait supplémenté en calcium à 10 et 20 mM respectivement, et de 8,05 $Pa.min^{-1}$ pour le lait de vache témoin à 22,63 et 26,92 $Pa.min^{-1}$ pour ceux enrichis en calcium à 10 et 20 mM, respectivement. De plus, l'enrichissement du lait en calcium permettait d'améliorer la fermeté de gels puisque G' passait de 18 Pa pour le lait de chamelle témoin à 26 Pa pour celui enrichi en calcium à 10 mM ; cependant, une valeur de G' similaire au lait témoin a été obtenu suite à l'ajout de 20 mM de calcium au lait de chamelle. Des résultats différents ont été observés sur le lait de vache car la fermeté de gels augmentait avec l'ajout de 20 mM calcium où G' passait de 121 Pa pour le lait témoin à 184 et 215 Pa pour ceux enrichis en calcium à 10 et 20 mM, respectivement. En revanche, un effet inverse a été observé pour les laits de vache et de chamelle supplémentés en phosphate (**cf. article 1, Figure 3 et 4a et b**).

Le préchauffage du lait à 50 et 70 °C pendant 10 minutes semblait avoir un effet négatif sur les caractéristiques de gels avec et sans ajout de minéraux (**cf. article 2, Tableau 2 et Figure 2**). En effet, une augmentation du temps de gélification et une diminution de la vitesse de gélification et de la fermeté de gels ont été observées pour le lait de chamelle en comparaison au lait cru (**Figure 16 a, b, c**) ; les mêmes tendances ont été obtenues pour le lait de vache. Cependant, l'effet du préchauffage a été plus prononcé pour le lait de chamelle que pour le lait de vache.

Il est bien connu que le lait de chamelle est instable aux traitements thermiques. Les causes de cette instabilité lors du chauffage à haute température ne sont, à ce jour, pas encore totalement connues (Farah et Atkins, 1992). Une des causes avancées serait les faibles quantités de caséine-κ et l'absence de β-lactoglobuline responsables de la stabilité du lait à haute température. Nos résultats sont en accord avec les travaux de Farah et Atkins (1992) puisque le traitement thermique du lait de chamelle à 70 °C pendant 10 minutes ne permettait pas sa gélification. Les détails concernant les résultats et la discussion de ces études sont présentés dans les **articles 1 et 2**.

II.2. Coagulation par voie acide

Dans le cadre de ce travail, la GDL a été utilisée pour l'acidification du lait de chamelle et de vache. En effet, la GDL est une lactone (ester cyclique) dérivant de l'acide gluconique obtenue à partir du D-glucose par fermentation oxydative. Son hydrolyse dans le lait conduit à former de l'acide gluconique qui à son tour libère des protons et du gluconate dans le milieu selon la réaction suivante :

$$\mathbf{C_6H_{10}O_6\ (GDL) + H_2O \leftrightarrow C_6H_{12}O_7\ (acide\ gluconique) \rightarrow C_6H_{11}O_7^-(gluconate) + H^+}$$

Cette libération de protons conduit à une acidification du lait.

Ce système de coagulation permet d'éviter certaines difficultés liées à l'utilisation des bactéries lactiques, dont l'activité bactérienne est conditionnée par le type de ferment utilisé et l'adaptabilité des ferments lactiques aux paramètres technologiques comme la température, la durée de l'acidification, etc. (Lucey et al. 1998b).

Dans la présente étude, le lait de chamelle a été collecté dans une station expérimentale située dans le désert marocain (Foum Lahcen, province de Tata) et le lait de vache a été obtenu d'une ferme régionale de Lille (France). L'acidification a été effectuée en utilisant la GDL (GDL, Merck schuchardt OHG, Hohenbrunn, Allemagne) à 3 % (m/v). Les cinétiques de coagulation ont été suivies pendant 180 minutes à 36 °C en utilisant le test de cisaillement dynamique et la spectroscopie de fluorescence frontale. Les principaux paramètres enregistrés au cours de la coagulation sont G', G" et tan δ. En tenant compte des valeurs de G', les paramètres suivants ont été déterminés : i) le temps de gélification défini comme étant le temps pour lequel G' = 1 Pa ; ii) la vitesse de gélification correspondant à la pente obtenue par régression linéaire de l'évolution de G' en fonction du temps à partir du temps de gélification et pendant une durée de 15 minutes ; et iii) la valeur de G' après 180 minutes de gélification.

Les résultats obtenus par voie acide sont similaires à ceux observés par voie enzymatique puisque des différences significatives entre les caractéristiques de gels obtenus à partir du lait de vache de ceux produits à partir du lait de chamelle ont été observées. A nouveau, la fermeté de gels du lait de vache est plus élevée que celle observée pour le lait de chamelle avec et sans ajouts de minéraux (calcium, phosphate). L'ajout du calcium à 20 mM diminuait le temps de gélification et améliorait la fermeté de gels, et ce pour les deux types de lait. Comparé au lait témoin, l'ajout de phosphate ne montrait pas un effet significatif ($p < 0,05$) sur les propriétés gélifiantes du lait de chamelle. Alors qu'un effet significatif ($p > 0,05$) a été observé pour le lait

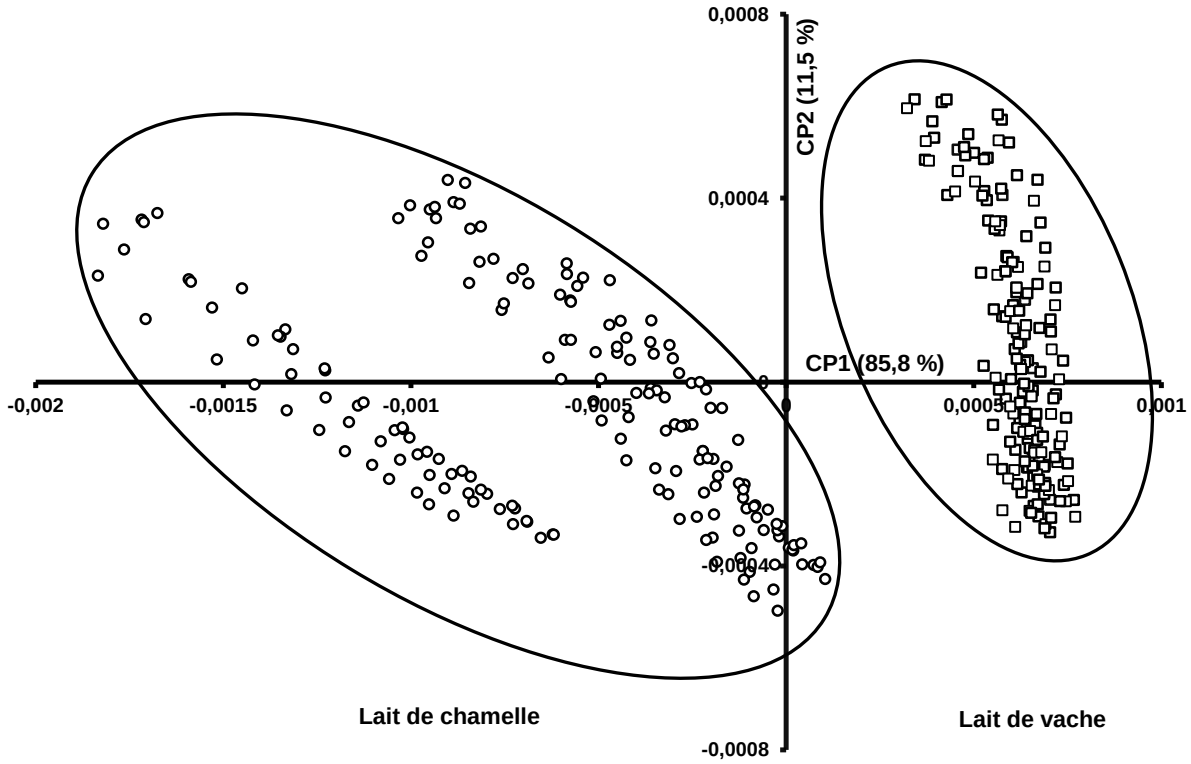

Figure 17 : Analyse en composantes principales réalisée sur les spectres normés de la vitamine A acquis au cours de la coagulation du lait de chamelle (○) et du lait de vache (□).

de vache (**cf. article 3, Tableau 1 et Figures 3a et b**). Les résultats et discussion de cette étude sont présentés dans **l'article 3**.

III. Spectroscopie de fluorescence frontale couplée aux techniques chimiométriques pour le suivi de la coagulation enzymatique du lait de chamelle en comparaison avec le lait de vache : corrélation avec les mesures rhéologiques

La spectroscopie de fluorescence est une méthode d'analyse rapide et non-destructive. Cette technique couplée à la chimiométrie a été utilisée avec succès pour le suivi de l'évolution de la structure et des interactions moléculaires durant la coagulation du lait de vache (Herbert et al. 1999 ; Blecker et al. 2012). En revanche, la potentialité de cette technique à détecter les changements structuraux des micelles de caséines au cours de la coagulation du lait de chamelle n'a pas été examinée à ce jour. Par conséquent, l'objectif de cette étude était d'évaluer le potentiel de la spectroscopie de fluorescence frontale couplée à des analyses statistiques multivariées pour déterminer les changements structuraux au niveau moléculaire durant la coagulation enzymatique du lait de chamelle en comparaison avec le lait de vache.

La coagulation a été réalisée à partir de lait de chamelle récolté en Tunisie (El Jem, Mahdia) et de lait de vache collecté d'une ferme régionale de Lille (France). La coagulation par voie enzymatique a été menée en utilisant la chymosine FAR-M®Sticks comme décrit précédemment (cf. II.1). Les cinétiques de coagulation ont été suivies pendant 45 minutes à 36 °C en utilisant le spectrofluorimètre Fluoromax-4 (Jobin Yvon, Horiba, NJ, Etats-Unis).

Les spectres d'émission de fluorescence du tryptophane des protéines (excitation : 290 nm ; émission : 305 – 450 nm) et d'excitation de la vitamine A (émission : 410 nm ; excitation : 252 – 390 nm) ont été enregistrés après 0, 10, 20, 30, 40 et 45 minutes de l'ajout de la chymosine au lait. Ces sondes ont été choisies dans le but d'obtenir des informations respectivement sur les modifications structurales des protéines et leurs interactions et sur l'état physique des triglycérides présents dans les globules gras.

L'application de l'ACP aux spectres normés du tryptophane acquis sur les laits de chamelle et de vache au cours de la coagulation a permis de les séparer clairement (**cf. article 4, Figure 3a**). Des résultats similaires ont été obtenus suite à l'ACP appliquée aux spectres de la vitamine A (**Figure 17**).

Dans un deuxième temps, l'ACP a été appliquée séparément à chaque type de lait et chaque condition expérimentale (lait cru, lait préchauffé à 50 et 70 °C pendant 10 minutes). L'ACP réalisée sur les spectres du tryptophane collectés sur le lait cru de chamelle dans

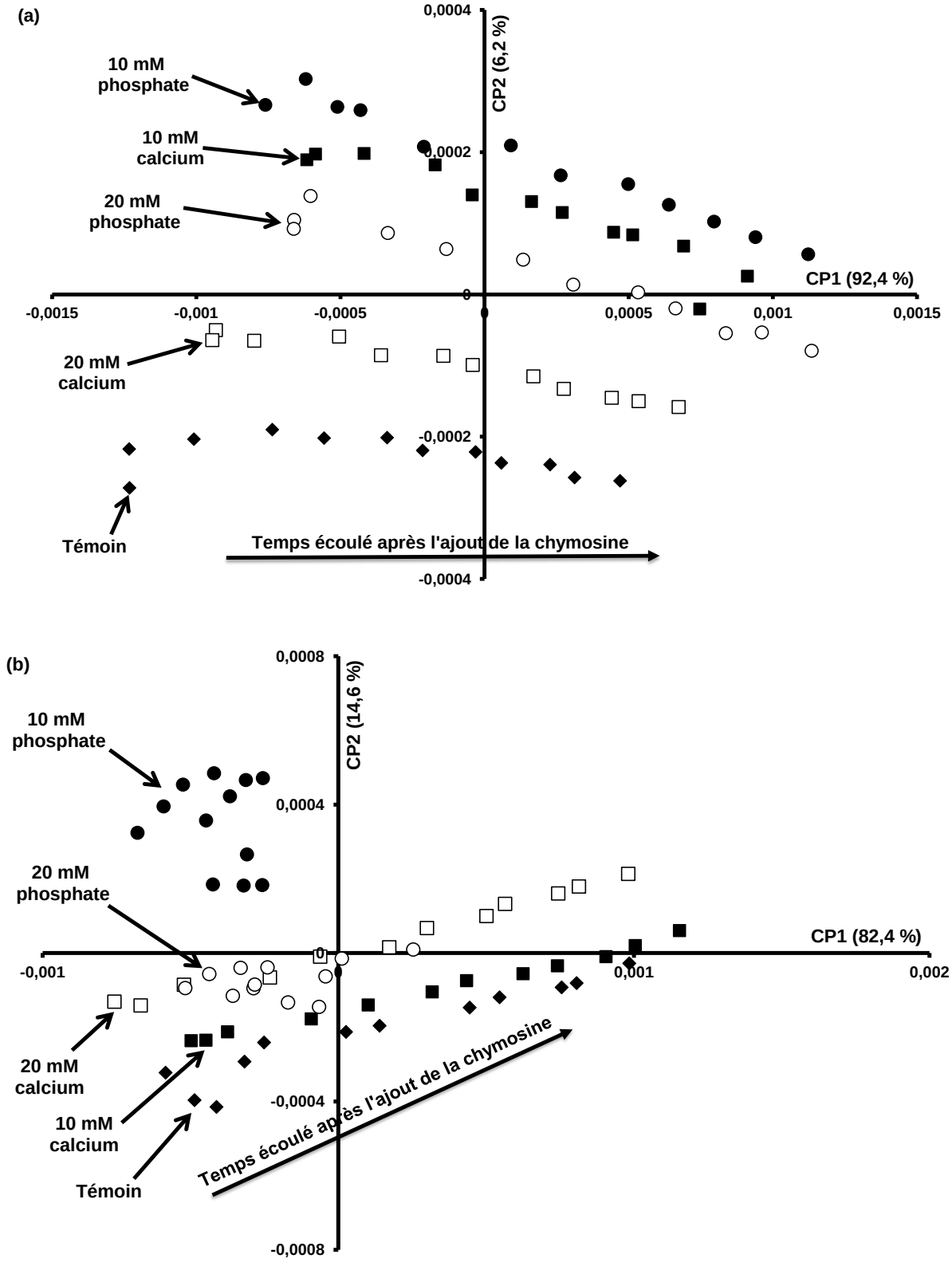

Figure 18 : Analyse en composantes principales réalisée sur les spectres normés du tryptophane enregistrés au cours de la coagulation du lait de chamelle préchauffé à 50 °C (a) et 70 °C (b).

différentes conditions expérimentales (témoin et enrichi avec du calcium et du phosphate à 10 et 20 mM) a permis de suivre les différentes étapes de la coagulation (**cf. article 4, Figure 3b**). L'ACP appliquée au lait de chamelle préchauffé à 50 °C a montré une bonne discrimination entre les spectres du lait témoin et ceux enrichis en minéraux à différentes concentrations (**Figure 18a**). En effet, selon la CP2 expliquant 6,2 % de la variance totale, les échantillons du lait témoin et ceux enrichis en calcium à 20 mM sont positionnés du côté négatif de l'axe, alors que ceux enrichis en calcium à 10 mM et en phosphate à 10 et 20 mM sont situées quasiment du côté positif de l'axe. De plus, une tendance de bonne discrimination des échantillons de laits en fonction du temps de coagulation a été observée. Concernant l'ACP réalisée sur les spectres du tryptophane du lait de chamelle préchauffé à 70 °C (**Figure 18b**), la CP2 représentant 14,6 % de la variation totale permet une bonne séparation entre les échantillons du lait témoin de ceux enrichis en calcium et phosphate. De plus, une discrimination nette en fonction du temps de coagulation a été observée pour les laits témoins et ceux enrichis en calcium qui montrent des trajectoires similaires. Cependant, les échantillons supplémentés en phosphate à 10 et 20 mM présentent une trajectoire différente. Cette différence pourrait provenir de l'effet négatif de l'ajout du phosphate sur la coagulation du lait comme cela a été observé au moyen du test de cisaillement dynamique. Des résultats quasi-similaires ont été observés avec la vitamine A.

En ce qui concerne le lait de vache, des résultats quasi-similaires ont été obtenus suite à l'application de l'ACP aux spectres du tryptophane et aux spectres de la vitamine A enregistrés pour chaque condition expérimentale : lait cru, lait préchauffé à 50 et 70 °C (**Figure 19 a, b, c**).

Dans un deuxième temps, nous avons voulu analyser conjointement les tableaux de données rhéologiques et de fluorescence acquis sur les échantillons de lait au cours de la coagulation. Les résultats obtenus indiquent une bonne discrimination des échantillons de lait en fonction du traitement thermique appliqué au lait de départ, mais aussi de la nature et de la concentration du minéral ajouté au lait (**cf. article 4, Figure 4a**). L'examen des poids spécifiques de chaque tableau de données montre que les paramètres rhéologiques contribuent faiblement à la formation de deux composantes communes, alors que les spectres de fluorescence du tryptophane et de la vitamine A semblent décrire des phénomènes similaires (**cf. article 4, Tableau 1**). Pour confirmer cette hypothèse, nous avons appliqué une ACCPS sur les spectres normés du tryptophane et de la vitamine A. Le plan défini par les composantes communes 2 et 4 montre une meilleure discrimination des échantillons du lait que celle obtenue avec l'ACCPS appliquée aux données rhéologiques, du tryptophane et de la vitamine A (**cf. article 4, Figure 5a**). A nouveau les variations spectrales acquises par chaque fluorophore sont dépendantes. Il en ressort que les interactions mises en œuvre concernent les globules gras et le

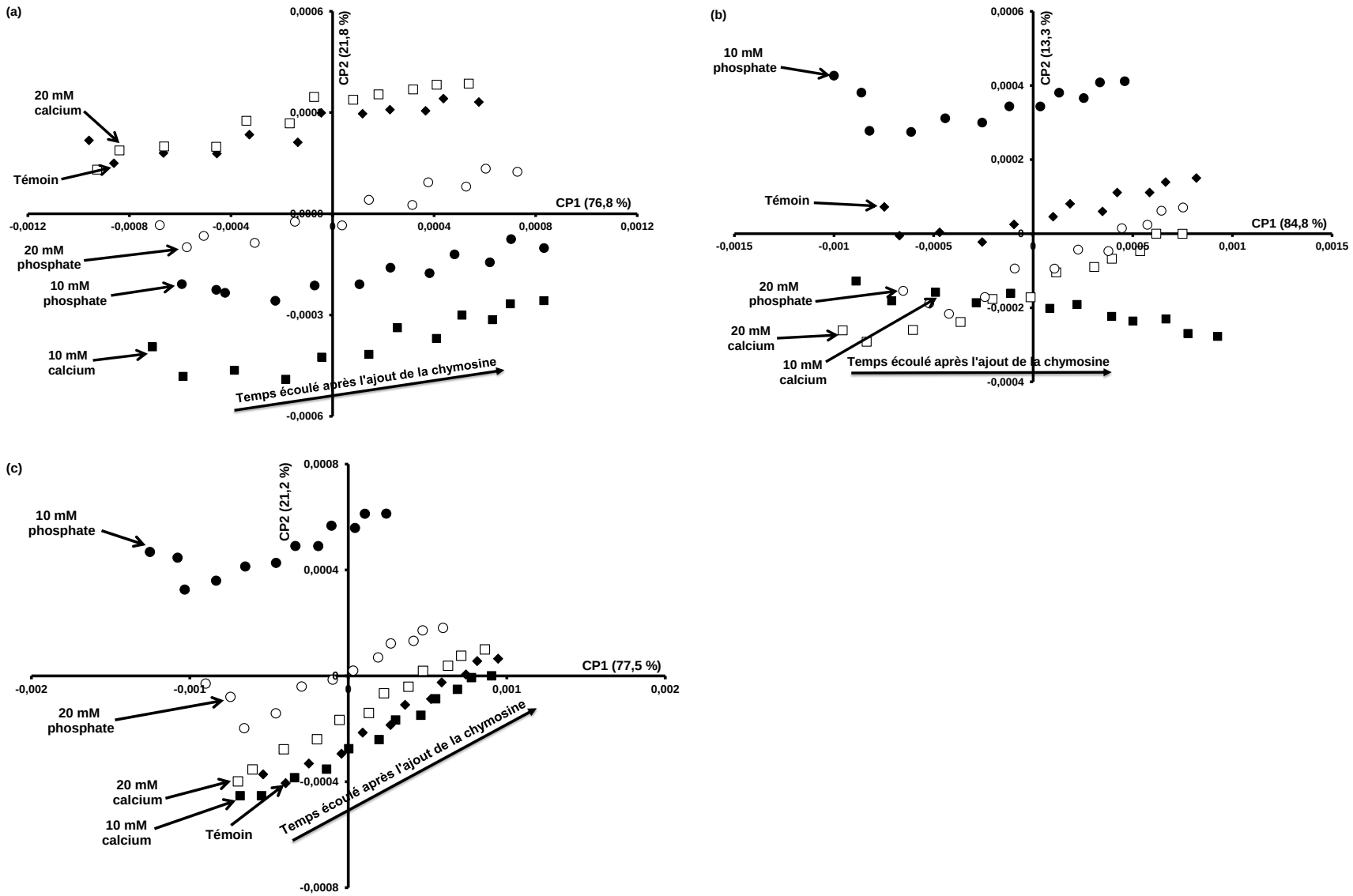

Figure 19 : Analyse en composantes principales réalisée sur les spectres normés du tryptophane enregistrés au cours de la coagulation du lait de vache cru (a), lait préchauffé à 50 °C (b) et à 70 °C (c).

réseau protéique provoquant des modifications dans les allures des spectres de vitamine A et des tryptophanes ; ces résultats sont en accord avec les travaux de Karoui et al. (2003a) et ceux de Herbert et al. (2000) qui ont observé sur des fromages à pâte molle que les interactions protéines-matières grasses induisaient des modifications dans l'allure des spectres de la vitamine A et du tryptophane.

Les résultats obtenus montrent que les spectres de fluorescence sont très sensibles aux modifications de la structure des micelles de caséines au cours de la coagulation enzymatique du lait. De par sa rapidité et sa sensibilité, cette méthode permet d'acquérir sur le même échantillon des spectres en fixant les longueurs d'onde d'excitation ou d'émission, ce qui n'est pas le cas pour le test de cisaillement dynamique. Les résultats et discussion de cette étude sont présentés dans **l'article 4**.

Fortifying camel and cow milk with calcium and phosphate and their impact on the rheological properties of rennet-induced coagulation

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Abstract

The rheological properties of rennet-induced coagulation of raw camel and cow milk enriched with calcium chloride ($CaCl_2$) and di-sodium hydrogen phosphate dihydrate ($Na_2HPO_42H_2O$) were studied using dynamic low amplitude oscillatory shear analysis. The final values of storage modulus (G′) and loss modulus (G″) of camel milk gels were significantly ($p < 0.05$) lower than those of cow milk gels. The addition of $CaCl_2$ at 10 mM to cow and camel milk and 20 mM to cow milk reduced significantly ($p < 0.05$) the gelation time and increased the gel firmness. However, the enrichment of milk with $Na_2HPO_42H_2O$ at 10 and 20 mM induced an opposite trend since a significant delay ($P < 0.05$) of the onset of gelation and a significant decrease of the gel firmness were observed.

Keywords: Rheology, Coagulation, Camel milk, Cow milk, Minerals.

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INTRODUCTION

Camel milk is very important in the human diet, especially, for the people living in the desert (arid) and semi-arid zones,[1] thanks to its richness in many compounds. For example, camel milk is known to have: i) a level of vitamin C three to five times higher than that in bovine milk;[1] and ii) a higher antibacterial and antiviral activity and many potential therapeutic properties than bovine milk.[2,3]

In the meantime, camel milk is known to present limit ability to the enzymatic coagulation i.e., rennet coagulation time is two to three times slower than cow milk treated under the same conditions.[4] This trend has been attributed to the special composition of the casein micelles.[5] Indeed, the κ-casein of camel milk, representing the micellar fraction reacting with the clotting enzymes has a different electro-potential than that of cow milk, causing lower electrophoretic mobility. Additionally, the low level of κ-casein and the large size of casein micelle of camel milk make the former milk difficult to coagulate.[6,7]

Calcium and phosphate are essential nutrients of the mineral fraction of camel and cow milk and their concentrations in milk contribute significantly to the gelation process. Glantz et al.[8] reported that the higher the level of ionic calcium in cow milk, the lower the gelation time and the higher the firmness of the gel, in agreement with others.[9,10] However, it should be kept in mind that there is a threshold of added calcium that varied according to studies beyond which, an increase of the positive charge of casein micelles caused an increase in the electrostatic repulsion and a weakness of the gel allowing in some case the absence of calcium-induced gelation.[11,12]

The mineral fractions present a key role in the formation of the coagulum and thus of the final product. Previous studies,[13,14] pointed out that the fortification of camel milk with calcium improved the gelation properties, in disagreement with the findings of Konuspayeva et al.[15] who did not observe any difference in the clotting time and curd firmness between milk supplemented with calcium and control (without added calcium). Regarding the enrichment of milk with phosphate, several authors pointed out that added phosphate to cow milk delayed the onset gelation and in some case prevents cow milk gelation.[12,16,17] This trend has been attributed to the decrease in the amount of ionic calcium leading to increase the negative charge of casein micelles.

Although several studies have been published on the effect of calcium and phosphate induced coagulation of cow milk, at our best knowledge, no research data has been conducted about the effect of added phosphate on raw camel milk gels. In addition, all the studies were performed separately on camel or cow milk gels. Therefore, the aim of this work was to determine the rheological properties of rennet-induced coagulation of raw camel milk gels enriched with calcium and phosphate at 10 and 20 mM compared to cow milk gels.

MATERIALS AND METHODS

Milk samples

Fresh camel milk (2 L distributed into plastic bottles of 30 mL capacity) was collected from an experimental farm located in the center of Tunisia (ElJem region, Mahdia governorate, Tunisia) by directly milking into a sterile milking bottle and the milk was transported using an icebox. Camels of Maghrebine genotype aged of 6 years were maintained on pasture feeding (yearly pasture of thorny plants and coquelicot) and supplemented with concentrate. They were inspected by a qualified shepherd on a daily basis, and routine animal care and vaccination procedures were conducted as prescribed by best practice protocols.

Fresh cow milk (2 L distributed into plastic bottles of 30 mL capacity) of

Holstein Friesian genotype was collected from a regional farm of Lille (France). Cows were maintained on pasture feeding and supplemented with a corn-based concentrate. Once arrival to the laboratory, milk samples were kept at −18 °C until analysis. All the analyses were made in duplicate.

Physicochemical analyses of camel and cow milk

The pH, protein, fat and dry matter contents of camel and cow milk were determined as described by a previous study.[18]

Size distribution of milk casein micelles

Skimmed milk was obtained by centrifugation at 3740 g for 15 min using a cream separator (Froilabo-SW14R, France). Number-based particle size distribution of skimmed milk was determined by using a SALD-2300 laser diffraction particle size analyzer in wet type mode (Shimadzu Scientific Instruments, Paris, France). Milk samples were directly injected into the dispersion cell (containing deionized water as dispersant) until a light intensity distribution more than 10 % was reached and the particle size distributions were measured. The refractive index of 1.55 was used according to the findings of Griffin and Griffin[19] who have used refractive index value of 1.57. For the dispersant, a blank was measured with deionized water at which the light intensity distribution is less than 10 %.

Samples preparation for the gelation

Milk samples (10 samples in total for each milk species) were thawed during 12 h at 4 °C and then kept at room temperature (~ 18 °C) for 15 min to avoid thermal shock and gently mixed. Milk samples were, after, placed in a water-bath equilibrated previously at 36 °C during 5 min. Then, calcium chloride anhydrous ($CaCl_2$, Merck, Darmstadt, Germany) and di-Sodium hydrogen phosphate dihydrate ($Na_2HPO_42H_2O$, Merck Darmstadt, Germany) powder at 10 and 20 mM were separately added to milk samples with stirring for 2 min. A volume of 6.25 µL of the rennet FAR-M®Sticks (12500 IMCU/stick) (Chr. Hansen A/S BoegeAllé, Hoersholm Denmark) prepared directly before the test from 1 g of powder FAR-M®Sticks diluted in 20 g of double distilled water was added to 25 mL of milk and rheological measurements were immediately performed. Control milk (i.e., raw milk samples without added $CaCl_2$ and $Na_2HPO_42H_2O$) was also prepared.

Rheological and pH measurements throughout rennet-induced coagulation

The experiments were performed using a controlled-strain rheometer (Physica MCR 301, Anton Paar Company, Germany) with low amplitude oscillation shear analysis and a temperature set at 36 °C by applying a Peltier plate. A volume of 20 mL was placed in two concentric cylinders, with an inner diameter of 26.66 mm, length of 40.02 mm, and a gap of 1.13 mm. A layer of liquid paraffin was placed onto the surface of milk sample to prevent evaporation during coagulation, and then measurement was started. The oscillation shear analyses were performed in the linear viscoelastic region during 45 min by applying a constant frequency of 1 Hz and a strain of 0.05 %. The gels were then subjected to a frequency sweep from 1 to 10 Hz (at 36 °C; 0.05 % strain), followed by an amplitude sweep from 0.05 to 150 % with a frequency of 1 Hz. Taking into account the elastic modulus (G'), the following parameters were determined: i) the gelation time defined as the time when G' = 1 Pa as reported by others;[20,21] ii) the gelation rate (defined as dG'/dt) corresponding to the slope obtained by linear regression of the evolution of G' *versus* time from the gelation onset over a period of 4 min; and iii) the asymptotic value of G' after 45 min of gelation (G'_{asym}). The pH was determined every 15 min throughout the whole rennet-induced coagulation period.

Statistical analysis

In order to detect difference between milk samples, rheological and physico-chemical parameters were compared using a one-way ANOVA ($p < 0.05$). ANOVA was applied using XLSTAT 2013 (Addinsoft SARL USA, New York, NY, USA) software.

RESULTS AND DISCUSSION

Physicochemical parameters of camel and cow milk

Physicochemical analyses conducted on raw milk showed a significant difference ($p < 0.05$) between pH values of camel and cow milk (i.e., 6.51 ± 0.13 and 6.42 ± 0.01, respectively). Dry matter, protein and fat contents of camel milk (110 ± 10.00, 28.58 ± 0.21 and 34.50 ± 0.51 $g.L^{-1}$, respectively) were significantly ($p < 0.05$) lower than those of cow milk (126.13 ± 8.48, 34.70 ± 0.55 and 37.50 ± 0.50 $g.L^{-1}$, respectively), in agreement with previous findings.[1,7,22,23]

During coagulation, the pH of raw camel and cow control milk gels changed slightly throughout the whole gelation reaching after 45 min 6.65 ± 0.03 and 6.45 ± 0.03, respectively (**Figs. 1a**, **b**). The addition of calcium to milk induced a significant decrease ($p < 0.05$) of pH values compared with control milk gels, irrespective of milk species A decrease of pH values of camel and cow milk gels enriched with 20 mM calcium was observed (**Figs. 1a, b**) attaining after 45 min of gelation 6.00 ± 0.06 and 5.65 ± 0.01, respectively, in agreement with others.[4,9,24] This decrease in the pH values of both types of milk is a manifestation of the calcium equilibrium between calcium phosphate salts and Ca^{2+}.[25]

In contrast, the enrichment of milk with phosphate resulted in a significant increase ($p < 0.05$) of pH values compared with control milk gels (**Figs. 1a, b**), since the pH value after 45 min of gelation was of 7.22 ± 0.10 and 6.90 ± 0.01, respectively for camel and cow milk gels enriched with 20 mM phosphate, in agreement with the findings of a previous study,[26] who attributed this trend to the increase of negative charge casein.

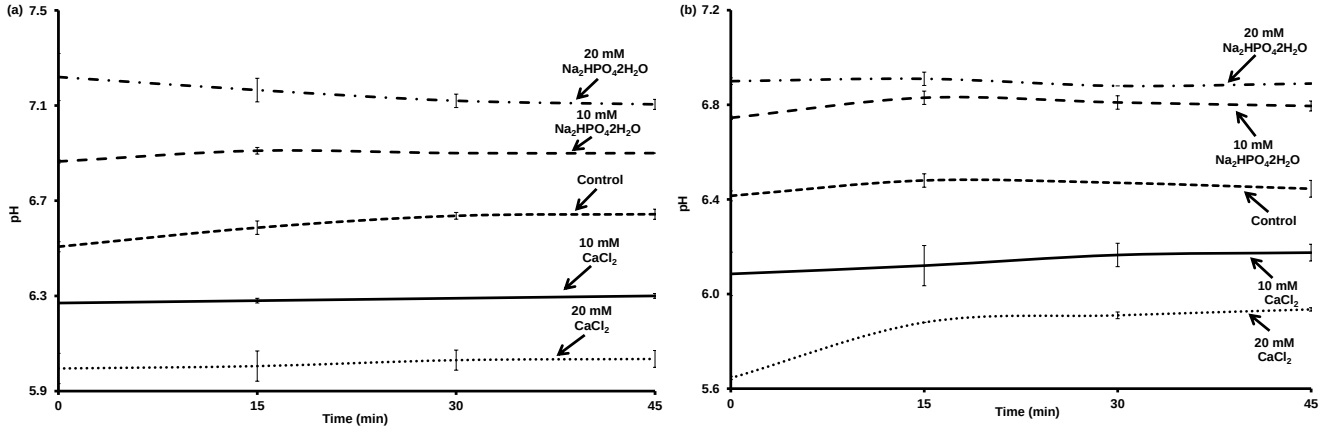

Figure 1: Evolution of pH values as a function of time for: (a) raw camel and (b) raw cow milk gels (data are means of duplicates with standard deviation).

Size distribution of casein micelles

The results exhibited a significant difference ($p < 0.05$) between the casein micelle sizes of camel milk that was of ~ 471 ± 2.50 nm, while it was only ~138 ± 1.50 nm for cow milk (**Fig. 2**). This result was in line with previous investigation,[7] reporting that the diameter of casein micelles of camel milk varied in the range 280-550 nm, larger than that of cow milk having a diameter in the 90-210 nm range. This difference could be attributed to the variation in the proportion of κ-casein, since the micelle size decreased with the increase of κ-casein proportion.[27] Some authors,[6,28] confirmed these findings since only 3.47 % of the total casein corresponds to κ-casein in camel milk, while it was ~ 13 % for cow milk.

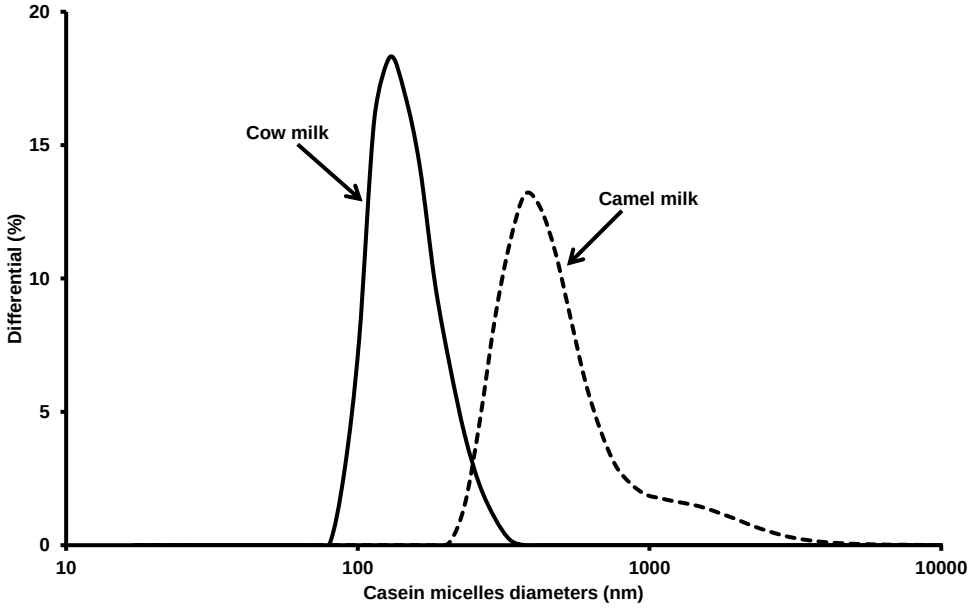

Figure 2: Diameters of casein micelles (nm) determined by laser light scattering on raw camel and cow milk (data are means of duplicates).

Rheological properties of calcium / phosphate-induced camel and cow milk gels

Calcium / phosphate-added milk induced the gelation of both camel and cow milk, irrespective of the calcium and phosphate concentration (**Figs. 3a, b, c**). With the added calcium, a reduction of the gelation time and an increase of the gelation rate and the G'_{asym} values were observed, while opposite trend was obtained with added phosphate. Although an improving in the gelation properties of both types of milk enriched with calcium has been observed, a different trend was noted for the two gel samples (**Figs. 3a, b, c and 4a, b**). This could be due to the low levels of protein and dry matter (110 and 28.58 $g.L^{-1}$, respectively) in camel milk compared to cow milk (126.13 and 34.70 $g.L^{-1}$, respectively), in agreement with others,[5,29] reporting that protein and dry matter contents in milk affected significantly the gelation properties.

The fortification of camel milk with 10 mM calcium allowed to obtain better gelation properties than the control milk since the gelation time decreased significantly ($p < 0.05$) from 218 sec for control milk gels to 131 sec, at which the gelation rate increased significantly ($p < 0.05$) from 1.02 to 1.96 Pa/min for control camel milk gels and those enriched with 10 mM calcium,

respectively (**Fig. 4a** and **Figs. 3a, b**). These results are in agreement with previous research data,[4,5] who reported that the addition of calcium to camel milk accelerated the renneting process, decreased the gelation time, and improved the renneting properties. This trend could be ascribed to the increase of calcium concentration in milk, inducing an increase of ash content and thus improving the renneting ability of the curd.

In contrast, no significant difference ($p < 0.05$) was observed between control gels and those supplemented with 20 mM calcium, which could be explained by the fact that excess calcium added to camel milk increased the positive charges of casein micelles, inducing charge repulsion and the formation of weaker gels compared to control gels.

Regarding cow milk gels, the gelation properties improved significantly ($p < 0.05$) with the increase of added calcium level. According to **Figs. 3a, b** and **Fig. 4b,** the higher the added calcium levels, the shorter the gelation time and the higher the gelation rate. Our results showed that compared with control milk gels presenting a gelation time of 143 sec with a gelation rate of 8.05 Pa/min, calcium added at 10 and 20 mM shortened significantly ($p < 0.05$) the gelation time (86 and 34 sec, respectively) and induced a significant ($p < 0.05$) rapid gelation rate (22.63 and 26.92 Pa/min, respectively). These variations could be explained by the increase of ionic calcium level in milk leading to increase the enzyme activity and casein micelles closeness during gelation process as described by others.[10,12,30]

According to **Figs. 3a, b, c**, and **Fig. 4a, b** added phosphate delayed significantly ($p < 0.05$) the gelation onset and reduced the gelation rate and the firmness of both types of milk gels. Indeed, compared to control camel milk gels, the addition of phosphate at 10 and 20 mM caused: i) a significant increase of the gelation time by a factor of 2.92 and 4.52, respectively; and ii) a decrease of the gelation rate since it passed from 1.02 Pa/min for control milk gels to 0.22 and 0.15 Pa/min for those enriched with phosphate at 10 and 20 mM, respectively.

The addition of phosphate to cow milk gels showed similar trend to that observed with camel milk gels, since significant delay of the gelation onset and reduction of the gelation rate ($p < 0.05$) was observed. We noticed also the formation of weak gels compared to control milk gels (**Fig. 4b** and **Figs. 3a, b, c**). Indeed, the gelation time of cow milk gels supplemented with 10 and 20 mM phosphate was of 625 and 889 sec, with a gelation rate of 1.77 and 1.27 Pa/min, respectively.

Despite no scientific data are available in the literature concerning the effect of added phosphate on the properties of raw camel milk gels, our results obtained with cow milk gels were consistent with others reporting that the addition of phosphate to recombined milk delay the gelation time,[16] and prevented the gelation of milk products.[31] The onset gelation delay and the decrease of gelation rate could be due to the complexation of calcium with added phosphate, leading to a decrease in the quantity of ionic calcium and consequently an increase in the negative charges of casein micelles.[26] In addition, the added phosphate to milk induced a decrease in the number of carboxyl residues, which promote the formation of the network, and increased the micellar diameter leading to the rehydration of the casein micelles.[17]

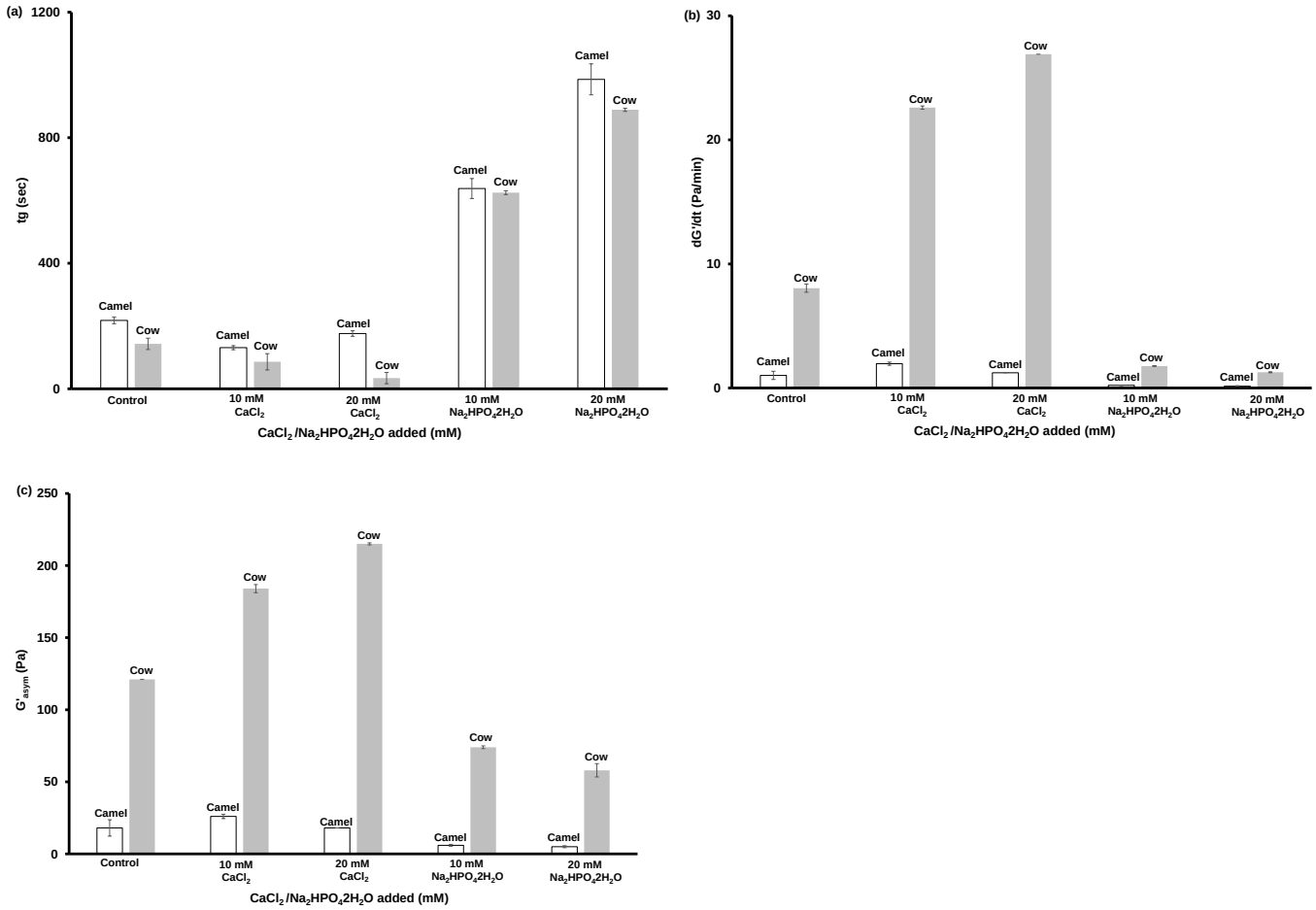

Figure 3: Gelation kinetics parameters including: (a) gelation time, (b) gelation rate, and (c) asymptotic value of storage modulus after 45 minutes of gelation (G'_{asym}) for control raw cow and camel milk gels and those enriched with calcium and phosphate (data are means of duplicates with standard deviation).

Evolution of storage and loss modulus of calcium/phosphate induced milk gels as a function of frequency

The evolution of G′ as a function of frequency and strain amplitude sweep tests are depicted in **Figs. 4c, d** and **5**. A slight increase of G' (**Figs. 4c, d**) and G" (data not shown) of calcium induced milk gels were observed with the increase of frequency in the 1-10 Hz range, irrespective of milk species. Considering each milk gel, separately, the difference between G' and G" is less than 1 log, particularly, for camel milk gels indicating that the structure of gels resulted probably from the formation of weak interactions between milk proteins.[32] In fact, when frequency increased from 1 to 10 Hz (**Figs. 4c, d**), G' values of control cow and camel milk gels were in the 116-163 Pa and 15-21 Pa range, respectively. The fortification of camel and cow milk with calcium resulted in an increase of both G' (**Fig. 4c, d**) and G" values (data not shown) as a function of applied frequency (i.e., 1-10 Hz range), in agreement with the findings of Ramasubramanian et al.[21] who observed an increase of G' values for calcium-induced cow milk gel with the increase of frequency from 0.1 to 10 Hz reflecting that bonds have less time to relax when the time scale of the applied stress is shorter.[33,34]

Over the entire used frequency range (1-10 Hz), the G' slopes obtained using a linear regression of control and calcium-

induced milk gels at different concentrations exhibited similar values (i.e. 0.15) for both camel (R^2 = 0.97) and cow (R^2 = 0.99) milk gels, indicating that the evolution of G' is related to the number of cross-linking caseins that reflected the more or less stabilized gels.[10] The G' slopes recorded with added phosphate at different levels of both types of milk gels were different. In the case of cow milk gels, the obtained slopes exhibited similar values to those observed with added calcium (i.e., 0.15 with R^2 of 0.99). The difference in the G' values which were found to be lower for gels obtained with milk enriched with phosphate could be attributed mainly to the decrease of formed calcium bridges between casein molecules. This effect was more pronounced for camel milk gels since the observed slopes (i.e., ~ 0.5 with R^2 of 0.93) were 3.3 times higher than those found for control camel milk gel reflecting the formation of a weaker gel.

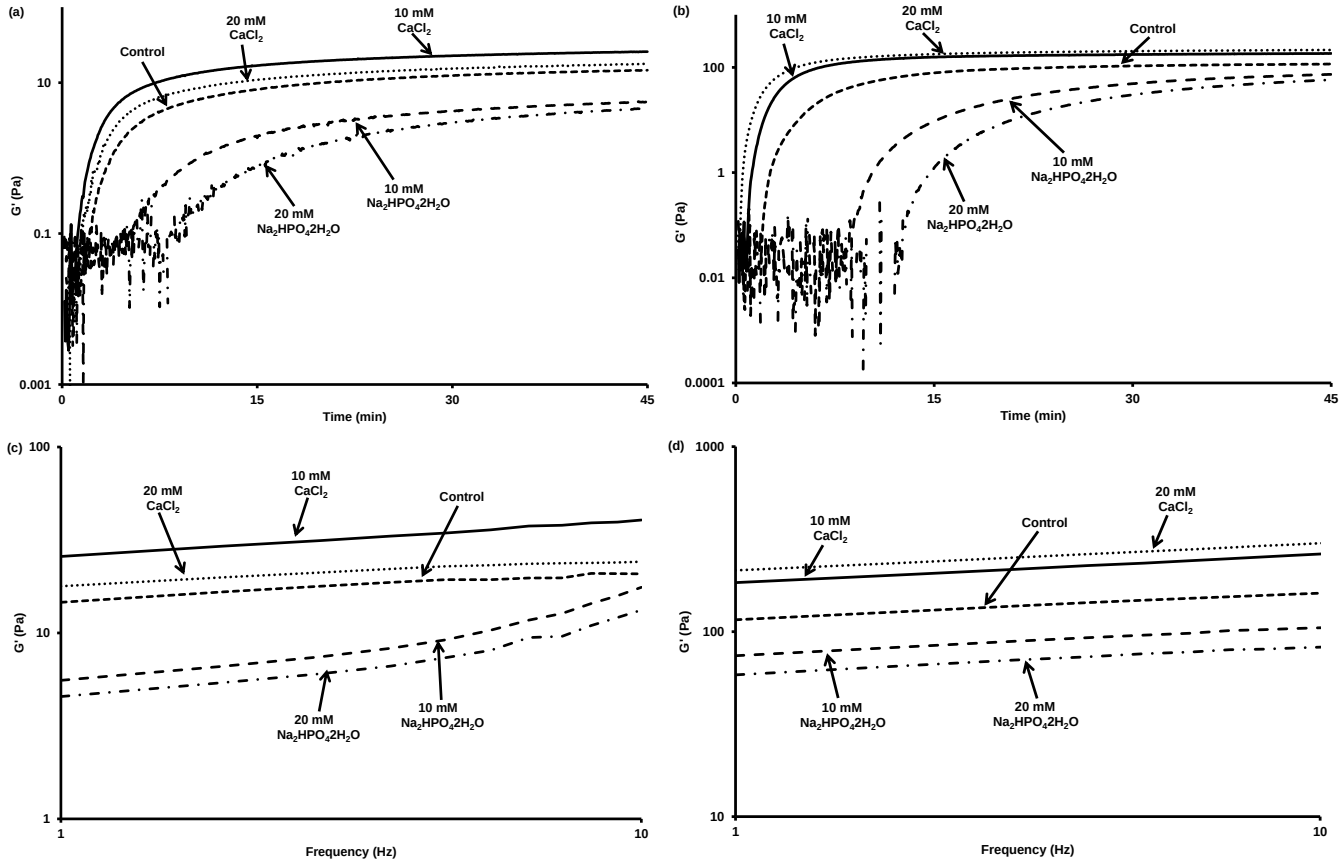

Figure 4: Storage modulus (G') of added calcium and phosphate milk as a function of: time (a, b) and frequency (c, d) of raw camel and cow milk gels (data are means of duplicates).

During the strain sweep tests (**Fig. 5a, b**), milk gels fortified with calcium showed higher breaking stress, regardless of the milk species; again, a different trend between both types of milk gels was observed. Camel milk gels enriched with 10 mM calcium exhibited the higher breaking stress, reaching values of ~ 7.9 Pa. Camel milk gels with added 20 mM calcium presented similar breaking stress values to that found with control milk gels (i.e., 4.4 Pa) (**Fig. 5a**). The breaking strain of control milk gels and those fortified with 10 and 20 mM calcium exhibited similar values of 48 %.

A different trend was observed for cow milk gels (**Fig. 5b**). The breaking stress of control milk gels and those added with 10

and 20 mM calcium was of 40.6, 69.45 and 80.95 Pa, respectively, while the breaking strain was of 103 % for control milk gels and 70 % for both added calcium concentrations. These results were in agreement with data described in a previous study,[21] who reported that the breaking stress of milk gels increased with the added level of calcium. This could be attributed to the increase of calcium amount in the casein micelles, inducing a higher number of formed calcium bridges in the protein network that contribute to the increase of the gels resistant to stress.[35]

The maximum value of breaking stress decreased with the added phosphate for both types of milk gels. However, this decrease was more pronounced for camel milk gels. Indeed, maximum breaking stress values of 2.16 and 2.03 Pa were observed with camel milk gels enriched with 10 and 20 mM phosphate presenting breaking strain of 48 and 70 %, respectively (**Fig. 5a**).

Regarding cow milk gels, the breaking stress was of 38.5 and 26.1 Pa with phosphate supplemented at 10 and 20 mM, while the breaking strain exhibited similar value of 103 % for both concentrations as depicted in **Fig. 5b**. These results agreed with those obtained by Guillaume et al.[17] who pointed out that the addition of phosphate reduced the ionic calcium content, causing a decrease in the bonds between the casein molecules and thus form a weaker gel that presented lower resistance to stress. The differences between camel and cow milk gel properties could be due to the variation in the protein levels of both types of milk (28.58 $g.L^{-1}$ in camel milk *versus* 34.70 $g.L^{-1}$ in cow milk), which increased the number of links and contact area in the gel network and thus the improving of the gel resistance to mechanical processes.

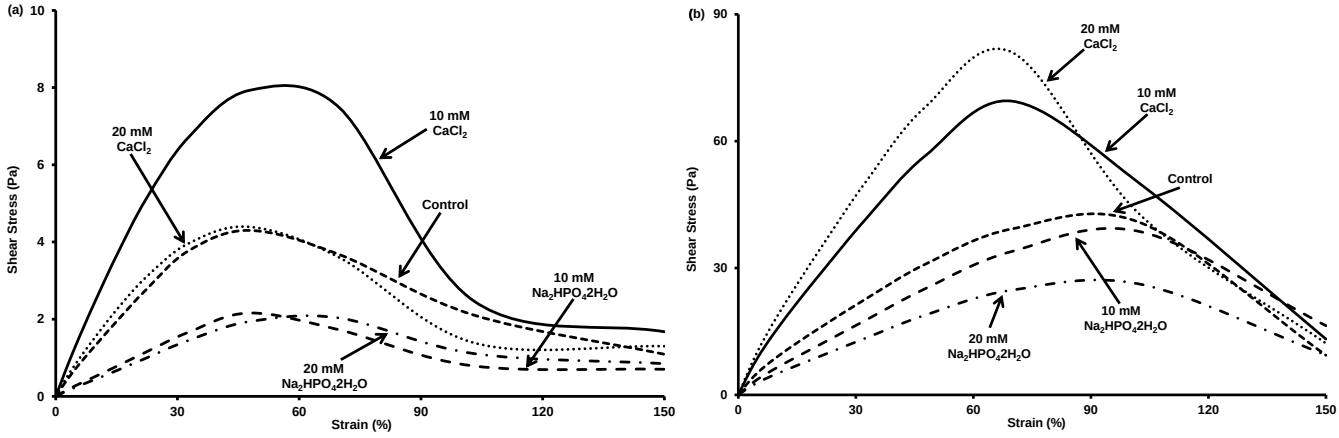

Figure 5: Shear stress as a function of strain of: (a) raw camel and (b) raw cow milk gels (data are means of duplicates).

Evolution of the final storage modulus of camel and cow milk gels as a function of the added level of calcium and phosphate

The evolution of G'_{asym} of camel and cow milk gels (**Fig. 6**) were in the following order: cow milk gels > camel milk gels. For a considered concentration of phosphate and calcium, cow milk gels presented significant higher G'_{asym} values ($p < 0.05$) than camel milk gels. Additionally, for a considered milk samples, G'_{asym} of calcium induced milk gels was significantly higher ($p < 0.05$) than those of phosphate induced milk gels. Indeed, the fortification of camel milk with phosphate resulted a significant decrease of G'_{asym} values compared to control camel milk gels and those enriched with calcium, since a decline of G'_{asym} values from 18, 18 and 26 Pa for control

camel milk gels and those supplemented with calcium at 20 and 10 mM, respectively to 6 and 5 Pa for milk gels incorporated with 10 and 20 mM phosphate, respectively was recorded (**Fig. 3c and 4a**). The G'_{asym} of cow milk gels fortified with phosphate at 10 and 20 mM showed values of 74 and 58 Pa, respectively and it passed to 121, 184 and 215 Pa for control milk gels and those added with 10 and 20 mM calcium, respectively (**Fig. 3c and 4b**). These results were in agreement with the finding of: i) Ramasubramanian et al.[21] who observed G'_{asym} values of 248.6 Pa with 20 mM added calcium to cow milk; and ii) Hailu et al.[14] who noted G'_{asym} values of 23 Pa for camel milk gel enriched with calcium at 1.8 mM. This trend could be ascribed to the reduction of the zeta potential of casein micelles, inducing a decrease of electrostatic repulsion and an increase of the calcium bridges formed between the casein micelles.[24,36]

The difference observed between camel and cow milk gels might be attributed to the variation in the physico-chemical parameters between both types of milk such as dry matter, protein and fat levels. Indeed, the low dry matter, protein and fat contents of camel milk in comparison with cow milk induced modifications in the properties of gel. In this context, Some authors,[5,13] pointed out that the low amount of dry matter and protein induced the formation of a weak curd due to the presence of lower number of gel-forming molecules that create fewer bonds per unit space. In addition, the low fat level of camel milk compared to cow milk provoked the formation of a weak gel since fat globules could interact with the gel matrix improving the firmness of milk gels.[5,37]

Furthermore, the size of the casein micelles affected the mechanical properties of the milk gels. It is well known that small casein micelles induced the formation of firmer gels,[38] in agreement with the results obtained in our study (**Fig. 2**) illustrating that the size of camel milk casein micelles (i.e., 471 nm) are significantly larger ($P < 0.05$) than those observed for cow milk (138 nm). This difference affected the clotting time and the characteristic of gels.[13] Indeed, for a considered condition, the G'_{asym} values of cow milk gels were higher than those of camel milk gels, in agreement with those of Logan et al.[39] who reported that the gel firmness of cow milk increased with small casein micelle size. This could be attributed to the increase of surface area provided by smaller casein micelle, leading to a greater availability of hydrolytic cleavage sites.

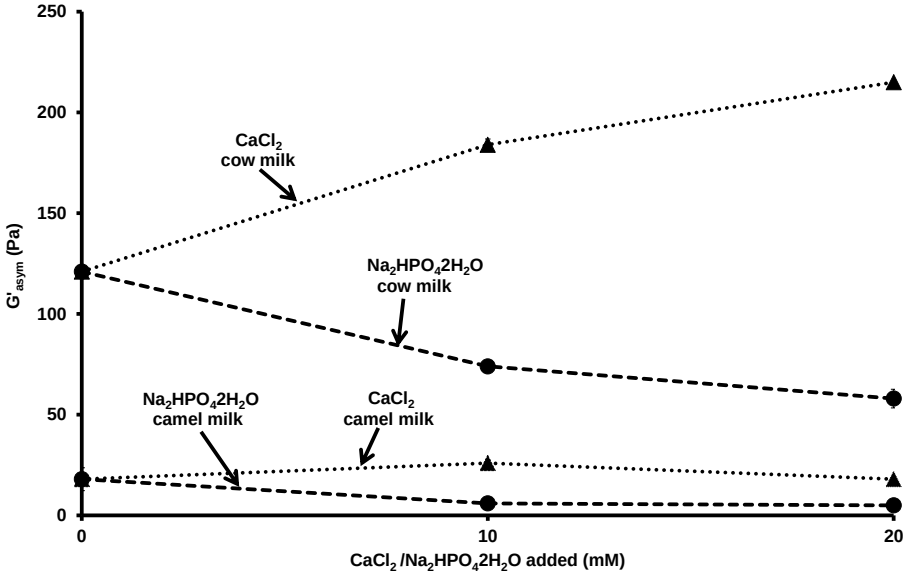

Figure 6: Effect of added calcium and phosphate on storage modulus after 45 minutes of gelation (G'_{asym}) of raw camel and cow milk gels (data are means of duplicates).

CONCLUSION

The study demonstrated that calcium and phosphate added at two concentrations induced the gelation of raw camel and cow milk. Calcium added at 10 mM improved the gelation properties of both types of milk gels, while calcium added at 20 mM to camel milk delayed the gelation time compared to that with 10 mM calcium. A different trend was observed for cow milk gels since the gelation properties were found to be improved with 20 mM added calcium. Phosphate added at different concentrations induced the formation of soft camel and cow milk gels compared to control milk gels.

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Effects of heating and mineral supplementation on the physical properties of rennet-induced coagulation of camel and cow milk gels

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Summary

The physical properties of rennet-induced coagulation of preheated camel and cow milk gels (50 and 70 °C for 10 min) enriched with calcium chloride ($CaCl_2$) and hydrogen phosphate dihydrate ($Na_2HPO_42H_2O$) were evaluated using the dynamic low amplitude oscillatory shear analysis. The storage modulus (G′) and loss modulus (G″) of camel milk gels showed significant ($p < 0.05$) lower values than those of cow milk gels. The increase of the intensity of camel milk preheating significantly affected the gelation properties of rennet-induced milk gels, while no effect was observed on the gelation properties of cow milk gels. The $CaCl_2$ added at 10 and 20 mM to preheated camel and cow milk reduced significantly ($p < 0.05$) the gelation time and increased the gel firmness. In contrast, $Na_2HPO_42H_2O$ added at 10 and 20 mM induced the formation of weak gels for preheated camel and cow milk at 50 °C, even no gelation for preheated camel milk at 70 °C.

Keywords: rheology; coagulation; milk; heat treatment; mineral salts

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Introduction

Camel milk represents one of the most interesting milk having an important role in human nutrition, particularly, for inhabitant in arid and semi-arid regions (Al-Haj & Al-Kanhal, 2010). For example, it was known that the level of vitamin C is three to five times higher than that in cow milk (Al-Haj & Al-Kanhal, 2010). In addition, camel milk contains higher levels of essential fatty acids and antimicrobial agents than all the other milk species and has potential therapeutic properties such as anti-diabetic as pointed out by Shori (2015). It is also considered as a promising new protein source for children allergic to cow milk due to the absence of β-lactoglobulin (El-Agamy et al. 2009). As indicated in **Table 1**, the major component of whey proteins in camel milk is similar to cow milk, except the absence of β-lactoglobulin in camel milk and peptidoglycan recognition protein in cow milk (Kappeler, 1998).

Camel milk is known to have limit ability for the enzymatic coagulation. Indeed, it exhibit a rennet-induced coagulation time two to three-fold longer compared with bovine milk (Farah & Bachmann, 1987). This behavior has been attributed to the specific structure of the casein micelles (El Zubeir & Jabreel, 2008). In fact, the low content of κ-casein and the large size of camel milk casein micelles compared with those of cow milk contributed largely to prolong the rennet-induced coagulation of camel milk (Kappeler et al. 1998).

Table 1: Detailed composition of casein and whey protein in camel and cow milk (Kappeler, 1998).

		Camel milk	
		Relative amount in total casein (%)	
Caseins	α_{s1}-CN	22	38
	α_{s2}-CN	9.5	10
	β-CN	65	39
	κ-CN	3.5	13
Whey proteins mg/L	α-la	>5000	600-1700
	PGRP	370	-
	Lactophorin	954	300
	Lactoferrin	220	140
	β-lg	-	<4000

PGRP: Peptidoglycan recognition protein

Heat treatment is an essential step of milk production adopted by the dairy industry either to improve desirable characteristics of the products, or to ensure its safety and shelf-life by reducing the microbial load (Donato & Guyomarc'h, 2009). However, this treatment results irreversible modifications for both the physical and chemical properties of milk (Vasbinder et al. 2003; Blecker et al. 2012).

In the literature, several studies were available regarding the impact of milk heating on the rheological properties of rennet-induced cow milk gels (Pomprasirt et al. 1998; Renault et al. 2000; Blecker et al. 2012). At the contrary, a very limited research studies was available in the literature on camel milk (Felfoul et al., 2017; Hattem et al. 2011). Felfoul et al. (2017) reported that heating camel milk at 80 °C for 60 min induced a complete disappearance of α-lactalbumin and peptidoglycan recognition protein and a decrease of 42 % of serum albumin concentration.

Regarding cow milk, it is well known that milk heat treatment at temperatures higher than 55 °C induced the denaturation of whey proteins causing an increase and a decrease of the gelation time and the curd firmness, respectively. This trend has been ascribed to the inhibition of the enzyme action on κ-casein resulting from the interaction of this casein with denatured whey proteins (particularly β-lactoglobulin) allowing the formation of whey protein/κ-casein complexes (Blecker et al. 2012).

Calcium and phosphate are essential elements of the mineral fraction of milk and their levels play a key role in the coagulation process. Gustavsson et al. (2014) found that high ionic calcium level in milk improved the gelation properties. This finding was, recently, confirmed by Ramasubramanian et al. (2014) reporting that added calcium to preheated cow milk at 90 °C for 10 min decreased the gelation time and increased the curd-firming rate. The authors ascribed these changes to the reduction of ionic calcium-induced pH of milk which induces a decrease of electrostatic repulsion between micelles allowing the formation of calcium bridges between caseins particles and the increase of calcium colloidal phosphate level. In contrast, the incorporation of phosphate in milk induced a delay in the onset gelation of the rennet-induced coagulation resulting from the decrease of the ionic calcium amount (Guillaume et al. 2004).

Although several studies have been published on the effect of milk pre-heating and its enrichment with calcium and phosphate on the physical properties of rennet-induced cow milk gels, to the best of our knowledge, the scientific literature do not contains information on the impact of added calcium and phosphate on the rheological properties of simultaneously preheated camel and cow milk gels, since all the studies were performed on cow or camel milk gels separately. In addition, no research has been conducted on the effect of added phosphate on preheated camel milk gels. Thus, the aim of this study was to monitor rheological properties of the rennet-induced coagulation of camel milk preheated gels at 50 and 70 °C and enriched with calcium and phosphate at 10 and 20 mM compared to cow milk gels.

Materials and methods

Milk samples

Fresh camel milk (2 liters distributed into plastic bottles of 30 mL capacity) was obtained from an experimental station located in the center of Tunisia (ElJem region, Mahdia, Tunisia) by directly milking into a sterile milking bottle and the milk was transported using an icebox. Camels of Maghrebine genotype aged of 6 years were maintained on pasture feeding (yearly pasture of thorny plants and coquelicot) and supplemented with concentrate. They were inspected by a qualified shepherd on a daily basis, and routine animal care and vaccination procedures were conducted as prescribed by best practice protocols.

Fresh cow milk (2 liters distributed into plastic bottles of 30 mL capacity) of Holstein Friesian genotype was obtained from a regional farm of France (Nord Pas de Calais, 59000 Lille, France). Cows were maintained on pasture feeding and supplemented with a corn-based concentrate.
Once arrival to the laboratory, milk samples were kept at -18 °C until analysis. All the analyses were made in duplicate.

Physicochemical analyses of camel and cow milk

The pH, protein, fat and dry matter contents of camel and cow milk were determined as described by Karoui & Dufour (2003).

Size distribution of milk casein micelles

Skimmed milk was obtained by centrifugation at 6000 rpm for 15 min (Froilabo-SW14R, 69330 Meyzieu, France). Number-based particle size distribution of skimmed milk was determined by using a SALD-2300 laser diffraction particle size analyzer in wet type mode (Shimadzu Scientific Instruments, 77448 Marne La Vallée, France). Milk samples were directly injected into the dispersion cell (containing deionized water as dispersant) until a light intensity distribution more than 10 % was reached and the particle size distributions were measured. The refractive index of 1.55 was used according to the findings of Griffin & Griffin (1985) who have used refractive index value of 1.57. For the dispersant, a blank was measured with deionized water at which the light intensity distribution is less than 10 %.

Samples preparation for the gelation

Twenty milk samples in total for each milk species were thawed during 12 h at 4 °C and then kept at room temperature (~ 18 °C) for 15 min and gently mixed. Milk samples were, after, placed in a water-bath preset at 50 °C or 70 °C for 10 min. The samples were then cooled under the tap water for 5 min and placed immediately in another water-bath equilibrated previously at 36 °C during 5 min.
Then, calcium chloride anhydrous ($CaCl_2$, Merck, 64271 Darmstadt, Germany) and di-Sodium hydrogen phosphate dihydrate ($Na_2HPO_42H_2O$, Merck, 64271 Darmstadt, Germany) powder at 10 and 20 mM were separately added to milk samples with stirring for 2 min. A volume of 6.25 µL of the rennet FAR-M®Sticks (12500 IMCU/stick) (Chr. Hansen A/S BoegeAllé, 2970 Hoersholm, Denmark) prepared directly before the test from 1 g of powder FAR-M®Sticks diluted in 20 g of distilled water was added to 25 mL of milk and measurements were immediately performed. Control milk (i.e., preheated milk samples at 50 and 70 °C without added calcium and phosphate) was also studied.

Rheological and pH measurements throughout rennet induced coagulation

The experiments were performed using a controlled-strain rheometer (Physica MCR 301, Anton Paar Company, 73760 Ostfildern, Germany) with low amplitude oscillation shear analysis and a temperature set at 36 °C by applying a Peltier plate. A volume of 20 mL milk was placed in two concentric cylinders, with an inner diameter of 26.66 mm, length of 40.02 mm, and a gap of 1.13 mm. A liquid paraffin layer was placed onto the surface of milk to prevent evaporation during coagulation, and then measurement was started. The oscillation shear analyses were performed during 45 min in the linear viscoelastic region by applying a constant frequency of 1 Hz and a strain of 0.05 %. The gels were then subjected to a frequency sweep from 1 to 10 Hz (at 36 °C; 0.05 % strain), followed by an amplitude sweep from 0.05 to 150 % with a frequency of 1 Hz frequency. Taking into account the elastic modulus (G'), the following parameters were determined: i) the gelation time defined as the time when G' = 1 Pa as reported by Klandar et al. (2007) and

Ramasubramanian et al. (2014); ii) the gelation rate (defined as dG'/dt) corresponding to the slope obtained by linear regression of G' *versus* time from onset of gelation over a period of 4 min; and iii) the asymptotic value of G' after 45 min of gelation (G'_{asym}).

The pH was determined every 15 min during 45 min of the rennet-induced coagulation.

Statistical analysis

In order to detect difference between milk samples, rheological and physico-chemical parameters were compared using a one-way ANOVA (p <0.05). ANOVA was applied using XLSTAT 2013 (Addinsoft SARL USA, New York, NY, USA) software.

Results and discussion

Physico-chemical properties of camel and cow milk and evolution of pH throughout gelation

Camel milk contains significantly lower amounts ($p < 0.05$) of dry matter, protein and fat (110 ± 10.00, 28.58 ± 0.21 and 34.50 ± 0.51 g/L, respectively) than those of cow milk that reached 126.13 ± 8.48, 34.70 ± 0.55, and 37.50 ± 0.50 g/L, respectively. At the contrary, the pH of camel milk was higher (6.51) than that of cow milk (6.42).

Throughout the whole gelation time, changes in pH of camel and cow control milk gels preheated at 70 °C were slightly achieving final values of 6.51 and 6.50, respectively (**Figs. 1a, b**), in agreement with those of Ramasubramanian et al. (2014) who indicated that milk preheating has no significant effect on the pH values of obtained gels. Similar results were observed for camel and cow milk gels preheated at 50 °C (data not shown).

A similar trend was observed following the addition of calcium and phosphate to preheated milk gels at 70 °C for both types of milk. Added calcium to milk induced a significant decrease ($p < 0.05$) of pH values compared with control milk gel; for milk fortified with 20 mM calcium, the pH reached after 45 min of rennet-induced coagulation of 5.95 ± 0.01 and 5.92 ± 0.02 for camel and cow milk gels, respectively (**Figs. 1a, b**), in agreement with others (Farah & Bachmann, 1987; Ramasubramanian et al. 2014). Indeed, excess amount of calcium ions added to milk displace H^+ ions from hydrogen phosphate, leading to the increase of the concentration of H^+ ions in milk gels and thus reducing pH as explained by the following equation (Lewis 2011):

$$3Ca^{2+} + 2HPO_4^{2-} \leftrightarrow Ca_3PO_4 + 2H^+$$

In contrast, added phosphate to preheated milk gels at 70 °C induced a significant increase of pH values ($p < 0.05$) compared with control milk gels, regardless of milk species (**Figs. 1a, b**). With the enrichment of milk with 20 mM phosphate, this increase reached after 45 min of rennet-induced coagulation, 6.98 ± 0.02 and 6.95 ± 0.01 for camel and cow milk gels, respectively, in agreement with Tsioulpas et al. (2010) who attributed this trend to the increase of negatively charged casein because of the reduction of milk ionic calcium. Similar results were observed for camel and cow milk gels preheated at 50 °C (data not shown).

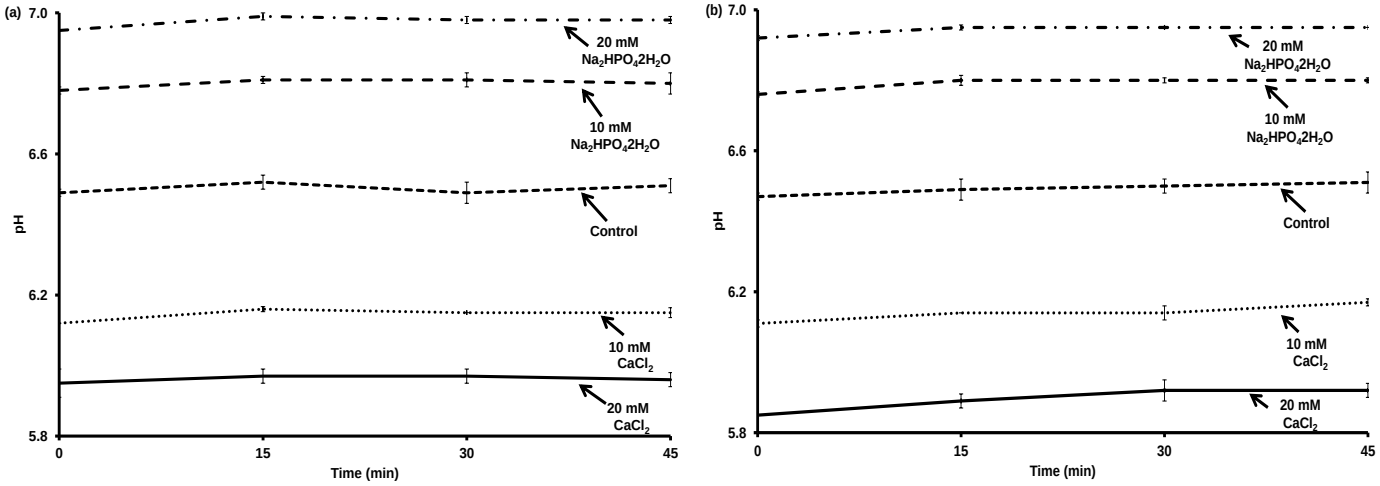

Figure 1: Evolution of pH as a function of time for (a) camel and (b) cow milk gels preheated at 70 °C for 10 minutes (data are means of duplicates with standard deviation).

Rheological properties of calcium / phosphate–induced milk gels

The gelation properties of camel and cow milk gels including the gelation time, the gelation rate and the G'_{asym} are illustrated in **Table 2**. Pre-heated cow milk enriched or not with calcium and phosphate allowed the formation of gels. A different trend was observed with camel milk since no gelation was observed for control camel milk and those enriched with phosphate (10 and 20 mM) preheated at 70 °C (**Fig. 2c** and **Table 2**). In addition, for a considered condition, the preheat-treatment (50 and 70 °C) of cow milk seemed not to have a significant effect of the gelation properties, which was not the case for camel milk.

It appears that some differences were observed between the two milk species. Indeed, cow milk gels enriched with 20 mM calcium showed the shortest ($p < 0.05$) gelation time and the highest gelation rate, while camel milk gels enriched with 10 mM calcium exhibited the shorted gelation time and the highest gelation rate (**Table 2**). This could be due to the absence of β-lactoglobulin and deficiency of κ-casein of camel milk (3.47 % of the total casein) compared to cow milk (13%) (Farah & Atkins, 1992; Al-Haj & Al-Kanhal, 2010).

Our findings were in agreement with those of: i) Montilla et al. (1995) who pointed out that the addition of 10 mM calcium to preheated cow milk gels at 70 °C for 3 min induced a decrease in the rennet clotting time. These findings were recently confirmed by Ramasubramanian et al. (2014) who observed a decrease and an increase of the clotting time and the gelation rate with added 20 mM calcium to cow milk gels preheated at 90 °C for 10 min compared with preheated milk gels without added calcium; and ii) Hattem et al. (2011) reporting that the gelation properties of preheated camel milk gels improved with the addition of calcium since the gelation time was of 20 min and 12 min for control preheated camel milk gels at 63 °C for 30 min and those supplemented with 1.8 mM calcium, respectively.

Only limited papers were available in the literature regarding the impact of preheated camel milk on the gelation properties of rennet-induced gel. Compared to our previous results obtained with camel raw milk gels (data not shown), the preheating of camel milk gels at 50 °C for 10 min induced: i) an increase of the gelation time by a factor of 2; and ii) a decrease of the gelation rate and G'_{asym} values of ~ 5 and 3.4 times, respectively, in agreement with the findings of Hattem et al. (2011) who reported that the gelation time of raw and preheated camel milk gels at 80 °C for 30 min was of 17 and 26 min,

respectively. This trend could be explained by the fact that denatured whey proteins (particularly α-lactalbumin) in heated camel milk react with κ-casein and inhibit access of the enzyme to the susceptible bond on κ-casein inducing: i) a delay in the gelation time, and/or ii) a decrease in the gelation rate and the formation of a weak gel.

According to **Table 2** and **Figs. 2a, b, c**, **d** added phosphate at 10 and 20 mM affected significantly ($p < 0.05$) the gelation properties of camel and cow milk gels. Compared with control milk gels, a significant delay ($p < 0.05$) of the gelation time and a significant decrease of both the gelation rate and gel firmness were observed with added phosphate to preheated camel and cow milk gels at 50 and 70 °C. The obtained results on the cow milk gels were in agreement with the finding of Guillaume et al. (2004) who reported that the addition of phosphate to milk delayed the gelation time. This trend could be explained by the decrease of the ionic calcium level resulting from the formation of a phosphocalcic complex between calcium and added phosphate (Udabage et al. 2001; Guillaume et al. 2004).

Compared to our previous results obtained with raw cow milk gels (data not shown), the preheating of cow milk gels at 50 and 70 °C induced an increase of the gelation time and a decrease of the gelation rate. This could be due to the denaturation of whey proteins, causing the formation of complex between denatured whey proteins and κ-casein *via* the formation of intramolecular disulphide bounds as pointed out by Blecker et al. (2012). Additionally, denatured whey protein could hamper the close approach and contact between casein micelles inducing the formation of a weak network, leading to the decrease of both the gelation rate and the gel firmness (Pomprasirt et al. 1998).

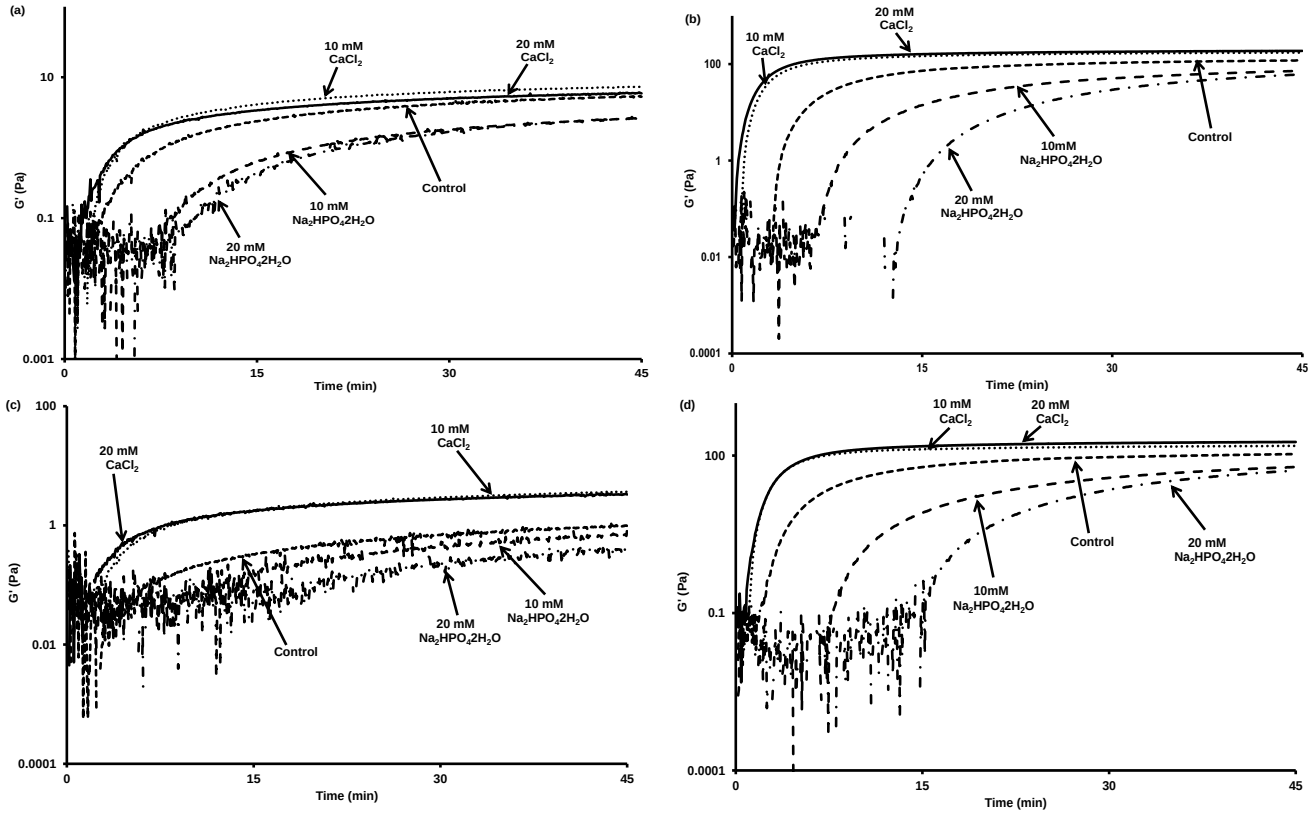

Figure 2: Storage modulus (G') of added calcium and phosphate milk samples as a function of time of: (a) camel, (b) cow milk gels preheated at 50 °C for 10 minutes, and (c) camel, (d) cow milk gels preheated at 70 °C for 10 minutes (data are means of duplicates).

Table 2: Gelation kinetic parameters of camel and cow milk samples preheated at 50 and 70 °C for 10 minutes for control milk gels and those enriched with calcium and phosphate.

	Milk type	Temperature °C	tg [sec]	dG'/dt [Pa/min]	G'_{asym} [Pa]
Control milk	**Camel**	**50**	459^{cB} ± 13.29	0.20^{aC} ± 0.01	5.28^{aC} ± 0.35
		70	-	-	0.97^{aF} ± 0.12
	Cow	**50**	221^{bJ} ± 3.75	7^{eK} ± 0.12	120^{eK} ± 1.50
		70	225^{bJ} ± 24.35	5.09^{dL} ± 0.45	107^{dK} ± 3.50
10 mM $CaCl_2$	**Camel**	**50**	236^{bC} ± 32.00	0.37^{abA} ± 0.03	7.33^{aA} ± 0.34
		70	454^{cB} ± 30.00	0.13^{aD} ± 0.01	3.65^{aD} ± 0.21
	Cow	**50**	58^{aK} ± 1.87	23.07^{gI} ± 0.87	173^{gI} ± 5.40
		70	84^{aK} ± 5.62	21.15^{fJ} ± 0.37	153^{fJ} ± 4.00
20 mM $CaCl_2$	**Camel**	**50**	251^{bC} ± 9.35	0.29^{aB} ± 0.01	5.89^{aB} ± 0.01
		70	484^{cdB} ± 18.75	0.12^{aDE} ± 0.004	3.30^{aD} ± 0.07
	Cow	**50**	41^{aK} ± 15.00	26.16^{hH} ± 0.12	188^{hH} ± 9.00
		70	60^{aK} ± 16.87	23.54^{gI} ± 2.41	182^{hHI} ± 6.15
10 mM $Na_2HPO_42H_2O$	**Camel**	**50**	1283^{fA} ± 138.75	0.08^{aEF} ± 0.01	2.63^{aE} ± 0.22
		70	-	-	0.84^{aFG} ± 0.15
	Cow	**50**	523^{cdI} ± 13.15	1.81^{cM} ± 0.03	72.50^{cL} ± 0.28
		70	591^{dI} ± 46.85	1.31^{cM} ± 0.10	60.47^{bLM} ± 6.43
20 mM $Na_2HPO_42H_2O$	**Camel**	**50**	1298^{fA} ± 5.30	0.07^{aF} ± 0.07	2.66^{aE} ± 0.28
		70	-	-	0.35^{aG} ± 0.08
	Cow	**50**	954^{eH} ± 39.40	1.29^{cM} ± 0.02	61.15^{bLM} ± 0.91
		70	1020^{eH} ± 33.72	1.07^{bcM} ± 0.02	52.00^{bM} ± 0.71

Values are means of 2 replicates with standard deviation, tg (sec); gelation time (second), dG'/dt; gelation rate (Pa/min), $G'_{asym;}$ asymptotic value of storage modulus (Pa) after 45 minutes of gelation, (-); no gelation.

Different small letters (a, b, c, d, e, f, g, h) represent statistical differences between the gelation properties of camel and cow milk ($p < 0.05$).

Different capital letters (A, B, C, D, E, F, G) and (H, I, J, K, L, M) represent statistical differences within the same type of milk as a function of added mineral for camel and cow milk respectively ($p < 0.05$).

Evolution of storage and loss modulus of calcium/phosphate induced milk gels as a function of frequency

The evolution of G' as a function of frequency and strain amplitude sweep are depicted in **Figs. 3-4**. In general, an increase in the G' (**Figs. 3a, b, c, d**) and G" (data not shown) values of calcium and phosphate induced milk gels was observed in the 1-10 Hz frequency range, regardless of milk species and the intensity of heating. These results agreed with the findings Pomprasirt et al. (1998) who observed that G' values of renneted gels made with recombined high total solids milk preheated at 70 °C for 3 min increased with increase of frequency from 0.001 to 1 Hz.

The differences between G' and G" were less than 1 log, particularly, for camel milk samples reflecting the formation of weak gels (Lapasin & Pricl, 1995). Indeed, an increase of G' values from 5.4 to 12.15 Pa for control camel milk gels preheated at 50 °C and from 6 to 12.02 Pa for those with added calcium at 20 mM was observed when the frequency passed from 1 to 10 Hz (**Fig. 3a**). A slight increase (i.e., from 7 to 7.52 Pa with increase of frequency from 1 to 10 Hz) was obtained for camel milk gels preheated at 50 °C with added 10 mM calcium, reflecting that G' values were independent of frequency and depended of the number of calcium bridges formed in the protein network (**Fig. 3a**). Similar trends were observed for G' (**Fig. 3c**) and G" values (data not shown) of preheated camel milk gels at 70 °C.

Over the entire used frequency range (from 1 to 10 Hz), the G' slope obtained using a linear regression of calcium-induced camel milk gels preheated at 50 and 70 °C exhibited lower values in comparison with control milk gels and those with added phosphate (**Figs. 3a, c**). For instance, slopes obtained from camel milk gels preheated at 70 °C were of ~ 0.32 (R^2=0.85) and ~ 0.50 (R^2= 0.91) for respectively milk gels supplemented with 10 and 20 mM calcium. While slopes of ~ 0.91 (R^2 =0.90), 0.87 (R^2 =0.93) and 1.29 (R^2= 0.95) were observed respectively for control milk gels and those added with 10 and 20 mM phosphate (**Fig. 3c**). These results reflected that the evolution of G' is related to the number of calcium bridges formed between casein molecules that increased with added calcium.

The obtained slopes from preheated camel milk gels are higher than those of preheated cow milk gels at 50 and 70 °C (**Figs. 3b, d**). This trend reflect the formation of higher cross-links between casein particles, leading to obtain more stabilized cow milk gels compared to camel milk gel. Indeed, the high quantity of protein in cow milk compared to camel milk (34.70 ± 0.55 versus 28.58 ± 0.21 g/L, respectively) increased the number of bonds and contact area in the gel network and thus the formation of more stabilized gel (Sandra et al. 2012).

The slope values of gels made from preheated milk at 50 °C were lower than those of preheated milk at 70 °C, indicating the formation of more stabilized gels for preheated milk at 50 °C. The results obtained from cow milk gels were in line with the findings of previous investigations (Lucey et al. 1998, 1999) reporting that log G' preheated milk gels at 75 and 80 °C for 30 min *versus* log frequency gave linear curves with a slope of ~ 0.15. Sandra et al. (2012) observed a lower slope value obtained with G' as a function of frequency following the addition of 1 mM calcium due to the formation of higher extent of cross-linking resulting from the formation of bridges between calcium and protein in the milk gels.

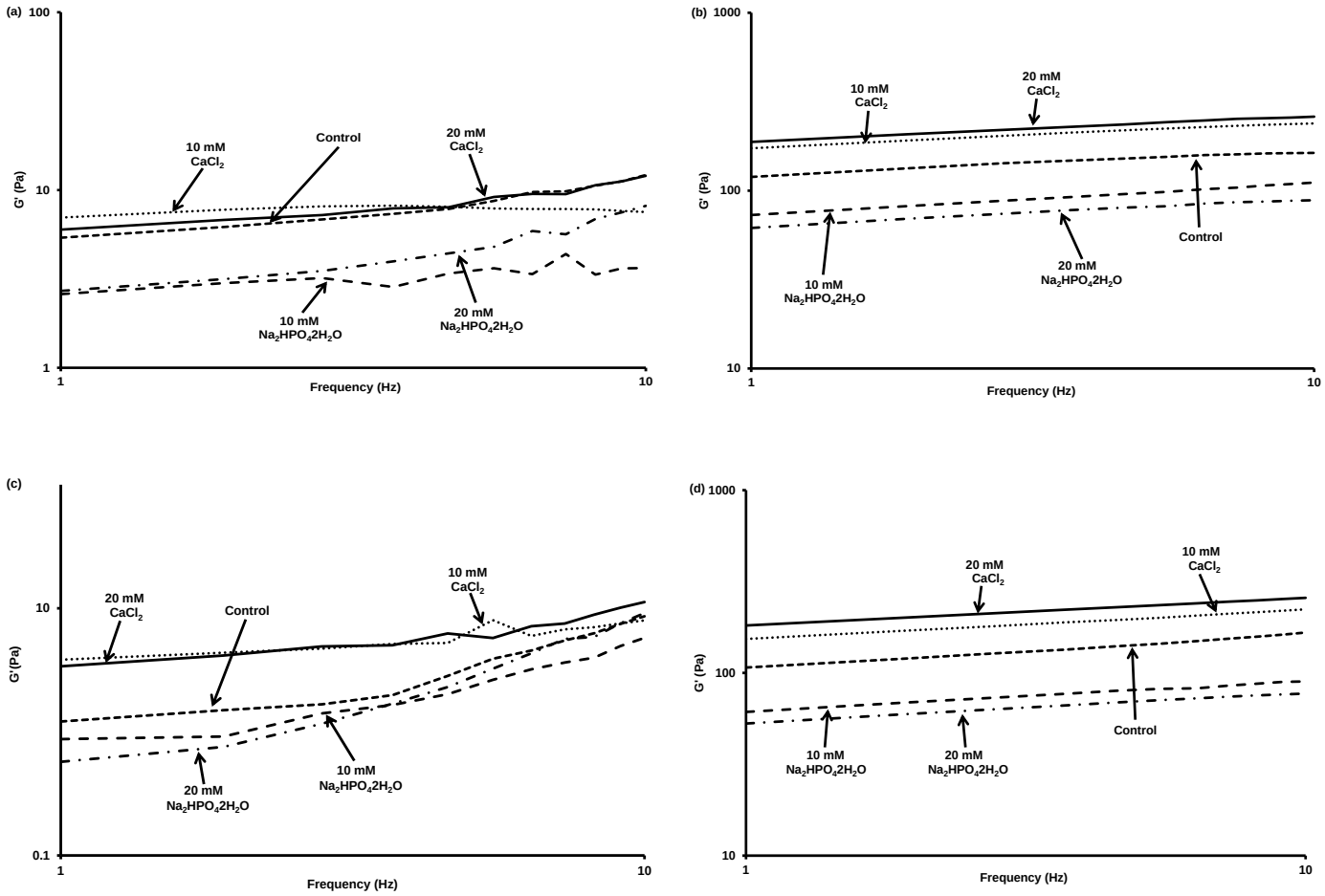

Figure 3: Storage modulus (G') of added calcium and phosphate milk samples as a function of frequency of: (a) camel, (b) cow milk gels preheated at 50 °C for 10 minutes, and (c) camel, (d) cow milk gels preheated at 70 °C for 10 minutes (data are means of duplicates).

During the strain sweep tests, the stress of milk gels increased the breaking point of the gels (**Figs. 4a, b, c, d**). Irrespective of the considered heat-treatment, the breaking stress of the cow and camel milk gels increased with added calcium. A decrease in the breaking stress values of camel and cow milk gels was recorded following the addition of phosphate mainly for camel milk gels preheated at 70 °C. For example, the maximum breaking stress of preheated control camel milk gels at 50 °C and those with 20 mM added calcium and phosphate exhibited values of 1.60, 2.54 and 1.34 Pa, respectively. The breaking strain with 20 mM added calcium and phosphate showed similar values (i.e. 48 %), while it was of 33 % for control milk gels (**Fig. 4a**). The maximum breaking stress of control cow milk gels preheated at 50 °C and those added with 20 mM calcium and phosphate was respectively of 51.10, 69.50 and 28.15 Pa. A similar breaking strain value of 70 % was obtained for control milk gels and those added with calcium at 20 mM, while values of 102 % were observed for added phosphate at 20 mM (**Fig. 4b**). Similar results were observed for preheated camel and cow milk gels at 70 °C (**Figs. 4c, d**), in agreement with the findings of Ramasubramanian et al. (2014) who found that the breaking stress of gels made from preheated milk at 90 °C during 10 min increased with calcium added at 20 mM, while the breaking strain was in the 70-80 % range. The increase of breaking stress could be explained by the increase of

calcium level in the casein micelles, inducing the formation of calcium bridges between caseins particles making the gels more resistant to stress (Lucey et al. 1997). In contrast, the decrease of gel breaking stress after the addition of phosphate could be ascribed to the reduction of colloidal calcium phosphate cross-links and the dispersion of casein particles (Ozcan et al. 2008).

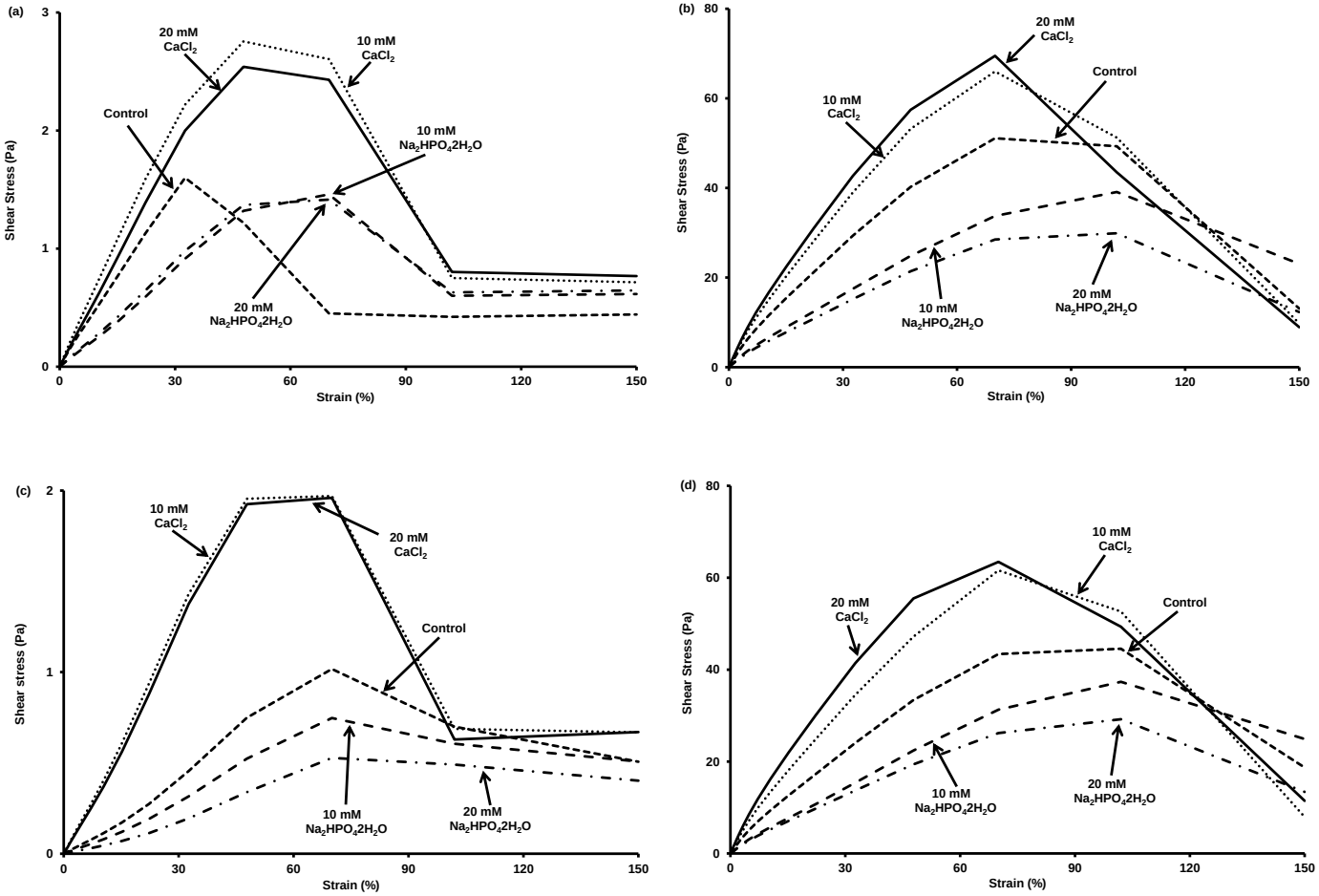

Figure 4: Shear stress of added calcium and phosphate milk samples as a function of strain of: (a) camel, (b) cow milk gels preheated at 50 °C for 10 minutes, and (c) camel, (d) cow milk gels preheated at 70 °C for 10 minutes (data are means of duplicates).

Comparison of the final storage modulus of camel and cow milk gels as a function of heat treatment and the level of added minerals

The G'_{asym} values of control preheated camel and cow milk gels at 50 and 70 °C and those supplemented with phosphate and calcium are depicted in **Fig.5**. The G'_{asym} values were in the following order: cow milk gels > camel milk gels. Indeed, irrespective of the applied temperature and the added amount of phosphate and calcium, cow milk gels presented significant higher values ($p < 0.05$) than camel milk gels. Moreover, the G'_{asym} values of phosphate and calcium induced camel milk gels obtained from milk preheated at 50 °C were significantly higher ($p < 0.05$) than those made from preheated milk at 70 °C (**Figs. 2a, c** and **Table 2)**; however, no significant differences were

observed between the G'_{asym} values of preheated cow milk gels at 50 and 70 °C. Additionally, for a considered milk species, the G'_{asym} values of added calcium gels were significantly higher ($p < 0.05$) than those of phosphate, regardless the intensity of heat treatment applied to milk. Compared with control milk gels, the fortification of preheated camel and cow milk at 50 and 70 °C with calcium induced a significant: i) increase of G'_{asym} and gelation rate values; ii) decrease of gelation time. The G'_{asym} of control camel milk pre-heated at 50 and 70 °C was of 5.28 and 0.97 Pa, respectively; it passed to 7.33 and 3.65 Pa for those enriched with 10 mM calcium, respectively. The G'_{asym} gel passed from 120 and 107 Pa for control cow milk gels preheated at 50 and 70 °C, respectively to 188 and 182 Pa for those with added 20 mM calcium, respectively (**Table 2**). These results confirmed the findings of Ramasubramanian et al. (2014) who observed G'_{asym} values of 248.6 Pa for gels made with preheated cow milk at 90 °C for 10 min and enriched with 20 mM calcium. This trend could be ascribed to the denaturation of β-lactoglobulin and α-lactalbumin in cow milk and α-lactalbumin in camel milk following the heating of milk leading to form whey protein network that strengthen the calcium-induced milk gel by increasing the cross-linking of the micelles during gelation (Riou et al. 2011). The difference between camel and cow milk gels could be ascribed to the difference in the: i) protein composition of both type of milk could be due to the absence of β-lactoglobulin in camel milk resulting in a poor stability of camel milk following thermal treatment (Farah & Atkins, 1992); ii) difference in the level of dry matter since Ramet (2001) and El Zubeir & Jabreel (2008) reported that the lower amounts of dry matter and protein in camel milk, the weaker the formed gel; this could be explained by the creation of less bonds per unit space allowing the formation of softer gel. Additionally, the low fat level of camel milk compared to cow milk induced the formation of weak curds as described by Lucey et al. (1998) and El Zubeir & Jabreel (2008) who pointed out that during coagulation fat globules interact with protein matrix improving the firmness of milk gels.

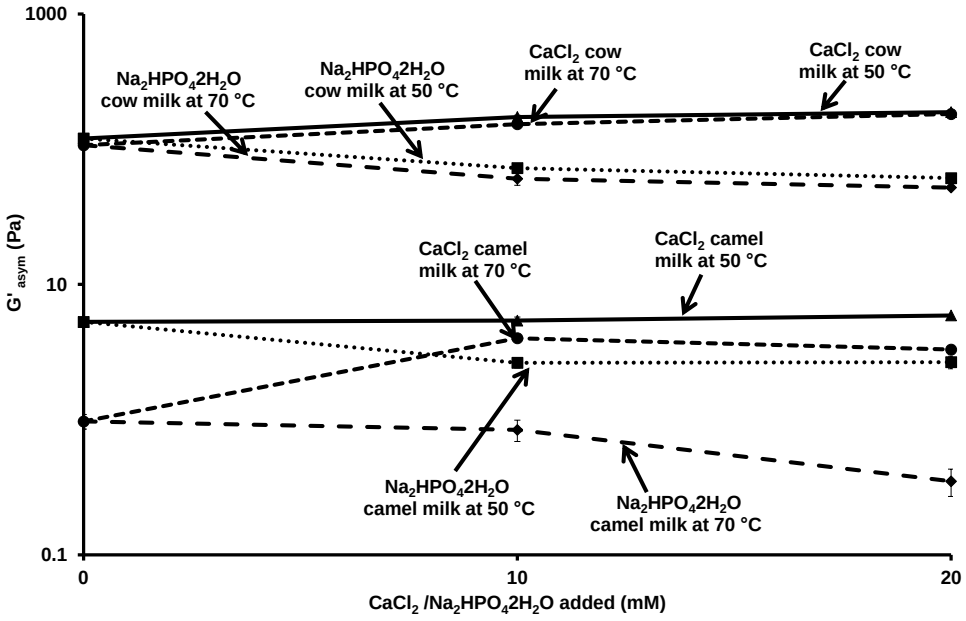

Figure 5: Effect of the milk preheating at 50 and 70 °C for 10 minutes and added calcium and phosphate on final storage modulus (G'_{asym}) of camel and cow milk gels (data are means of duplicates).

Furthermore, the size of the casein micelles affected the mechanical properties since smaller casein micelles increased the gels firmness and shortened the gelation times (Glantz et al. 2010). In the present study, the size of camel milk casein micelles was significantly ($P < 0.05$) larger (i.e., 471 ± 2.50 nm) than that of cow milk (i.e. 138 ± 1.50 nm), in agreement with the findings of Bornaz et al. (2009) who observed a diameter of casein micelles of camel milk in the 280-550 nm range, larger than that of cow milk (i.e., 90-210 nm). This difference induced a delay in the gelation time and reduced the gel firmness (Ramet, 2001) due to the increase of surface area provided by smaller casein micelle that leads to a greater availability hydrolytic cleavage sites (Niki et al. 1994).

In summary, the present study showed differences between the gelation properties of camel and cow milk gels preheated at 50 and 70 °C. A significant difference was observed between the gelation properties of preheated camel milk gels at 50 °C and those obtained from camel milk gels preheated at 70 °C. In contrast, no difference was noticed between the gelation characteristics of preheated cow milk gels at 50 and 70 °C. The addition of calcium at 10 and 20 mM improved the gelation properties of camel and cow milk gels compared to control milk gels, regardless of the intensity of heat treatment. Whereas, added phosphate at 10 and 20 mM to preheated milk gels at 50 and 70 °C induced the formation of softer camel and cow milk gels, even no gelation for preheated camel milk at 70 °C.

Despite the fact that the different measurements were performed by using frozen-thawed milk samples, a recent study conducted on caprine milk exhibited that the frozen of milk for up to 2 months at −27 °C did not present significant effect on the coagulation properties (Kljajevic et al. 2016).

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Article 3

ORIGINAL ARTICLE

Rheological and physical properties of camel and cow milk gels enriched with phosphate and calcium during acid-induced gelation

Mohammad Kamal[1] · Mohammed Foukani[2] · Romdhane Karoui[1,2,3,4]

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Abstract The rheological properties of acid-induced coagulation of camel and cow milk gels following the addition of calcium chloride ($CaCl_2$) and hydrogen phosphate dehydrate ($Na_2HPO_4*2H_2O$) were investigated using a dynamic low amplitude oscillatory rheology. For a considered condition, the final values of storage modulus (G′) and loss modulus (G″) of camel milk gels were significantly lower than those of cow milk gels. The increase of the added $CaCl_2$ levels improved significantly the gelation properties of camel and cow milk gels, since a reduction in the gelation time and an increase in the gel firmness were observed. Following the addition of $Na_2HPO_4*2H_2O$ at 10 and 20 mM, no significant effect on the gelation rate and the firmness of camel milk gels was observed, while, a significant decrease in the gelation rate and firmness were observed for cow milk gels.

Keywords Rheology · Acid coagulation · Camel milk · Cow milk · Mineral salts

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Introduction

Milk acidification is traditionally induced by using bacterial cultures and/or glucono-δ-lactone (GDL) which converts lactose into lactic acid (Sadeghi et al. 2014). The hydrolysis of GDL leads to the formation of gluconic acid inducing a decrease of pH (Koh et al. 2002). With this decrease: (1) the solubilization of the colloidal calcium phosphate gradually begins and becomes fully soluble at a pH of ~5.20 (Le Graet and Brule 1993); and (2) the surface charges of the casein micelles decreased, leading to reduce the steric and electrostatic stabilization of milk and the formation of gel at pH ~4.80 (Lucey et al. 1997).

Camel milk is mostly consumed as fluid contrary to other types of milk (Yagil et al. 1984). This could be explained by the difficulty of the camel milk transformation regardless of the gelation type i.e., acid, enzymatic or mixed (Attia et al. 2000; El Zubeir and Jabreel 2008). This trend has been attributed by the authors to the casein micelles structure. Indeed, the low content of κ-casein and the large size of casein micelles of camel milk compared with cow with cow milk contribute to prolong the coagulation time (Al Haj and Al Kanhal 2010; Kappeler et al. 2003). Additionally, the presence of several natural antimicrobial agents in camel milk such as lysozyme, lactoferrin, lactoperoxidase and immunoglobulin limit the effect of lactic acid bacteria (Ramet 2001).

Calcium and phosphate are considered as important elements of the mineral fraction of cow and camel milk with total concentrations varying in the 25–35 mM for both types of milk (Bornaz et al. 2009; Gaucheron 2005). These mineral components are in equilibrium state between the micellar and the serum phase and contribute significantly to the formation of coagulum (Ramasubramanian et al. 2008).

In addition, the addition of calcium significantly increased the curd yield (Makhal et al. 2015).

In the literature, the rheology properties of acid-induced cow milk protein gels following the addition of calcium have been studied (Gastaldi et al. 1994; Guillaume et al. 2002; Ramasubramanian et al. 2008). One of the main conclusions of these studies was that added calcium decreased the gelation time and improved the firmness of the gels. Regarding the effect of phosphate on the acid-induced coagulation of cow milk, few studies have been published (Mizuno and Lucey 2007; Ozcan et al. 2008). The authors pointed out that the enrichment of milk with phosphate delayed the onset gelation and decreased the gel firmness.

Although several studies were published regarding the effect of calcium and phosphate on the acid gelation properties of cow milk gels, to the best of our knowledge, very limited scientific report were available in the literature concerning the effect of these minerals on camel milk gels. Thus, in the present work, rheological properties of the GDL-induced coagulation of camel and cow milk gels following the addition of $CaCl_2$ and $Na_2HPO_4*2H_2O$ at two concentrations (10 and 20 mM) will be studied.

Materials and methods

Milk samples

Fresh camel milk (2 L distributed into plastic bottles of 30 mL capacity) was obtained from an experimental station located in region Foum Lahcen, province Tata (Moroccan desert). Camels of Crezni genotype, aged of 6 years with two child births were used. Camels were kept on pasture feeding and supplemented with barley and clover.

Fresh cow milk (2 L distributed into plastic bottles of 30 mL capacity) of Holstein–Friesian genotype was collected from a regional farm of Lille (France). Cow were maintained on pasture feeding and supplemented with a corn-based concentrate.

Once arrival to the laboratory, milk samples were kept at −18 °C up to analysis. All the analyses were conducted in duplicate (2 samples for each condition).

Physicochemical analyses of camel and cow milk

The pH, protein, fat and dry matter contents of camel and cow milk were determined as described by Karoui and Dufour (2003). Prior to pH measurements, the pH meter (Hach sensION + pH3 Laboratory) was calibrated with standard pH solutions prepared using buffer capsules of pH 7 and 4.

Distribution of casein micelles size of milk

Skimmed milk was obtained by centrifugation at 3740 g for 15 min (Froilabo-SW14R, France). Number-based particle size distribution of skimmed milk was obtained with SALD-2300 laser diffraction particle size analyzer (Shimadzu Scientific Instruments, France) using wet type measurement. Milk was directly injected into the dispersion cell (containing deionized water under agitation) until a light intensity distribution more than 10% was reached and the particle size distribution was determined. The refractive index of 1.55 was used according to the findings of Griffin and Griffin (1985) who have used refractive index of 1.57. For the dispersant, a blank was measured with deionized water at which the light intensity distribution is less than 10%.

Samples preparation for the gelation

Milk samples (10 samples in total for each milk species) were thawed during 12 h at 4 °C and then kept at room temperature (~18 °C) for 15 min to avoid thermal shock. Milk samples (25 mL for each sample) were placed in a water-bath equilibrated previously at 36 °C for 5 min. Then, calcium chloride anhydrous ($CaCl_2$, Merck, Darmstadt, Germany) and di-Sodium hydrogen phosphate dehydrate ($Na_2HPO_4*2H_2O$, Merck Darmstadt, Germany) powders were separately added to the milk at two concentrations (10 and 20 mM). Milk coagulation was performed by progressive acidification with GDL (Gluconodelta-lactone «$C_6H_{10}O_6$», Merck schuchardt OHG, Hohenbrunn, Germany) added at 3% (w/v) with stirring for 2 min and rheological measurement was immediately performed. Control milk (without added $CaCl_2$ and $Na_2HPO_4*2H_2O$) was also studied.

Rheological and pH measurements throughout acid induced coagulation

The experiments were performed using a controlled-strain rheometer (Physica MCR 301, Anton Paar Company, Germany) with low amplitude oscillation and a temperature set at 36 °C by applying a Peltier plate. A volume of 20 mL was placed in two concentric cylinders, with an inner diameter of 26.66 mm, length of 40.02 mm, and a gap of 1.13 mm. A layer of liquid paraffin was placed onto the surface of milk sample to prevent evaporation during coagulation, and then measurement was started. The oscillation experiments were performed by applying a constant frequency of 1 Hz and a strain of 0.05% during 180 min that was in the linear viscoelastic range of these types of gels network. Then, a frequency sweep from 1 to 10 Hz was performed by applying a strain of 0.05%,

followed by an amplitude sweep from 0.05 to 150% with a frequency of 1 Hz. Taking into account the elastic modulus (G′), the following parameters were assessed: (1) the gelation time defined as the time when $G' = 1$ Pa as reported by Lucey et al. (1998a) and Ramasubramanian et al. (2014); (2) the gelation rate defined as dG′/dt corresponding to the slope obtained by linear regression of G′ versus time from onset of gelation over a period of 15 min; and (3) the asymptotic value of G′ after 180 min of gelation (G'_{asym}).

The pH was determined every 5 min during 180 min.

Statistical analysis

In order to detect difference between milk samples, rheological parameters were compared using a one-way ANOVA ($p < 0.05$). ANOVA was applied using XLSTAT 2013 (Addinsoft SARL USA, New York, NY, USA) software.

Results and discussion

Physicochemical parameters of camel and cow milk

Physicochemical properties of camel and cow milk showed that dry matter, protein and fat contents of camel milk (108.30 ± 10.74, 27.32 ± 0.06 and 34.50 ± 0.50 g L^{-1}, respectively) were lower than those of cow milk (120.50 ± 1.45, 33.75 ± 0.16 and 37.50 ± 0.71 g L^{-1}, respectively), in agreement with others (Al Haj and Al Kanhal 2010; Hailu et al. 2016; Jumah et al. 2001).

The added GDL induced a decrease of the pH values reaching 3.48 and 4.02 after 180 min for camel and cow milk gels, respectively. This decline in the pH values for both types of milk gels is related to the added GDL, in agreement with the findings of Lucey et al. (1998a).

Compared with control camel and cow milk gels, the addition of $CaCl_2$ and Na_2HPO_4*$2H_2O$ to milk induced change in the pH, particularly for added Na_2HPO_4*$2H_2O$ (Fig. 1a, b). For a considered time, an increase and a decrease of pH values were observed for milk gels enriched with phosphate and calcium. After 180 min of added GDL, milk samples added with $CaCl_2$ presented pH values of 3.46 and 3.84 for camel and cow milk gels, respectively, while those added with Na_2HPO_4*$2H_2O$ presented higher pH values of 3.75 and 4.45 for respectively camel and cow milk gels.

Size distribution of casein micelles

The size distribution of casein micelles of camel and cow milk are presented in Fig. 2. The casein micelles size of camel milk was of 468 ± 1.00 nm, while it was only of

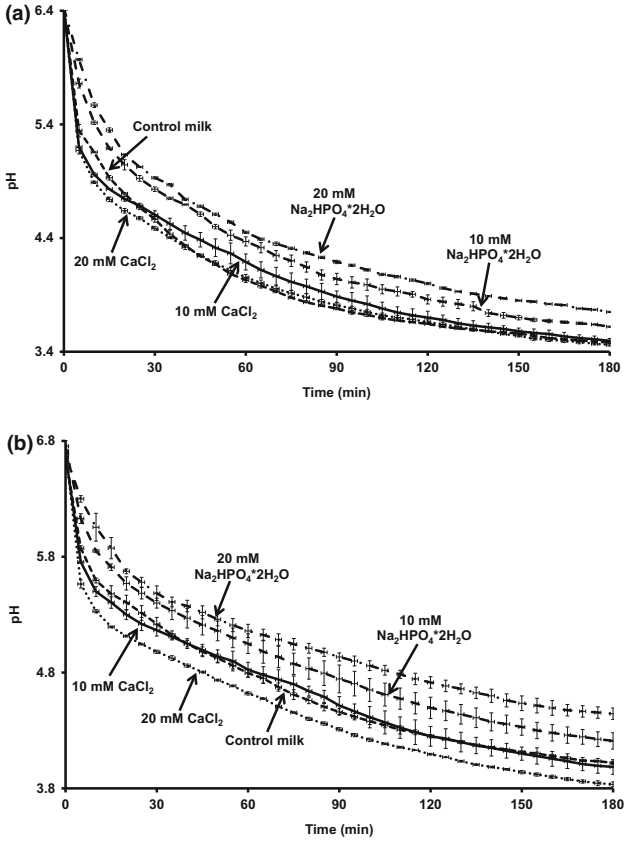

Fig. 1 Evolution of pH values as a function of time of **a** camel and **b** cow milk gels (data are means of duplicates with standard deviation)

137 ± 1.50 nm for cow milk. This result was in agreement with the findings of Bornaz et al. (2009) reporting that casein micelles diameter of camel milk varied in the 280–550 nm range, larger than that of cow milk having a diameter in the 90–210 nm range. This difference between the both types of milk might be explained by the variation in the physico-chemical composition (e.g., κ-casein

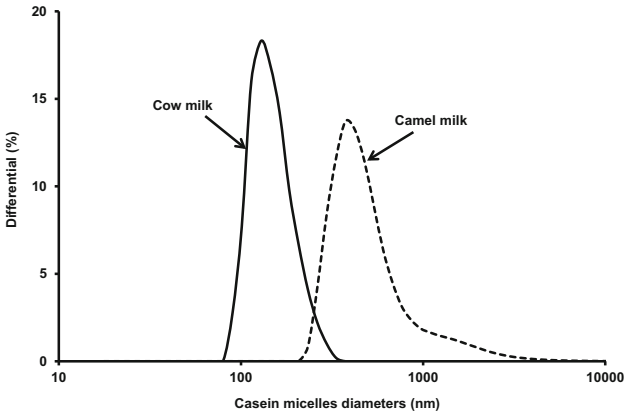

Fig. 2 Diameters of casein micelles (nm) of camel and cow milk determined by laser light scattering (data are means of duplicates)

content, etc.) where a smaller micelle size was known to be associated with a higher amount of κ-casein ~3.47 and 13% for camel and cow milk, respectively as pointed out by others (Davies and Law 1980; Kappeler et al. 2003; Walsh et al. 1998).

Rheological properties of calcium/phosphate-induced camel and cow milk gels

The evolution of G′ following the addition of $CaCl_2$ and $Na_2HPO_4*2H_2O$ to cow and camel milk gels as a function of time are shown in Fig. 3a, b. Calcium/phosphate-added milk induced the gelation of both camel and cow milk, irrespective of the $CaCl_2$ and $Na_2HPO_4*2H_2O$ concentration. For both types of gels: (1) the higher the calcium concentration, the higher the gel firmness; and (2) the higher the phosphate concentration, the lower the gel firmness (Fig. 3a, b). The G'_{asym} of control camel milk gels showed average value of 1.61 Pa and increased significantly ($p < 0.05$) to 2.23 and 3.81 Pa with the addition of $CaCl_2$ at 10 and 20 mM, respectively (Fig. 3a; Table 1). The low elasticity and the weakness of camel gels could be ascribed to the lack of network structure through the hydrophobic interactions which involved low energy and little resistant to mechanical treatment (Mahaut et al. 2000).

The gelation time and gelation rate of the calcium/phosphate-induced camel milk gels compared with control milk gels were determined (Table 1). As observed, shorter gelation time and higher gelation rate were observed for camel milk gels with added calcium (20 mM) since a significant decrease of gelation time ($p < 0.05$) and an increase in the gelation rate compared with control camel milk gels were observed with added $CaCl_2$ at 20 mM. These modifications in the gelation time and gelation rate with added $CaCl_2$ could be attributed to the increase of ionic calcium concentration inducing: (1) a pH decrease resulting from the negative charge decrease at the surface of caseins; and (2) the formation of more bridges between casein proteins and calcium (Koutina et al. 2016; Ramasubramanian et al. 2012).

As observed in Fig. 3a and Table 1, no significant differences ($p < 0.05$) were noticed between the gelation rates and G'_{asym} values of control camel milk gels and those added with $Na_2HPO_4*2H_2O$ (10 and 20 mM). However, the addition of $Na_2HPO_4*2H_2O$ at 20 mM induced a

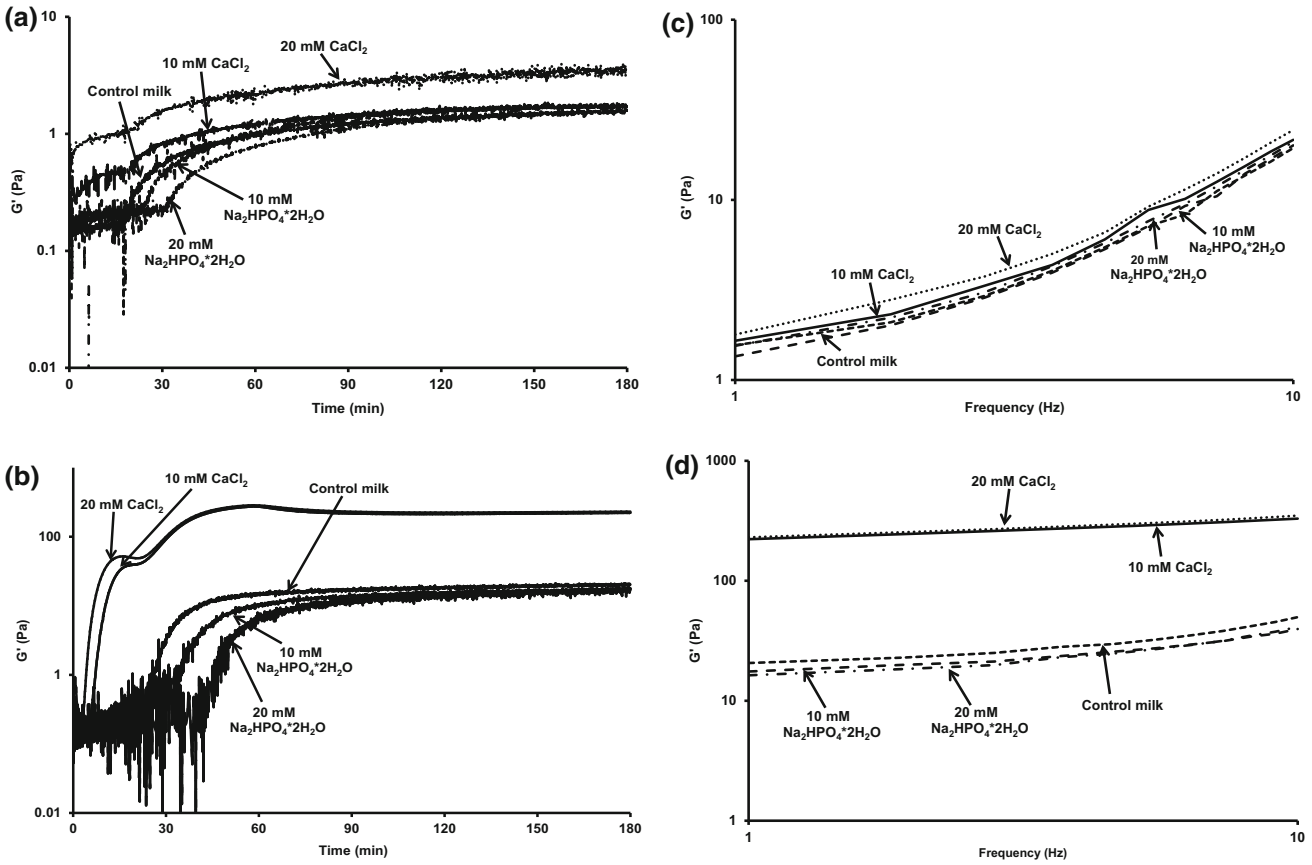

Fig. 3 Evolution of storage modulus (G′) as a function of time (**a**, **b**) and frequency (**c**, **d**) of camel and cow milk gels added with $CaCl_2$ and $Na_2HPO_4*2H_2O$ (data are means of duplicates)

Table 1 Gelation kinetic parameters of control camel and cow milk and those added with $CaCl_2$ and $Na_2HPO_4*2H_2O$

Milk type	Control milk		10 mM $CaCl_2$		20 mM $CaCl_2$		10 mM $Na_2HPO_4*2H_2O$		20 mM $Na_2HPO_4*2H_2O$	
	Camel	Cow	Camel	Cow	Camel	Cow	Camel	Cow	Camel	Cow
tg [min]	58.500 ± 2.125^{b}	26.375 ± 0.188^{d}	45.423 ± 2.563^{c}	7.250 ± 0.000^{f}	17.100 ± 1.854^{e}	4.282 ± 0.398^{f}	62.235 ± 3.093^{b}	32.406 ± 4.468^{d}	77.063 ± 0.750^{a}	45.094 ± 1.104^{c}
dG′/dt [Pa/min]	0.009 ± 0.001^{e}	0.645 ± 0.035^{c}	0.010 ± 0.000^{e}	3.087 ± 0.118^{b}	0.047 ± 0.001^{e}	3.855 ± 0.007^{a}	0.013 ± 0.002^{e}	0.360 ± 0.042^{d}	0.009 ± 0.003^{e}	0.354 ± 0.021^{d}
G'_{asym} [Pa]	1.610 ± 0.110^{e}	20.280 ± 1.103^{c}	$2.230 \pm 0.020^{e,f}$	222.650 ± 1.061^{b}	3.805 ± 0.500^{e}	230.800 ± 0.141^{a}	1.752 ± 0.081^{e}	17.460 ± 1.937^{d}	1.534 ± 0.020^{e}	16.722 ± 1.250^{d}

Values are means of 2 replicates with standard deviation, tg (min); gelation time (minute), dG′/dt; gelation rate (Pa/min), G'_{asym}; asymptotic value of storage modulus (Pa) after 180 min of gelation

Values with different superscripts (a, b, c, d, e, f) for a considered line differ significantly with respect to gelation properties of both camel and cow milk ($p < 0.05$)

significant delay ($p < 0.05$) of the gelation time compared with control camel milk gels and those added with 10 mM phosphate. This trend could be probably attributed to the formation of a phosphocalcic complex between calcium and added phosphate as pointed out by Tsioulpas et al. (2010).

A similar trend was observed for cow milk gels following the addition of $CaCl_2$; however, the effect of calcium was more pronounced in cow milk gels than that of camel milk gels (Fig. 3b; Table 1). From the obtained results, it could be concluded that G'_{asym} values exhibited higher significant values ($p < 0.05$) with the increase of added $CaCl_2$ concentration. Indeed, according to Table 1, the highest G'_{asym} value was observed following the addition of 20 mM $CaCl_2$ (i.e., 231 Pa), while it was only of 20.28 Pa for control cow milk gels, in agreement with others who observed after 360 min of skim cow milk gels acidification G'_{asym} values of 319 (Lucey et al. 1998a) and 244 Pa (Oh et al. 2007). In addition, the difference in G'_{asym} values between control and those enriched with calcium could be attributed to the increase of the micellar calcium phosphate amount leading to higher establishment of calcium bridges between milk proteins and the formation of more elastic gel (Guillaume et al. 2002). From Fig. 3b, it was observed that the G′ values of gels added with $CaCl_2$ increased rapidly during the first 18 min, corresponding to pH value of ~5.20. This trend could be related to the dissociation and rearrangement of casein that increase with the solubilization rate of colloidal calcium phosphate (Gastaldi et al. 1996). After the first 18 min, the G′ values showed small change with the decrease of pH from 5.20 to 4.70 that could be attributed to the reincorporation of casein into the micelle structure (Gastaldi et al. 1996). In the 24–62 min range, the G′ values increased, where the pH value was of ~4.70. Beyond 62 min, the G′ values showed a slight decrease during ~10 min, and after remains constant until the end of gelation (180 min). Regarding the gelation time and gelation rate of cow milk gels, a similar trend was observed for camel milk. Indeed, the increase of the added $CaCl_2$ levels to 20 mM reduced significantly the gelation time ($p < 0.05$) and increased the gelation rate compared with control milk (Table 1). These modifications could be explained by the increase of the neutralization rate of the negatively charged casein, resulting from the increase in ionic calcium amount (Dalgleish 1983).

The impact of $Na_2HPO_4*2H_2O$ added to cow milk gels was found more pronounced than that of camel milk gels, since a significant delay in the gelation onset, a reduction of the gelation rate, and the formation of a weak gels were obtained with the increase of the added $Na_2HPO_4*2H_2O$ levels. Indeed, from Fig. 3b and Table 1, it could be observed that the gelation rate and G'_{asym} values decreased

significantly ($p < 0.05$) with the increase of Na_2HPO_4*2-H_2O concentration in comparison with control milk gels (Table 1). The decline of curd-firming rate and G'_{asym} values could be explained by the decrease in the colloidal calcium phosphate cross-linking leading to the dispersion of casein as described by Mizuno and Lucey (2007) and Ozcan et al. (2008) reporting that the addition of low concentrations of tetrasodium pyrophosphate to milk induced slow protein gelation. As shown in Table 1, the gelation time increased significantly ($p < 0.05$) with the increase of the level of added phosphate. This trend could be attributed to the formation of a phosphocalcic complex, leading to decrease the calcium ions and thus increase the negative charges of casein micelles (Tsioulpas et al. 2010).

The evolution of G′ as a function of frequency and strain amplitude sweep tests are reported in Figs. 3 and 4. According to Fig. 3c, d, an increase in the G′ values of calcium-induced milk gels was observed with the increase of frequency from 1 to 10 Hz, irrespective of milk species. Considering each milk gels, separately, the difference between G′ and G″ (data not shown) was less than 1 log, particularly for camel milk gels, indicating the formation of weak gels (Lapasin and Pricl 1995). Indeed, when frequency increased from 1 to 10 Hz, G′ values of control camel and cow milk gels varied from 2 to 19 Pa and from 22 to 46 Pa, respectively (Fig. 3c, d). The obtained results were in agreement with the findings of Martin et al. (2009) who reported that G′ and G″ of GDL-induced skim milk gels increased in the 0.016–1.60 Hz range.

Compared with control milk gels, the incorporation of calcium at 10 and 20 mM induced an increase in the G′ and G″ values as a function of applied frequency (i.e., 1–10 Hz range) (Fig. 3c, d). These results were in agreement with those of Ramasubramanian et al. (2014) who observed an increase of G′ for calcium-induced cow milk gels with increase of frequency from 0.1 to 10 Hz, reflecting that the formed bonds in the gels matrix have less time to relax when the time scale of the applied stress is shorter (Mishra et al. 2005). Similar results for control milk gels were obtained with added phosphate (Fig. 3c, d). For example, with added Na_2HPO_4*$2H_2O$ at 20 mM, an increase in the G′ values from 1.55 to 21 Pa and from 16.31 to 41.27 Pa was observed with increasing frequency from 1 to 10 Hz for camel and cow milk gels, respectively.

Over the entire frequency range (from 1 to 10 Hz), the slopes obtained using a linear regression of log G′ versus log frequency exhibited similar values i.e., ~1.17, $R^2 = 0.96$ for all camel milk samples indicating that the evolution of G′ depend on the frequency, and reflecting the formation of a weaker gel structure (Fig. 3c). Those of cow milk gels showed lower values, where slopes of ~0.17 ($R^2 = 0.99$) and ~0.35 ($R^2 = 0.92$) were observed with 10 and 20 mM calcium-induced milk gels, and control milk gels, respectively (Fig. 3d). Similar results were obtained with added phosphate (Fig. 3c, d).

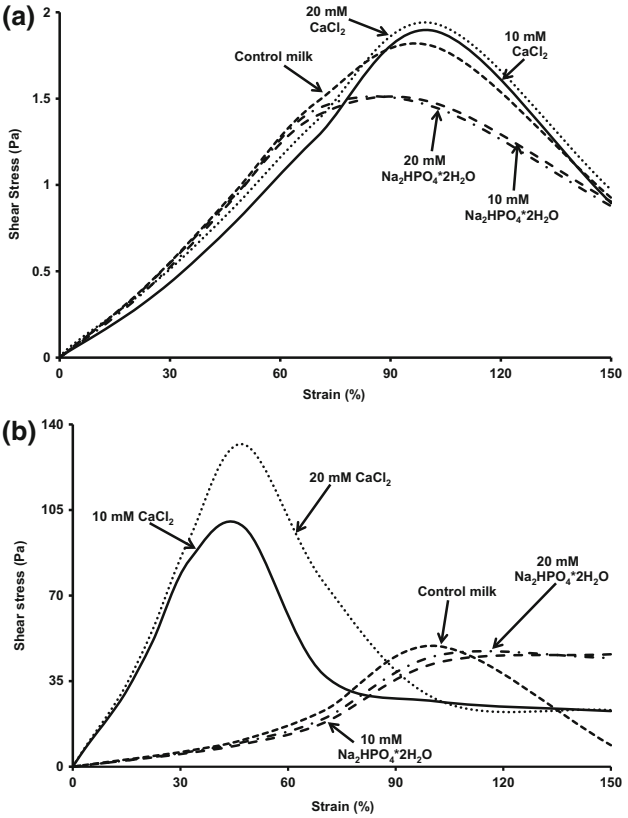

Fig. 4 Evolution of shear stress as a function of strain of **a** camel and **b** cow milk gels added with $CaCl_2$ and Na_2HPO_4*$2H_2O$ (data are means of duplicates)

The low slopes obtained from cow milk gels reflects that the evolution of G′ values was independent of frequency, indicating the formation of gels with higher firmness that could be related to the increase of the number of formed bonds in the gel matrix. No data was available concerning the slopes obtained for camel milk gels. However, those obtained from cow milk gels were in line with the literature (Lucey et al. 1998a; Oh et al. 2007). Indeed, Sandra et al. (2012) observed fair slope values as a function of frequency following the addition of $CaCl_2$ suggesting a higher extent of cross-linking as a result of calcium bridges formed in the milk gels. Figure 4a, b illustrates that the highest breaking stress values of camel and cow milk gels were obtained with added $CaCl_2$ (10 and 20 mM). Indeed, the breaking stress of camel milk gels exhibited a maximum value of 1.89 and 1.94 Pa with 10 and 20 mM added $CaCl_2$, while it was only of 1.80 for control milk gels (Fig. 4a). The breaking strain showed similar values of 102% for the three cases.

A similar trend was observed for cow milk gels (Fig. 4b), since the breaking stress for control milk gels

and those added with 10 and 20 mM $CaCl_2$ was respectively of 49.31, 97.92 and 131.75 Pa, with a breaking strain of 102% for control milk gels and 48% for those with added $CaCl_2$. The increase of the breaking stress with the addition of $CaCl_2$ could be explained by the increase of the insoluble calcium level of casein micelles, leading to decrease the large pores and increase the strength interactions and bonds between caseins particles (Lucey et al. 1997).

Maximum breaking stress values of 1.47 and 1.45 Pa and a breaking strain of 102% were observed, respectively for 10 and 20 mM phosphate-induced camel milk gels (Fig. 4a). For cow milk gels (Fig. 4b), the maximum breaking stress following the addition of 10 and 20 mM Na_2HPO_4*$2H_2O$ showed similar values of ~46 Pa, with a breaking strain of 150 and 102%, respectively. The decrease in the breaking stress compared to added calcium could be ascribed to the reduction in the number of colloidal calcium phosphate cross-links, and the dispersion of casein particles as reported by Ozcan et al. (2008).

Comparison of the final storage modulus of camel and cow milk gels as a function of added mineral levels

Changes in G'_{asym} values of Na_2HPO_4*$2H_2O$ and $CaCl_2$-induced camel and cow milk gels were in the following order: cow milk gels > camel milk gels (Fig. 5). Indeed, for a considered concentration of Na_2HPO_4*$2H_2O$ and $CaCl_2$, the G'_{asym} of cow milk samples exhibited significant higher values ($p < 0.05$) than those obtained with camel milk gels. Furthermore, the G'_{asym} of calcium-induced milk gels was significantly higher ($p < 0.05$) than that of phosphate-induced milk gels. The difference in the physico-chemical composition of both types of milk such as dry matter, protein and fat might be considered as the principal reason of this variation. In fact, the low contents of dry matter, protein and fat in camel milk compared with cow milk induced some modifications in the gelation properties. In this context, Jumah et al. (2001) reported that the lower levels of dry matter and protein of camel milk, the weaker acid-induced gel. This could be explained by the fact that: (1) the lower fat content of camel milk induced the formation of a weak gel since fat globules could interact with the protein matrix improving the firmness of recombined milk gels (El Zubeir and Jabreel 2008; Lucey et al. 1998b); and/or (2) the larger size of camel casein micelles affect the rheological properties of milk gels (Glantz et al. 2010), in agreement with the results obtained in Fig. 2 indicating that casein micelles size of camel milk (468 nm) is larger than that of cow milk (137 nm).

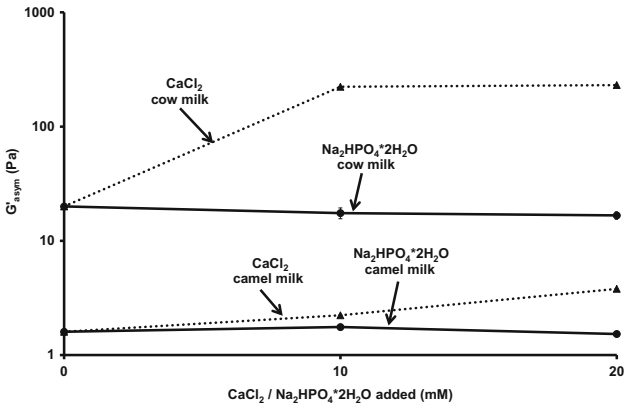

Fig. 5 Effect of added $CaCl_2$ and Na_2HPO_4*$2H_2O$ on asymptotic value of G′ after 180 min of gelation (G'_{asym}) of camel and cow milk gels (data are means of duplicates)

Conclusion

The results of the present study showed some difference between the rheological properties of acid-induced camel and cow milk gels. The increase of the added $CaCl_2$ concentration from 10 to 20 mM reduced significantly the gelation time and increased the firmness for both camel and cow milk gels compared with control milk gels.

The addition of Na_2HPO_4*$2H_2O$ at 10 and 20 mM exhibited a slight effect of the gelation properties of camel milk gels compared with control milk gels. This effect was more pronounced for cow milk gels, since a significant delay in the gelation onset and decreases of the gel firmness were observed. However, the detailed mechanism by which added $CaCl_2$ and phosphate affects the rheological properties of acid-induced camel milk gels needs to be further investigated.

The different measurements were performed on frozen-thawed milk samples and it would be interesting to test these results on fresh milk samples although a recent study conducted on caprine milk pointed out that milk freezing up to 2 months at −27 °C did not present significant effect on the coagulation properties (Kljajevic et al. 2016).

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Rennet-induced coagulation of raw and heated camel and cow milk gels determined by instrumental techniques: effects of added calcium and phosphate

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Abstract

BACKGROUND: The potentiality of the front-face fluorescence spectroscopy and rheological measurements combined with chemometric tools to characterise the structure evolution during coagulation of raw and preheated camel and cow milk at 50 and 70 °C with/without added calcium and phosphate was evaluated. Tryptophan and vitamin A fluorescence spectra were collected during the gelation of milk at 0, 10, 20, 30, 40, and 45 min after the addition of rennet-induced coagulation. In parallel, the storage modulus (*G′*), loss modulus (*G″*) and tan delta (tan δ) were determined using low amplitude oscillation shear analysis.

RESULTS: The principal component analysis (PCA) applied to the normalised tryptophan spectra allowed the gels made with camel milk to be differentiated from those of cow milk on the one hand, and to monitor protein structure modifications during the gelation, on the other hand. The common components and specific weights analysis (CCSWA) applied jointly to the fluorescence and rheological data sets permitted a clear separation of raw milk gels from those preheated at 50 and 70 °C.

CONCLUSION: The front-face fluorescence spectroscopy method coupled with multi-variate statistical analyses showed a high capacity for studying changes in the micelle structure throughout the rennet-induced coagulation process.

Keywords: coagulation; milk; fluorescence; rheology; chemometric

INTRODUCTION

The combination of rennet-induced coagulation with heat treatment and supplementation with calcium is a common practice that is applied in the dairy industry for producing several dairy products. Heat treatments applied to milk at temperatures higher than 55 °C induce the denaturation of whey proteins which in turn interact with κ-casein forming complexes that are stabilised either by the formation of intermolecular disulfide bonds and/or hydrophobic interactions. The denaturation level of whey proteins depends on milk species and the applied temperature/time.

It is well known that camel milk is more heat resistant than cow milk.[1] The presence of denatured whey proteins hinder the second phase of the rennet-induced coagulation causing an extension of the coagulation time and a decrease of the gel firmness.[2–4] In addition, protein–protein and protein–fat globule interactions affect the rheological properties of gels and thus their syneresis behaviour. Although several studies have been published on the rennet-induced coagulation of cow milk, only limited studies are available regarding rennet-induced coagulation of camel milk.[5–7] Previous research studies conducted on rennet-induced coagulation of cow milk showed that the properties of the gel depend on the treatment applied to milk.[8–13] A positive effect on cow milk gel properties was noted following the addition of calcium,[14] while a less conclusive impact was observed in the case of camel milk gel.[7]

Camel milk is known for its high nutritional value, but it is described as a matrix that is difficult to process into dairy products such as yogurt and cheese.[15]

Taking into account that the structure of the gel at the molecular level affects the texture of the final product, it would be interesting to control its structure. In this context, fluorescence and infrared methods have been employed to monitor rennet-induced coagulation of cow milk.[16–21] However, to the best of our knowledge no papers have been published describing the ability of front-face fluorescence spectroscopy to monitor rennet-induced coagulation of camel milk. The presence of fluorophores in camel milk makes fluorescence spectroscopy an ideal method to evaluate the molecular changes occurring during rennet-induced coagulation. For example, fluorescence properties of aromatic amino acids of

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proteins,[22] vitamin A[23] and riboflavin[24] in dairy products have been explored and provided useful information at the molecular scale.

The objective of this study was to investigate the potential of front-face fluorescence spectroscopy in combination with chemometric tools to monitor the structure evolution throughout rennet-induced coagulation of raw and preheated camel milk supplemented with calcium and phosphate compared to cow milk.

MATERIALS AND METHODS

Milk samples

Fresh camel milk was collected from an experimental farm bulk milk tank (ElJem region, Mahdia governorate, Tunisia). After the camels had been milked, the milk was homogenised to avoid variations in the composition between the samples, then sub-samples into 30-mL containers and it was transported to France using an icebox. Camels of the Maghrebine genotype, aged 6 years, were maintained on pasture feeding (annual pasture of thorny plants and coquelicot) and supplemented with concentrate. Animals were inspected by a qualified camel herder on a daily basis, and routine animal care and vaccination procedures were conducted as prescribed by best practice protocols. Fresh cow milk (2 L distributed into plastic bottles of 30 mL capacity) of Holstein Friesian genotype was collected from a regional farm bulk milk tank (Lille, France). Cows were maintained on pasture feeding and supplemented with a corn-based concentrate. After arrival at the laboratory, milk samples were kept at −18 °C until analysis (2 weeks).

Samples preparation for the gelation

Milk samples were thawed by holding at 4 °C for 12 h and then kept at room temperature (∼18 °C) for 15 min to avoid thermal shock. The samples were then placed in a water-bath preset at 50 °C or 70 °C for 10 min. Milk samples were then directly cooled under the tap water for 5 min and placed immediately in another water bath equilibrated previously at 36 °C for 5 min.

Anhydrous calcium chloride ($CaCl_2$) powder (Merck, Darmstadt, Germany) at 1.11 and 2.22 g L^{-1} and di-sodium hydrogen phosphate dehydrate powder ($Na_2HPO_4 \cdot 2H_2O$; Merck) at 1.78 and 3.56 g L^{-1} were separately added to milk samples with stirring for 2 min. A volume of 8.75 µL of the rennet FAR-M®Sticks (12500 IMCU/stick) (Chr. Hansen A/S BoegeAllé, Hoersholm, Denmark) prepared from 1 g of powder FAR-M®Sticks diluted in 20 g of double distilled water was added to 35 mL of milk and measurements were immediately recorded. Raw milk (i.e. samples without preheating at 50 and 70 °C) and control milk corresponding to milk (raw, preheated at 50 or 70 °C) without added phosphate and calcium were also prepared.

Determination of the pH during the gelation

The pH was measured at 0, 15, 30 and 45 min during milk gelation using a digital pH meter (WTW pH 330i Taschen-pH-Meter; WTW GmbH, Nanterre, France). Prior to pH measurements, the pH meter was calibrated with standard pH solutions prepared using buffer capsules of pH 7 and 4.

Rheological measurements throughout rennet-induced coagulation

The experiments were performed using a controlled-strain rheometer (Physica MCR 301; Anton Paar Company, Ostfildern, Germany) with low amplitude oscillation shear analysis and a temperature set at 36 °C using a Peltier plate. A volume of 20 mL was placed in two concentric cylinders, with an inner diameter of 26.66 mm, length of 40.02 mm, and a gap of 1.13 mm. A layer of liquid paraffin was placed onto the surface of milk sample to prevent evaporation during coagulation, and then measurement was started. The oscillation experiments were performed for 45 min in the linear visco-elastic region by applying a constant frequency of 1 Hz and a strain of 0.05%.

Fluorescence measurements

Fluorescence spectra were recorded using a Fluoromax-4 spectrofluorometer (Jobin Yvon, Horiba, NJ, USA). The incidence angle of the excitation radiation was set at 60° to ensure that reflected light, scattered radiation, and depolarisation phenomena were minimised. The spectrofluorometer was equipped with a thermostated cell and the temperature was controlled by a Haake A25 AC 200 temperature controller (Thermo-Scientific, Courtaboeuf, France). Milk samples were poured into a 3-mL quartz cuvette and fluorescence spectra were recorded at 36 °C. The excitation and emission wavelengths were set at 290 and 410 nm, respectively, to record the emission spectra of tryptophan residues (305–450 nm) and the excitation spectra of the vitamin A (252–390 nm). Each spectrum was recorded twice at 0, 10, 20, 30, 40, and 45 min during gelation.

Mathematical analysis of data

Rheological and pH parameters were compared using a one-way ANOVA ($P < 0.05$). ANOVA was applied using XLSTAT 2013 (Addinsoft SARL USA, New York, NY, USA) software.

In order to reduce scattering effects and to compare samples, fluorescence spectra were normalised by reducing the area under each spectrum to a value of 1 according to others.[25,26] Specifically, the shift of the peak maximum and the peak width changes in the spectra were considered following normalisation. The PCA was applied to the normalised spectra to monitor the changes occurring during the gelation process. The PCA transforms the original variables into new axes called principal components (PCs). This statistical multi-variate treatment was earlier used to observe similarities among different samples[27,28] reducing the dimension to two or three PCs, while keeping most of the original information found in the data sets.

In a second step, common components and specific weight analysis (CCSWA) was applied to the whole data sets. The objective of this technique is to describe several data sets observed for the same samples. The CCSWA takes into account the maximum inertia (total variance) of the data sets (tryptophan, vitamin A, and rheological data sets). The technique consists of determining a common space of representation for all the tables. Each table has a specific weight associated with each dimension for this common space. A large difference between the values of the specific weights for a given dimension would express the fact that this dimension reveals physical phenomena, which is visible by one method and not by the others. The CCSWA deals with analysis of co-inertia that is the total variance in data sets and enable the overall data collected to be described by taking into account the relation between the different data sets. Samples can be drawn by projection on the planes defined by each couple of the $q_1, q_2, \ldots, q_n$ dimensions. Orthogonal spectral patterns related to the q_n dimension can be calculated. The PCA and CCSWA were performed using MATLAB Software (Version 6, Release 12; The MathWorks S.A.S, Sèvres Cedex, France).

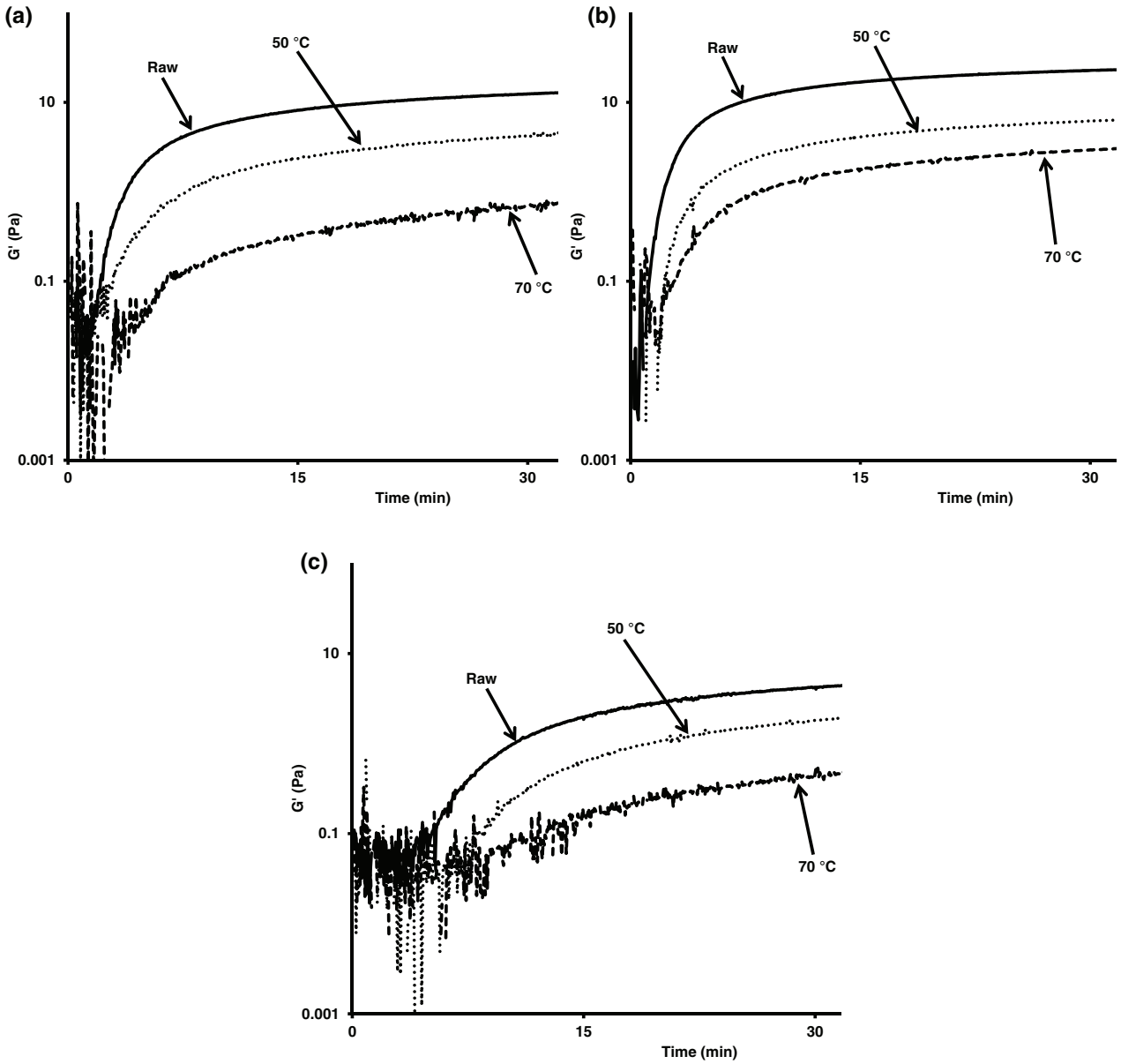

Figure 1. Storage modulus (G') as a function of time for gels made with raw and preheated control camel milk (a) and those with added 1.11 g L^{-1} calcium (b) and 1.78 g L^{-1} phosphate (c). Control milk: Milk with no added calcium and phosphate.

RESULTS AND DISCUSSION

Evolution of pH throughout the renneting of cow and camel milk

The pH values of gels made with control raw camel and cow milk changed slightly throughout the whole gelation reaching values of 6.66 and 6.64 after 45 min of rennet-induced coagulation, respectively. Irrespective of milk species, the addition of calcium and phosphate to milk induced a decrease and an increase of pH values of gels, respectively compared to those made with control milk. Indeed, the supplementation of milk with 2.22 g L^{-1} calcium caused significant ($P < 0.05$) reductions in the pH with values of 6.16 and 6.14 being recorded for gels made with camel and cow milk respectively after 45 min of rennet-induced coagulation, which is consistent with previous investigations.[15,29]

On the contrary, the fortification of milk with 3.56 g L^{-1} phosphate induced a significant increase ($P < 0.05$) of pH values of formed gels, irrespective of milk type. Indeed, the pH of gels made with camel and cow milk was of 7.12 and 7.01, respectively after 45 min of rennet-induced coagulation. This trend could be related to the increase of negative casein charge.[30] Similar results were observed for gels produced with camel and cow milk preheated at 50 and 70 °C (data not shown).

Rheological properties

Calcium/phosphate added to milk at different concentrations showed similar trends for both types of gels. An example of the evolution of G' as a function of time for control camel milk gels and those added with 1.11 g L^{-1} calcium and 1.78 g L^{-1} phosphate is presented in Fig. 1a to c. As observed, significant higher values of G' ($P < 0.05$) were observed for gels made with raw milk samples irrespective of the added calcium and phosphate levels, while gels obtained with milk samples preheated at 70 °C presented significantly lower values of G' ($P < 0.05$). The same trend was observed for gels produced with cow milk (data not shown).

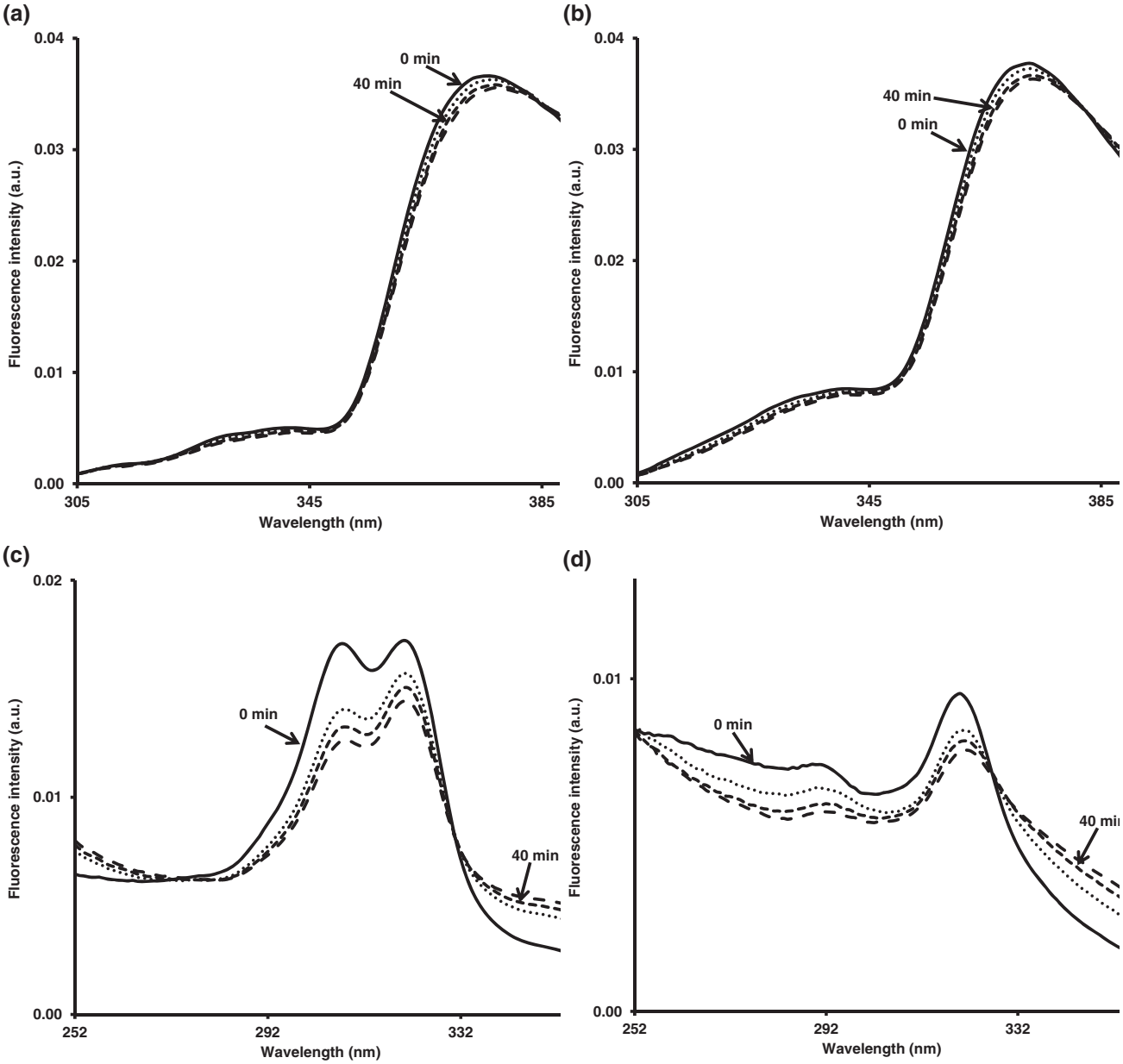

Figure 2. Normalised tryptophan emission spectra of gels made with control raw camel milk (a) and cow milk (b), and vitamin A excitation spectra of gels made with control raw camel milk (c) and cow milk (d) acquired at 0, 20, 40, and 45 min of rennet-induced coagulation. Control milk: Milk with no added calcium and phosphate.

The decrease of the gel firmness after heat-treatment of milk could be attributed to the denaturation of whey proteins, leading to hampering of the interaction between casein micelles inducing the formation of a weak network.[31] It could be concluded that the resulting gels presented different rheological properties, suggesting different organisation of the protein networks at the molecular level.

Formation of a network structure during the rennet-induced coagulation of camel and cow milk

Fluorescence spectroscopy is a very sensitive technique providing useful information about the structural evolution during milk coagulation. Indeed, monitoring tryptophan residues should allow the investigation of the protein structure and its environment, while vitamin A, located in fat globules, determines the physical state of the triglycerides and protein–lipid interactions.[20]

Evolution of camel and cow milk fluorescence spectra during the gelation

Example of the normalised tryptophan emission spectra of rennet-induced coagulation of gels made with control raw milk scanned between 305 and 450 nm at 0, 20, 40 and 45 min are presented in Fig. 2a and b. The spectra exhibited a shoulder ~ 340 nm and a maximum ~ 375 nm that change slightly during the rennet-induced coagulation. Indeed, throughout coagulation, both a red shift of the maximum emission of tryptophan and a decrease in the fluorescence intensity at 375 nm were observed. This change could be attributed to the: (1)

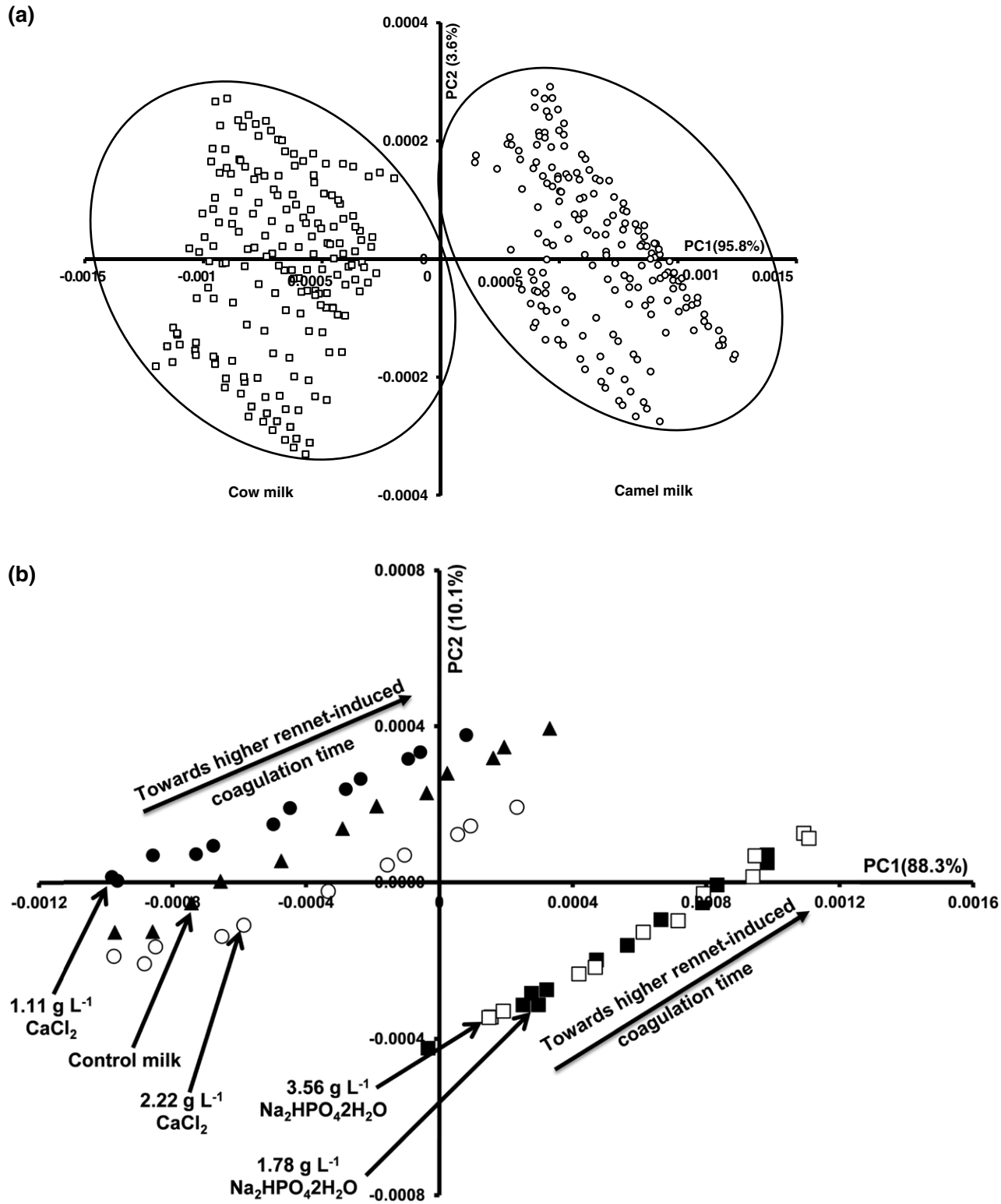

Figure 3. Principal component analysis similarity map determined by principal components 1 (PC1) and 2 (PC2) of tryptophan fluorescence spectra of (a) gels made with camel and cow milk and (b) gels made with raw camel milk. Control milk: milk with no added calcium and phosphate.

modification of tryptophan environment due to the exposure of tryptophan to solvent as illustrated by the red shift;[19,32,33] and (2) modification in the protein–protein, protein–water, and/or protein–lipid interactions.[16,17] Similar emission spectra were observed on the gels obtained with control milk samples preheated at 50 and 70 °C and those added with minerals (data not shown).

The vitamin A excitation spectra scanned on gels made with control raw milk are illustrated in Fig. 2c and d. The vitamin A spectra of gels made with camel milk showed two peaks located ~307

and 320 nm (Fig. 2c), while those produced with cow milk (Fig. 2d) exhibited maxima at 290 and 320 nm. The spectra changed during coagulation indicating modification in the environment of vitamin A due to lipid–lipid and lipid–protein interactions and/or the modification in the physical state of triglyceride.[23,34,35] Similar excitation spectra were observed on the gels obtained with control milk samples preheated at 50 and 70 °C and those added with minerals (data not shown).

In order to extract information from the data sets, PCA was applied, separately, to the normalised tryptophan and vitamin A fluorescence spectra. The map defined by the two first PCs of the PCA is shown in Fig. 3a. Considering the PC1, accounting for 95.8% of the total variance, a clear discrimination between the gels produced with camel and cow milk was observed, irrespective of the preheat treatment applied to milk and the added minerals. These results reflected that tryptophan modifications occurring during rennet-induced coagulation were different in camel and cow milk. This hypothesis was confirmed by the PCA applied to vitamin A spectra since similar results to those observed with tryptophan were observed (data not shown).

In a second step, PCA was performed separately for each species and each condition (i.e. raw, preheated milk at 50 and 70 °C). Figure 3b shows the similarity map of the first two PCs of the PCA applied to the tryptophan spectra scanned on the gels made with raw camel milk. As observed, gels made with control camel milk and those added with calcium at 1.11 and 2.22 g L^{-1} presented mostly negative score values, while those supplemented with phosphate exhibited positive scores. Although a clear separation was observed on the similarity map between gels obtained with camel milk samples enriched with 1.11 and 2.22 g L^{-1} calcium, an overlapping was observed for gels fortified with phosphate at 1.78 and 3.56 g L^{-1} suggesting that the increase of the added phosphate level to milk (from 1.78 g L^{-1} to 3.56 g L^{-1}) did not affect the molecular structure of tryptophan, that was not the case of calcium. One explanation could arise from the ability of calcium to bending with tryptophan (that was not the case of phosphate) causing changes in the tryptophan fluorescence spectra. However, for each group of gels, a clear discrimination of rennet-induced coagulation as a function of time was observed. Similar results were noted for the gels made with raw cow milk (data not shown).

From these results, it may be concluded that the enrichment of camel and cow milk with either calcium or phosphate induced changes at the molecular level of the formed gels that impacted the texture at the macroscopic level as indicated by the rheology measurements. Similar results were obtained for gels produced with camel and cow milk preheated at 50 and 70 °C (data not shown).

Regarding the PCA applied to the vitamin A spectra, only a slight discrimination was obtained between gels produced with milk samples supplemented with calcium and phosphate (data not shown). This could be explained by the fact that the most important modifications occurring during rennet-induced coagulation concern the protein matrix, which accounts for the high potential of tryptophan fluorescence spectra to monitor changes at the molecular level of rennet-induced coagulation.

Joint analysis of tryptophan, vitamin A and rheological data sets: CCSWA

The CCSWA method was applied to the three normalised data sets including tryptophan, vitamin A and rheological parameters. The map defined by the first two common components gave different weights: the first common component expressed 81.7% of the inertia of tryptophan and a tiny part of vitamin A (12.9%) and rheological (9.3%) data set (Table 1). On the contrary, the second common component expressed 81.8% of the inertia of the vitamin A and a tiny part of rheological parameters (1.6%) and tryptophan (1.2%).

Table 1. Saliences of the first two common components q_1 and q_2 of the common components and specific weights analysis (CCSWA) performed on the tryptophan, vitamin A and rheological data recorded on gels made with raw and preheated camel milk at 50 and 70 °C

	Common component (%)	
Parameter	q_1	q_2
Rheological data	9.32	1.56
Tryptophan	81.67	1.22
Vitamin A	12.93	81.83

From Table 1, it could be concluded that the weight of two data sets stakeholders in the constitution of the common components 1 and 2 are similar: tryptophan fluorescence spectra expressed 82.89% of the inertia for common components 1 and 2, quite similar to that found with vitamin A (94.76% of the inertia). It proves that the spectral variations recorded by each fluorophore on gels made with raw and preheated camel milk enriched or not with calcium and phosphate are inter-related. We concluded that for the first two common components, similar phenomena are observed by each probe: this is only possible if the interactions concerned the protein network and fat globules which cause changes in both the shape of tryptophan and vitamin A spectra. These results confirmed a previous study,[36] which pointed out that protein–fat interactions induced changes in the shape of tryptophan and vitamin A spectra.

The plane defined by the common components 1 and 2 is presented in Fig. 4a. Considering q_2, gels made with raw camel milk presented negative score values, while those produced with preheated camel milk at 50 and 70 °C were located mostly on the positive side. From these results, it could be concluded that preheat treatment applied to milk induced modifications in the protein structure and protein–fat globule, protein–protein and protein–water interactions of the obtained gels, allowing CCSWA to distinguish between gels produced with raw camel milk from those preheated at 50 and 70 °C. Similar results were observed with gels made with cow milk (data not shown). Additionally, for each group (gels produced with raw milk or preheated milk at 50 and 70 °C), a trend of a clear discrimination was observed as a function of time.

The examination of the spectral pattern of tryptophan associated with q_1 (Fig. 4b) illustrated that the most interesting band discriminating between gels occurs at approximately 365 nm. The spectral pattern 1 showed enlargement of spectra located on the positive side corresponding to gels acquired at the final stage of rennet-induced coagulation. This could be due to the modification of the environment of tryptophan during rennet-induced coagulation that became more hydrophilic resulting from the changes of protein structure.[19,37] The spectral pattern 2 is too difficult to analyse. However, it showed a red shift to higher wavelengths for gels obtained with milk preheated at 50 and 70 °C that was due to the change of the tryptophan conformation.

The spectral pattern of the vitamin A associated with q_1 (Fig. 4c) was characterised by a negative band located ~299 nm and a positive one ~324 nm. The spectral pattern 2 exhibited two negative

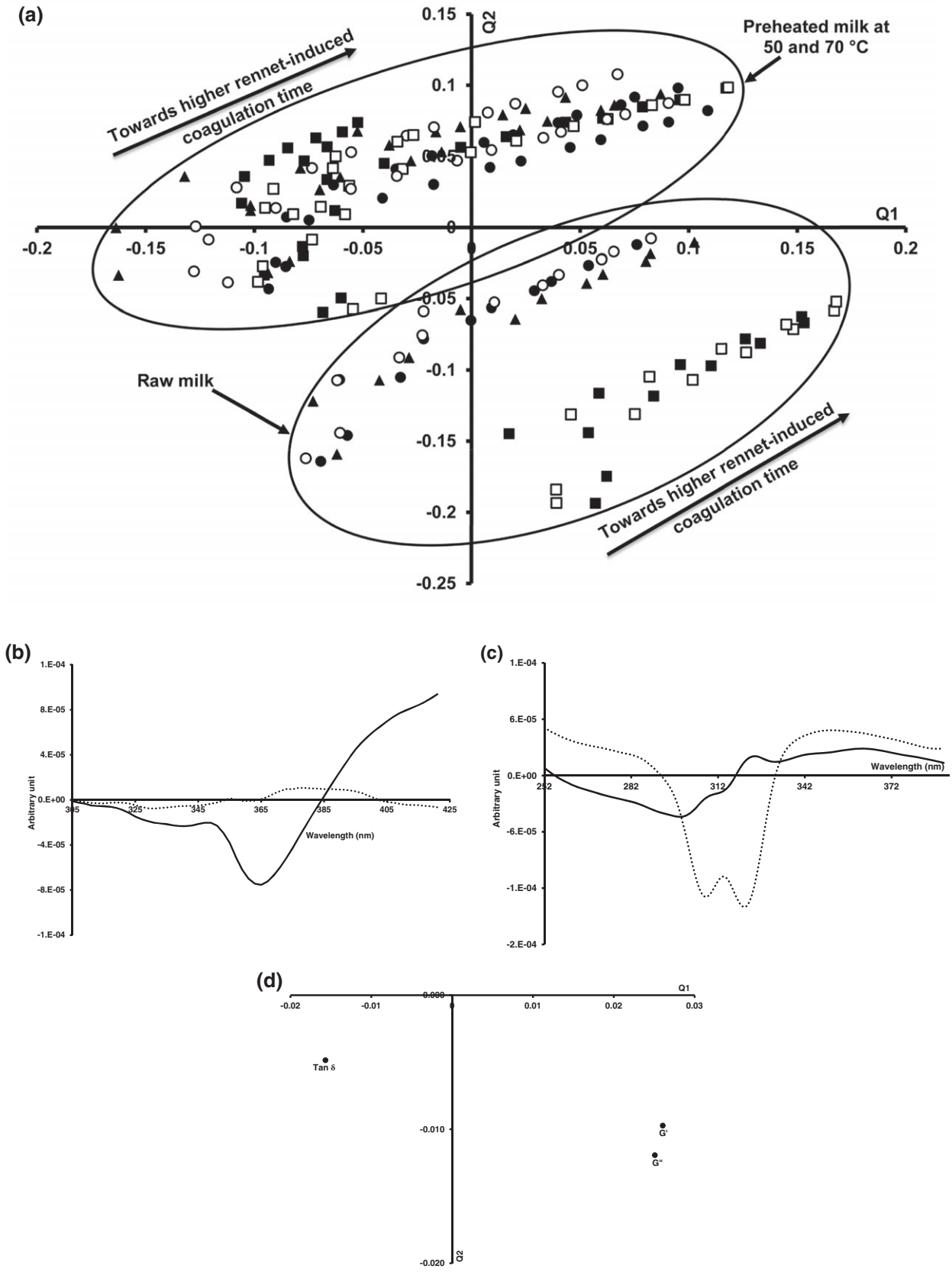

Figure 4. (a) Common components and specific weights analysis (CCSWA) similarity map defined by the common components 1 and 2 of gels made with raw and preheated camel milk at 50 and 70 °C applied to the tryptophan, vitamin A and rheological data sets for control (▲), 1.11 g L^{-1} calcium (●), 2.22 g L^{-1} calcium (○), 1.78 g L^{-1} phosphate (■) and 3.56 g L^{-1} phosphate (□); spectral patterns of tryptophan (b) and vitamin A (c) associated with the common components 1 (____) and 2 (.....), and rheological data (d) associated with the common components 1 and 2. Control milk: Milk with no added calcium and phosphate.

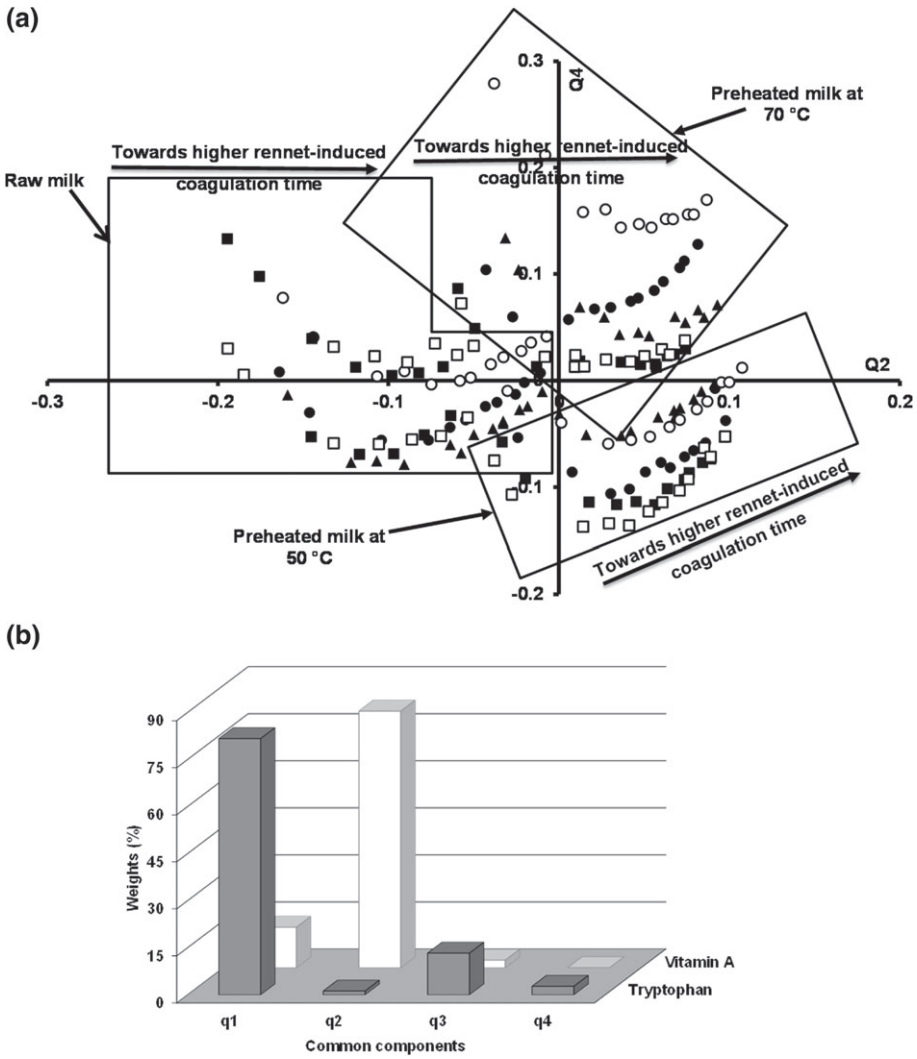

Figure 5. (a) Common components and specific weights analysis (CCSWA) similarity map defined by the common components 2 and 4 of gels made with raw and preheated camel milk at 50 and 70 °C applied to the tryptophan and vitamin A data sets for control (▲), 1.11 g L^{-1} calcium (●), 2.22 g L^{-1} calcium (○), 1.78 g L^{-1} phosphate (■) and 3.56 g L^{-1} phosphate (□); (b) weights for the common components q_1, q_2, q_3 and q_4 of CCSWA performed on tryptophan and vitamin A fluorescence spectra. Control milk: Milk with no added calcium and phosphate.

bands located ~309 and 320 nm (Fig. 4c). The changes in the spectral patterns 1 and 2 could be due to modification of the viscosity of fat, protein–lipid, and/or lipid–lipid interactions of samples during coagulation.[24,38]

The rheological data associated with q_1 and q_2 (Fig. 4d) showed that gels made with raw milk presented higher G' and G'' values than those produced with milk preheated at 50 and 70 °C.

Regarding the weights associated with q_1 and q_2, it appeared that the separation of samples was based on vitamin A and tryptophan fluorescence spectra. To confirm this hypothesis, a new CCSWA was applied to the normalised tryptophan and vitamin A spectra (Fig. 5a). The similarity map divided the samples into three groups. The first group contained gels made with raw milk samples having negative scores according to q_2. The second group contained gels formed with preheated milk at 70 °C and presenting positive scores according to q_4. The third group contained all the gels made with milk preheated at 50 °C that presented positive and negative scores according to q_2 and q_4, respectively. The gels were clearly separated according to heat treatment applied to milk. Indeed, a clear separation of gels made with preheated milk at 50 °C from those preheated at 70 °C was observed. This was not obtained with the CCSWA applied to fluorescence and rheology data sets, since gels made with preheated milk at 50 and 70 °C were overlapped on the map.

Regarding the weights associated with q_2 and q_4 (Fig. 5b), it could be concluded that the information contained in the tryptophan and vitamin A spectra referred to similar phenomena. Thus, the fat globule–protein interaction is the only common phenomenon observed from the vitamin A and tryptophan fluorescence spectra. Similar results were observed with gels made with cow milk (data not shown).

CONCLUSIONS

The results presented in this work demonstrate the potential for front-face fluorescence spectroscopy to investigate changes in the micelle structure, aggregation of particles and interactions of casein with water during the rennet-induced coagulation process. Many of the structural modifications occurring in micelle structure and interaction can thus be revealed. Indeed, a clear

separation of gels according to heat treatment applied to milk and the added calcium and phosphate levels was observed. Tryptophan and vitamin A spectra combined with CCSWA have the potential to monitor molecular changes in complex product such as milk. The analysis of tryptophan and vitamin A spectral patterns resulting from CCSWA showed that rennet-induced coagulation could be viewed as a progressive process since a trend to a clear separation of gels as a function of time was observed. The CCSWA applied to the vitamin A and tryptophan spectra allowed a global characterisation of the modifications of protein matrix and lipid throughout gelation. Fluorescence and rheological data scanned on rennet-induced coagulation of cow and camel milk give information on the molecular and macroscopic levels that could be used as complementary tools.

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Partie 2

Potentiel de la spectroscopie de fluorescence frontale couplée à la chimiométrie pour l'étude des changements structuraux du lait de chamelle au cours du chauffage

Partie 2 : Potentiel de la spectroscopie de fluorescence frontale couplée à la chimiométrie pour l'étude des changements structuraux du lait de chamelle au cours du chauffage

Ce chapitre a fait l'objet d'une publication :

- Kamal, M., & Karoui, R. (2016). Monitoring of mild heat treatment of camel milk by front-face fluorescence spectroscopy. *LWT-Food Science and Technology* (article 5, **79, 586–593**).

I. Introduction

Le lait est un produit fragile et facilement altérable ce qui est dû à la complexité de sa structure physique et la grande diversité de ses constituants chimiques. Dès la réception du lait en usine et avant sa transformation, des traitements technologiques sont généralement appliqués pour en assurer sa stabilité. Le traitement thermique est généralement la première étape appliquée par l'industrie laitière pour améliorer la qualité de ce produit et allonger sa durée de conservation en réduisant la charge microbienne initiale présente dans le lait (Donato et Guyomarc'h, 2009). Différentes techniques ont été utilisées pour évaluer la qualité du lait suite à un traitement thermique comme les méthodes physico-chimiques, chromatographiques, microbiologiques et sensorielles. Cependant, la plupart de ces techniques sont destructives, coûteuses et longues à mettre en œuvre. Par conséquent, il nous a semblé judicieux de développer des méthodes rapides, non-destructives, non-invasives et peu coûteuses afin d'étudier les changements moléculaires qui peuvent avoir lieu lors d'un traitement thermique du lait. Ainsi, la spectroscopie de fluorescence frontale a été étudiée comme un outil de suivi des changements induits dans le lait de chamelle lors de traitements thermiques modérés.

II. Spectroscopie de fluorescence frontale couplée aux outils chimiométriques comme outil de caractérisation du traitement thermique appliqué au lait de chamelle

Le lait de chamelle fait partie de l'alimentation de base des populations vivant dans les zones arides et semi-arides (Asres et Yusuf, 2014). Il est riche en différents nutriments (protéines, lipides, lactose, minéraux, etc.) et possède une haute valeur biologique grâce à ses teneurs élevées en composés antimicrobiens tels que le lysozyme, la lactoferrine et les immunoglobulines (El-Agamy et al. 1998). Sa conservation peut être atteinte en appliquant différents traitements thermiques tels que la pasteurisation et la stérilisation permettant de

prolonger sa durée de vie de quelques jours à plusieurs mois (Felfoul et al. 2015). Cependant, ce traitement provoque des changements dans les propriétés physiques, chimiques et sensorielles du lait comme, par exemple, la dénaturation des protéines du lactosérum, la dégradation du lactose, de certaines vitamines et enzymes (El-Agamy, 2000a ; Mohamed et El-Zubeir, 2014 ; Sakkas et al. 2014). Comme l'a montré une recherche bibliographique, peu de techniques analytiques ont été utilisées pour déterminer les changements moléculaires qui ont lieu dans le lait de chamelle suite à différents traitements thermiques. Parmi ces méthodes, la spectroscopie de fluorescence frontale couplée aux outils chimiométriques a démontré sa capacité comme une méthode rapide et non-destructive pour contrôler les changements moléculaires des composants d'un lait traité thermiquement (Dufour et Riaublanc, 1997 ; Kulmyrzaev et al. 2005). En revanche, la potentialité de cette technique pour déterminer les modifications induites dans le lait de chamelle lors d'un traitement thermique n'a pas été testée.

L'objectif de cette étude fut d'explorer le potentiel de la spectroscopie de fluorescence frontale combinée aux méthodes statistiques multivariées pour caractériser les changements structuraux au niveau moléculaire du lait de chamelle suite à différents traitements thermiques.

L'étude a été effectuée en utilisant du lait de chamelle obtenu d'une ferme expérimentale tunisienne (El Jem, Mahdia). Une série d'échantillons de lait a été préparée en chauffant le lait cru dans un bain-marie préréglé à 55, 60, 65, 70 et 75 °C. Pour ce faire, des aliquots de 3 mL de lait ont été prélevés et transférés dans des tubes à bouchons vissés. Les tubes étaient disposés dans un support dans le bain-marie réglé à une température donnée. Pour chaque température, un tube était prélevé à 0,5, 1, 5, 10 et 30 minutes et refroidi immédiatement dans un bain d'eau glacée pendant 5 minutes, puis conservé à température ambiante pendant 15 minutes. Un échantillon de lait cru n'ayant subi aucun traitement thermique a été utilisé comme témoin.

Les spectres d'émission de fluorescence du NADH, des PFRM (Produits Fluorescents de la Réaction de Maillard) et d'excitation de la vitamine A ont été enregistrés sur les échantillons en utilisant le spectrofluorimètre Fluoromax-4 (Jobin Yvon, Horiba, NJ, Etats-Unis). Les spectres du NADH ont été acquis pour appréhender l'état d'oxydation de cette coenzyme dans le lait traité thermiquement. Quant aux spectres des PFRM et de la vitamine A, ils ont été choisis dans le but d'obtenir des informations sur la vitesse de formation des produis fluorescents de la Réaction de Maillard et l'état physique des triglycérides présents dans les globules gras, respectivement.

Les résultats de cette étude présentés dans **l'article 5** ont permis de déceler une augmentation du maximum de l'intensité de fluorescence des PFRM au voisinage de 480 nm avec l'augmentation de la température appliquée au lait (**cf. article 5, Figure 1c**). En revanche,

une diminution de l'intensité de fluorescence des maximas du NADH (480 nm) et de la vitamine A (320 nm) a été notée avec l'augmentation de l'intensité du traitement thermique (**cf. article 5, Figures 1a et b**). Cette réduction de l'intensité de fluorescence du NADH et de la vitamine A pourrait être expliquée par : i) l'oxydation du NADH à des hautes températures ; et ii) la diminution de la viscosité de la phase solvant (les triglycérides) avec l'augmentation de l'intensité du traitement thermique induisant des modifications au niveau des interactions protéine - globules gras.

L'application de l'ACCPS à l'ensemble des spectres de fluorescence a permis de séparer les échantillons du lait de chamelle en fonction de l'intensité du traitement thermique et du temps de chauffage (**cf. article 5, Figure 3a**).

Il en ressort que la technique de spectroscopie de fluorescence couplée à l'ACCPS présente un fort potentiel pour évaluer le traitement thermique appliqué au lait de chamelle et pour suivre les changements de ses constituants au niveau moléculaire.

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Monitoring of mild heat treatment of camel milk by front-face fluorescence spectroscopy

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ABSTRACT

The potentiality of the front-face fluorescence spectroscopy coupled with chemometric tools to characterize changes in camel milk following thermal treatments in the 55–75 °C temperature range from 0.5 min up to 30 min was studied. Nicotinamide adenine dinucleotide (NADH), fluorescent Maillard reaction products (FMRP) and vitamin A fluorescence spectra were collected on camel milk samples. Using principal component analysis (PCA), the vitamin A spectra allowed some discrimination between milk samples according to heat treatment intensity and holding time. The best results was obtained by applying common components and specific weights analysis (CCSWA) to the three spectral data sets, since a clear differentiation between camel milk samples preheated at 70 and 75 °C from the others was achieved. Apparent activation energy of camel milk determined from NADH, FMRP and vitamin A spectra was of 85.1, 84.2 and 47.3 kJ mol^{-1}, respectively. The spectral patterns of the CCSWA model enabled us to get information about the molecular changes occurring in camel milk fluorophores during heat treatment.

1. Introduction

Milk plays an important role in the human diet thanks to its balanced composition insuring the essential nutriments including proteins, carbohydrates, fats, vitamins, and so on (Haug, Hostmark, & Harstad, 2007). Camel milk represents one of the main sources of nutrients in many parts of the world, especially, in the arid and semi-arid areas (Al-haj & Al-kanhal, 2010). From a nutritional point of view, it has been reported that the level of vitamin C in camel milk is three to five times higher than that in bovine milk (Al-haj & Al-kanhal, 2010). Additionally, camel milk has many therapeutic properties since it has been used as anti-carcinogenic (Magjeed, 2005) and anti-diabetic (Agrawal, Budania, Sharma, Gupta, & Kochar, 2007) agents.

Thermal processing of milk is considered as one of the essential steps of milk production. The purpose of milk heat treatment is to improve the quality of this biological fluid and prolong its shelf life by either partial destruction of microorganisms or complete sterilization of milk (Hattem, Manal, Hanna, & Elham, 2011; McKinnon, Yap, Augustin, & Hermar, 2009; Mohamed & El Zubeir, 2014). However, thermal treatment affect functional properties of milk proteins and induce changes in their physical, chemical, and sensorial properties such as the degradation of lactose, denaturation of whey proteins, destruction of some vitamins and enzymes, hydrolysis of proteins and lipids, and so on (Donato & Guyomarc'h, 2009; Elagamy, 2000; Farah, 1986; Sakkas, Moutafi, Moschopoulou, & Moatsou, 2014).

In the literature, various conventional methods has been employed to monitor the quality of heated milk such as physico-chemical, chromatographic, microbiological, and sensory measurements (Christensen & Reineccius, 1992; Clare et al., 2005; Elagamy, 2000; Elliott, Dhakal, Datta, & Deeth, 2003; Hattem et al., 2011; Mayer, Raba, Meier, & Schmid, 2010; Sakkas et al., 2014; Ul Haq et al., 2013, 2014). Although the above mentioned techniques are considered as reference ones, they are destructive, costly, time-consuming, and require sophisticated analytical equipment and skilled operators (Dufour & Riaublanc, 1997). Thereby, more attention has been paid to the development of non-invasive and non-destructive techniques to be used as rapid

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screening tools for the evaluation of the quality of milk (Kamal & Karoui, 2015). Front-face fluorescence spectroscopy (FFFS) is a rapid method, relatively inexpensive, and gives a great deal of information with only one test. This technique is known to be sensitive and non-destructive, and enable to provide information on the presence of fluorescent molecules and their environment in all types of biological samples containing double conjugated bonds (Christensen, Becker, & Frederiksen, 2005; Karoui et al., 2005b; Karoui, Dufour, Schoonheydt, & De Baerdemaeker, 2007a). In dairy products, several intrinsic fluorescent components including tryptophan, vitamin A, aromatic amino acids and nucleic acids, fluorescent Maillard reaction products (FMRP), NADH, and so on were found to be sensitive to their environment (Andersen & Mortensen, 2008; Dufour & Riaublanc, 1997; Strasburg & Ludescher, 1995).

The application of FFFS in the research fields of dairy products is usually related with the use of multivariate statistical analyses. For example, this technique coupled with chemometric tools has been used to: i) monitor the structural changes occurring in milk proteins and their physicochemical environment during milk coagulation and heat treatment (Blecker, Habib-Jiwan, & Karoui, 2012; Boubellouta & Dufour, 2008; Dufour & Riaublanc, 1997; Hougaard, Lawaetz, & Ipsen, 2013; Kulmyrzaev, Levieux, & Dufour, 2005); ii) determine the quality of different varieties of cheeses during ripening (Karoui, Dufour, & De Baerdemaeker, 2007b; and iii) authenticate milk (Karoui, Martin, & Dufour, 2005a) and Emmental cheeses according to their geographic origin (Botosoa & Karoui, 2013).

Compared to other milk types, camel milk is known to have high biological value: absence of β-lactoglobulin, high percentage of vitamin C which is three to five times higher than that of cow's milk, high contents of anti-microbiologic agents, due to the presence of lysozyme, lactoperoxydase, lactoferrin, immunoglobulin, and bacteriocins produced by lactic acid bacteria (Elagamy, Nawar, Shamsia, Awad, & Haenlein, 2009).¶All these characteristics contributed to the stability of camel milk, since its stability is maintained for 5 days at 30 °C, while cow's milk is completely destabilized after 48 h at the same temperature.

At our best knowledge, only limited studies were carried on the effect of heat treatment of camel milk constituents. For example, Elagmy (2000) compared by using electrophoresis method, the impact of heat treatment in the 65–100 °C range for 10, 20 and 30 min on the quality of whey proteins, similar to the study conducted by Farah (1986). Recently, Mohamed and El Zubeir (2014) determined the impact of two heat treatments (63 °C and 72 °C for 30 min and 15 s, respectively) on the microbiological quality of camel milk and reported that heat treatment improves the microbial quality and extends the shelf life of camel milk.

No data are present in the literature on the effect of camel milk heat treatment on its structure. Thus, the objective of this study was to explore the potential of FFFS combined with multivariate statistical methods to characterize changes occurring in milk by the application of mild heat treatment (55–75 °C temperature range from 0.5 min up to 30 min).

2. Materials and methods

2.1. Milk samples

Fresh camel milk (2 L distributed into plastic bottles of 30 mL capacity) was obtained from an experimental station located in the center of Tunisia (ElJem region in Mahdia governorate). Camels of Maghrebine genotype, aged of 6 years were maintained on pasture feeding (yearly pasture of thorny plants and coquelicot) and supplemented with concentrate. The animals were inspected by a qualified shepherd on a daily basis, and routine animal care and vaccination procedures were conducted as prescribed by best practice protocols.

Fresh cow milk of Holstein Friesian genotype was collected from a regional farm of Lille (France). Cows were maintained on pasture feeding and supplemented with corn-based concentrate. Once arrival to the laboratory, milk samples were kept at −18 °C until analysis.

2.2. Samples preparation

Milk samples were thawed during 12 h at 4 °C and then kept at room temperature (~18 °C) for 10 min to avoid thermal shock. Series of milk samples were prepared by heating raw milk in a water-bath (Haake A25 AC 200 temperature controller, Thermo-Scientific. France) preset at 55, 60, 65, 70 and 75 °C for 0.5, 1, 5, 10 and 30 min at each temperature considered. Heated milk samples were cooled in ice water bath for 5 min and left at room temperature for 15 min. Raw milk was also studied.

2.3. Fluorescence spectroscopy

Fluorescence spectra were recorded using a Fluoromax-4 spectrofluorometer (Jobin Yvon, Horiba, NJ, USA). The incidence angle of the excitation radiation was set at 60° to ensure that reflected light, scattered radiation, and depolarization phenomena were minimized. The spectrofluorometer was equipped with a thermostated cell and the temperature was controlled by a Haake A25 AC 200 temperature controller (Thermo-Scientific. France). Milk samples were placed in 3 ml quartz cuvette and fluorescence spectra were recorded at 20 °C. The emission spectra of NADH (360–600 nm) and FMRP (380–680 nm) were recorded with the excitation wavelengths set at 340 and 360 nm, respectively. The excitation spectra of the vitamin A (252–390 nm) were scanned with the emission wavelength set at 410 nm. For each sample, 2 spectra were acquired.

2.4. Mathematical analysis of data

In order to reduce scattering effects and to compare samples, fluorescence spectra were normalized by reducing the area under each spectrum to a value of 1 according to others (Karoui, Bosset, Mazerolles, Kulmyrzaev, & Dufour, 2005c; Karoui et al., 2007a; Leriche et al., 2004). Specifically, the shift of the peak maximum and the peak width changes in the spectra were considered. PCA was applied to the normalized spectra to investigate differences between milk samples. The PCA transforms the original variables into new axes, or principal components (PCs). This statistical multivariate treatment was earlier used to observe similarities among different samples (Karoui & Dufour, 2003), reducing the dimension to two or three PCs, while keeping most of the original information found in the data sets.

In a second step, common components and specific weight analysis (CCSWA) was applied to the whole data sets. The objective of this technique is to describe several data sets observed for the same samples. The CCSWA takes into account the maximum inertia (total variance) of the data sets (NADH, FMRP and vitamin A spectra). It consists of determining a common space of representation for all the data sets. Each table (NADH, FMRP and vitamin A spectra) has a specific weight associated with each dimension for this common space. A large difference between the values of the specific weights for a given dimension would express the fact that this dimension reveals physical phenomena, which is visible by one method and not by the others. The CCSWA deals with analysis of co-inertia that is the total variance in data sets and enable the overall

data collected to be described by taking into account the relation between the different data sets (Karoui et al., 2006; Mazerolles, Devaux, Dufour, Qannari, & Courcoux, 2002). Similarity maps of the samples can be drawn by projection on the planes defined by each couple of the q_1, q_2, …,q_n dimensions. Orthogonal spectral patterns related to the q_i dimension can be calculated. PCA and CCSWA were performed using MATLAB. The methodology of MATLAB software environment has been used with success in several applications (Khasraghi, Sefidkouhi, & Valipour, 2015, Valipour, 2012 and 2016; Valipour, Banihabib, & Behbahani, 2013; Valipour, Sefidkouhi, & Eslamian, 2015).

3. Results and discussion

3.1. Evolution of fluorescence spectra of camel milk as a function of applied heat treatment

The NADH is an enzyme cofactor naturally found in the milk, having fluorescence properties with absorption and emission maxima set at 340 and 470 nm, respectively (Lacowicz, 2006). An example of normalized NADH emission spectra recorded between 360 and 600 nm after excitation set at 340 nm for raw and heated camel milk samples at different temperatures is shown in Fig. 1a. The spectra exhibited three peaks located ~450, 480 and 510 nm. A decrease in the fluorescence intensity was observed following heat-treatment. Indeed, raw camel milk presented the highest fluorescence intensity (FI) at 480 and 510 nm, while those heated at 75 °C for 30 min presented the lowest intensity. The peaks ~480 and 510 nm could be attributed to NADH and flavin adenine dinucleotide (FADH), respectively. Although, no publication was found on the use of fluorescence spectroscopy for studying camel milk, our results are in line with those of Kulmyrzaev et al. (2005) who observed for heated cow milk in the 57–72 °C range, a maximum emission of NADH and FADH at 460 and 518 nm, respectively.

Fig. 1b illustrates an example of normalized vitamin A excitation spectra scanned between 252 and 390 nm after emission set at 410 nm of raw and heated camel milk samples at different temperatures. As observed, the spectra exhibited two maxima located around 307 and 320 nm that changed drastically with the intensity of heat treatment, in agreement with the findings of Dufour and Riaublanc (1997). The $FI_{320\ nm}/FI_{307\ nm}$ ratio showed some differences from one milk to another, and decreased with the increase of temperature for all the investigated milk. This trend could be explained by: i) the decrease of the viscosity of triglycerides during heating; and/or ii) the modification in the protein-fat globule interactions during heat treatment (Boubellouta & Dufour, 2008). Indeed, it has been shown that vitamin A properties are sensitive to the changes in the solvent viscosity (Karoui, Laguet, & Dufour, 2003).

Maillard reaction products are fluorescent intermediary substances formed in milk during heating and resulting from chemical reaction between amino groups and reducing sugar (Van Boekel, 1998). Several intermediary products formed in heated milk have been reported in the literature such as keto and aldoseamine compounds (Hodge, 1953), maltol (Potter & Patton, 1956), pyrrole and imidazole derivatives (Birlouez-Aragon, Sabat, & Gouti, 2002),

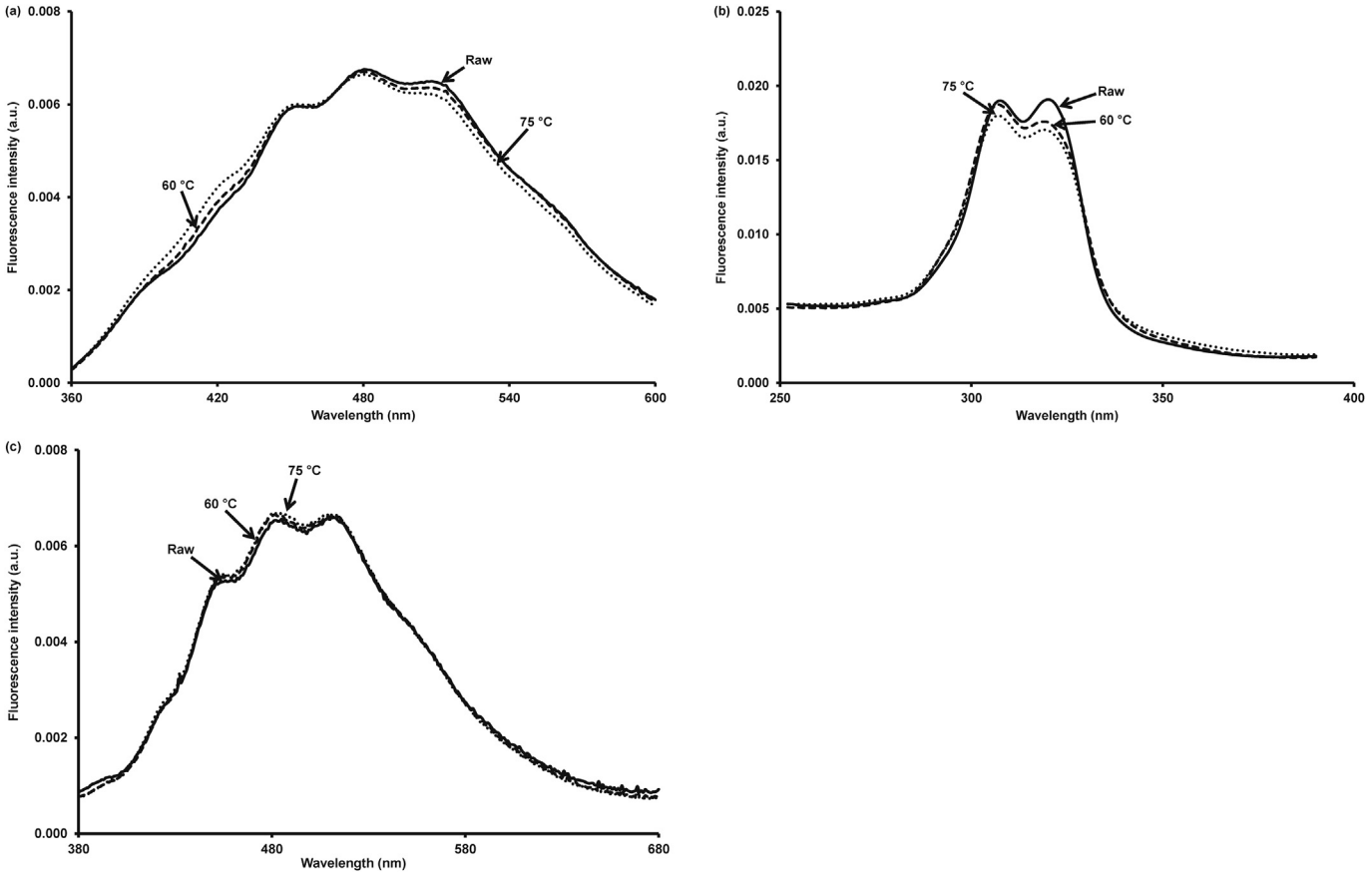

Fig. 1. Normalized fluorescence spectra of NADH (a), vitamin A (b), and fluorescent Maillard reaction products –FMRP- (c) acquired on raw camel milk and those heated at 60, and 75 °C for 30 min.
Kamal and Karoui (2015).

pyranones (Ledl, Hiebl, and Severin (1983), and so on.

An example of normalized FMRP emission spectra recorded between 380 and 680 nm following excitation set at 360 nm for raw and heated camel milk samples at different temperatures is presented in Fig. 1c. As observed, the shape of emission spectra exhibited maxima located ~450, 480 and 510 nm. Additionally, an increase in the fluorescence intensity was observed with the increase of the heating intensity, reflecting the formation of FMRP products. Indeed, the highest fluorescence intensity at 480 nm was observed for milk heated at 75 °C for 30 min, while the lowest intensity was noted for raw milk. These results were in agreement with the finding of Schamberger and Labuza (2006) reporting that the fluorescence intensity of FMRP increased with higher time-temperature combinations of skim milk.

Fluorescence spectra acquired on camel milk are sensitive to heat treatment and holding time, and valuable information on the physicochemical changes in the milk constituents, particularly on heat induced protein denaturation, could be derived from fluorescence spectra.

3.2. Discrimination based on the fluorescence spectra recorded on raw and heated camel milk

As mostly of the investigated spectra presented similar shapes, and cannot thus visually easily be distinguished, multidimensional statistical techniques such as PCA and CCSWA allows to extract useful information from spectral data bases (Karoui et al., 2007a).

PCA was applied to the NADH fluorescence spectra and the similarity map defined by the PC1 and PC2 accounted for 95.2% of the total variance (data not shown). Some discrimination between milk samples heated at 55 and 60 °C from the others was observed according to the PC2 accounting for 25.8% of the total variance.

Better discrimination was obtained by applying the PCA to the emission spectra collected after excitation set at 360 nm (Fig. 2) and emission set at 410 nm (data not shown), since a clear separation between milk samples heated at 55 and 60 °C from the others was observed. Fig. 2 showed that milk samples preheated for 0.5, 1 and 5 min regardless of the heat treatment intensity presented positive scores according to PC1 accounting for 53.8% of the total variance, while the remaining samples were located on the negative side. The first two numbers correspond to heating temperature in °C and the next two numbers mean heating time in min.

3.3. Joint analysis by CCSWA of the three spectral data tables recorded on raw and heated camel milk

The data sets obtained from fluorescence method contain information about the changes occurring at the molecular level of camel milk induced by the intensity of heat treatment and holding time. Therefore, better discrimination of milk samples as a function of time-temperature combination could be obtained by jointly analyzing spectral data sets. This combined analysis can be performed using CCSWA technique (Karoui et al., 2007a).

The CCSWA method was applied to the three normalized data sets containing NADH, vitamin A and FMRP fluorescence spectra. The values of the saliences corresponding to the first three common

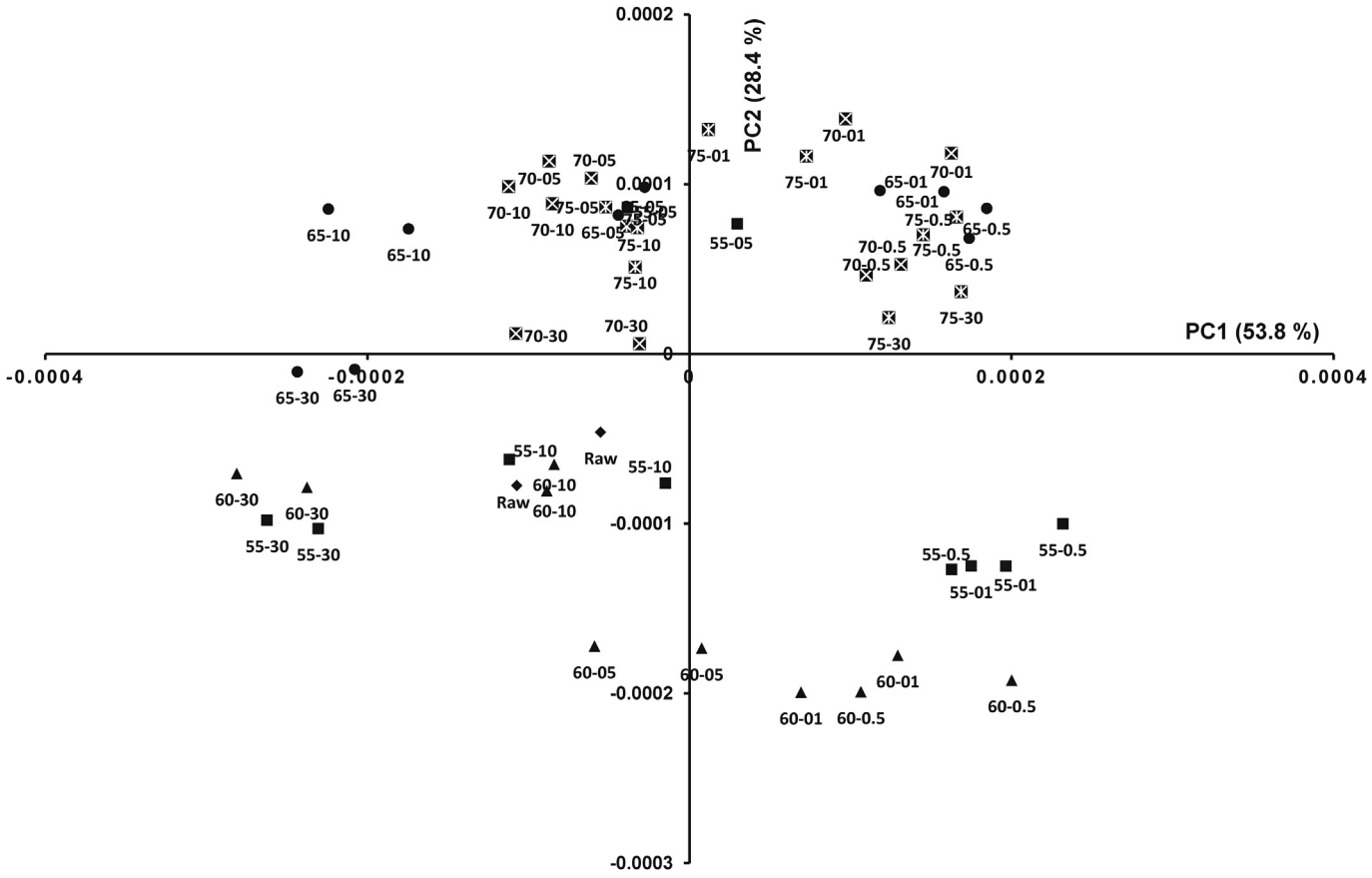

Fig. 2. Principal component analysis similarity map determined by principal components 1 (PC1) and 2 (PC2) of fluorescent Maillard reaction products –FMRP- of raw camel milk and those heated at 55, 60, 65, 70 and 75 °C at different times.
Kamal and Karoui (2015).

Table 1
Saliences of the first three common components q_1, q_2 and q_3 of the common components and specific weights analysis (CCSWA) performed on the NADH, vitamin A and fluorescent Maillard reaction products (FMRP) spectra recorded on raw and heated camel milk.

	Common component (%)		
	q_1	q_2	q_3
NADH	68.54	21.24	3.61
FMRP	7.17	52.35	10.11
Vitamin A	23.71	20.55	34.87

Kamal and Karoui (2015).

components q_1, q_2 and q_3 are presented in Table 1.

The first common component expressed 68.54% and 23.71% of the inertia of NADH and vitamin A spectra, respectively, and a relatively small part (7.17%) of the inertia of the FMRP. On the contrary, the second common component expressed 52.35% of the inertia of the FMRP spectra and 21.24% and 20.55% of the inertia of the NADH and vitamin A spectra, respectively. From the obtained results, it appears that the spectral data recorded using, on the one hand, NADH and vitamin A and, on the other hand, FMRP were independent and that the common components 1 and 2 were related to different phenomena observed by each intrinsic probe.

The plane defined by the first two common components q_1 and q_2 is shown in Fig. 3a. A clear discrimination was observed between milk samples according to the intensity of heat treatment. Indeed, the q_1 divided the samples into three groups. First group contained the raw milk samples. Second group contained camel milk samples heated at 55, 60, and 65 °C, while samples heated at 70 and 75 °C were placed in third group. In addition, for a considered heat treatment, a clear differentiation between milk samples as a function of holding time was observed. From the obtained results, it could be concluded that the discrimination of camel milk samples according to heat treatment and holding time was better than those obtained from the PCA performed separately on NADH, FMRP, and vitamin A fluorescence spectra.

The spectral patterns corresponding to the common components provide information about the characteristic peaks which are the most discriminating for the samples observed on the map. The examination of the spectral pattern of NADH associated with the q_1 (Fig. 3b) showed an opposition between a positive peak located ~415 nm and a negative peak ~510 nm. Milk heated at 70 and 75 °C exhibited the lowest intensity at 510 nm indicating more oxidation of NADH for these milk samples. The q_2 showed a red shift of the maximum emission of NADH.

The spectral patterns of the FMRP associated with the common components q_1 and q_2 are presented in Fig. 3c. The spectral pattern 1 showed a positive peak located ~420 nm for milk samples preheated at 70 and 75 °C. It could be concluded that heat treatment at 70 and 75 °C allowed the formation of more FMRP, in agreement with the findings of Andersen and Mortensen (2008) and Birlouez-Aragon et al. (2002) who reported that the maximum emission of FMRP is in the 415–440 nm range. The examination of the spectral pattern 2 showed an opposition between a positive peak at

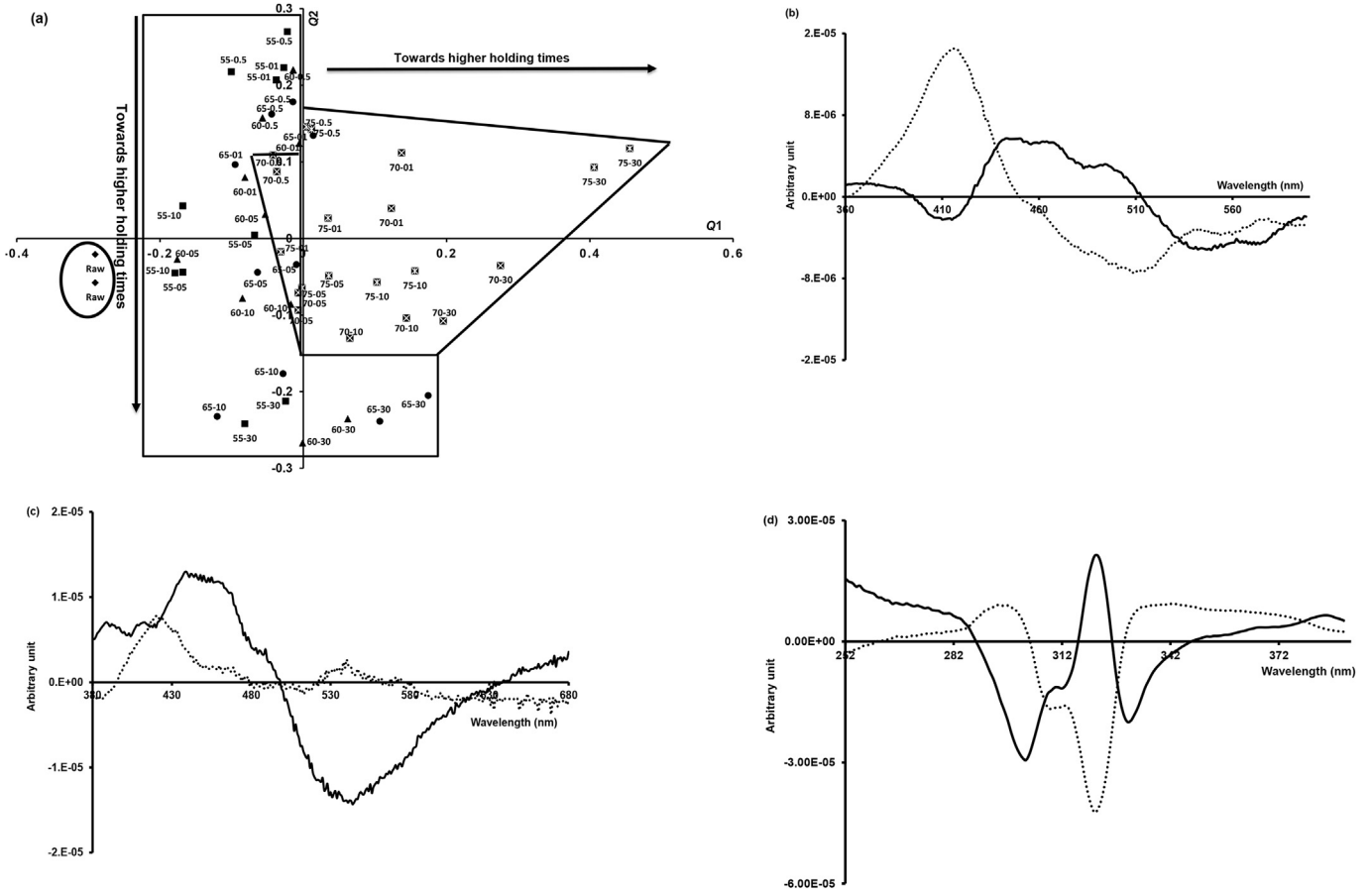

Fig. 3. (a) Common components and specific weights analysis (CCSWA) similarity map defined by the common components 1 and 2 of raw and heated camel milk; spectral patterns of NADH (b), fluorescent Maillard reaction products –FRMP- (c), and vitamin A (d) associated with the common components 1 (····) and 2 (—).
Kamal and Karoui (2015).

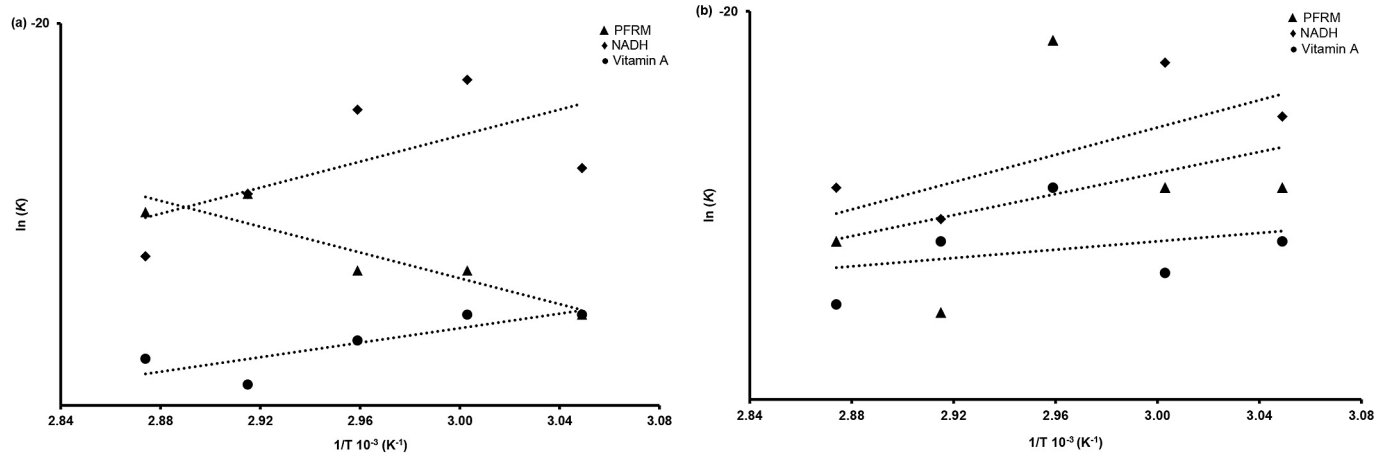

Fig. 4. Arrhenius plot of NADH, vitamin A and fluorescent Maillard reaction products –FMRP- of (a) camel and (b) cow milk heated in the 55–75 °C range for 0.5 min up to 30 min. Kamal and Karoui (2015).

430–460 nm and a negative peak at 540 nm.

The spectral pattern of the vitamin A associated with the common component q_1 (Fig. 3d) was characterized by a positive peak at 295 nm, and a negative one at 322 nm. The q_2 exhibited a red shift of vitamin A spectrum from 322 to 330 nm that could be related to change of the physical state of the triglycerides in the fat globules and/or the interactions of the fat-globule membrane with the protein network as reported by Boubellouta and Dufour (2012) and Karoui et al. (2003).

From the obtained results, it could be concluded that the approach based on using the three data sets allowed to manage, in a very efficient way, all the spectroscopic information collected on camel milk subjected to heat treatment and holding time. The CCSWA method sums all the information occurred during heating of milk on two common components (q_1 and q_2) taking into account the relationship between the different data tables.

3.4. Determination of activation energy

To compare the variation in the fluorescence intensity of camel milk samples subjected to different heat treatments during different holding times, a kinetic study was carried out. It should be kept in mind that chemical changes in milk are often the result of many separate reactions, each with its own energy of activation. All these reactions may depend in different ways on the reaction conditions. Consequently, an activation energy obtained from the temperature dependence of a reaction rate should be considered as an apparent average one. The formation of FMRP and oxidation of NADH could be described by zero-order kinetics in systems with proteins, while destruction of vitamin A could be considered as first-order. The activation energy was estimated using Arrhenius model (Eq. (1)):

$$K_T = A_0 \exp^{(-Ea/RT)} \quad (1)$$

Where K_T is reaction rate constant, A_0 is the pre-exponential Arrhenius factor, R is the ideal gas constant, T is the absolute temperature, E_a is the apparent activation energy of the process.

The logarithm of the rate constant as a function of the reciprocal of the absolute temperature enabled the activation energies of camel and cow milk to be calculated (Fig. 4a, b). The average values of the activation energy for the different fluorophores heated in the 55–75 °C temperature range were between 47 and 85 kJ mol^{-1} for camel milk, while it was in the 22–73 kJ mol^{-1} for cow milk (Table 2). For each fluorophore, the activation energy of camel milk was found to be higher than that of cow milk. This difference might be attributed to the variation in the physico-chemical properties between both types of milk. Indeed, it has been reported that the stabilization of camel milk to the heat treatment was higher than that of cow milk. This trend has been explained by the faster cow milk proteins denaturation compared to camel milk proteins (Laleye, Jobe, & Wasesa, 2008). Additionally, camel milk contained significantly higher concentrations of anti-microbial factors such as lysozyme, lactoferrin and immunoglobulin than cow milk (Elagamy, 2000).

At our best knowledge, no research was available in the literature concerning the determination of the activation energy of camel milk. The different fluorophores gave different significant activation energy values. The results obtained with FMRP were: i) in agreement with those of Morales, Romero, and Jimenez-Perez (1996) who found activation energy of 84 kJ mol^{-1} for preheated cow milk in the 90–140 °C, quite similar to our results where activation energy of 73 kJ mol^{-1} was observed in the present study; and ii) in disagreement with the findings of Schamberger and

Table 2
Comparison of activation energies calculated in this study and in previous reports.

Fluorescence parameters Emission (Em) and Excitation (Ex)	Camel milk	Cow milk	Temperature range (°C)	References
	Activation energy (kJ. mol^{-1})	Activation energy (kJ. mol^{-1})		
NADH (Ex: 340, Em: 360–600)	85.10	73.22	55–75	This study
FMRP (Ex: 360, Em: 380–680)	84.20	55.87	55–75	This study
Vitamin A (Ex: 252–390, Em: 410)	47.33	22.38	55–75	This study
Ex: 347, Em: 415		84	90–140	Morales et al. (1996)
Ex: 360, Em: 440		190	120–140	Schamberger and Labuza (2006)

Kamal and Karoui (2015).

Labuza (2006) who observed value of 190 kJ mol^{-1}. This difference could be due to the fact that activation energy was determined on raw skim milk in the study of Schamberger and Labuza (2006).

4. Conclusion

Front-face fluorescence spectroscopy along with chemometric tools has a great potential to evaluate the quality of camel milk subjected to different heat treatment and holding time. The FMRP spectra appeared to be the most sensitive probe to detect changes in camel milk as a function of heat treatment and holding time. Indeed, camel milk heated at 70 and 75 °C exhibited the highest intensity of FMRP spectra confirming the findings of Kulmyrzaev and Dufour (2002) reporting the efficiency of fluorescence spectroscopy to predict lactulose and furosine levels of cow milk submitted to different heat treatment. The fluorescence spectroscopy could be considered as a rapid and non-destructive screening tool for differentiating between milks according to heating intensity and time. In order to be used as on line screening tool for controlling thermal processing of camel milk, it would be interesting to validate the obtained results on camel milk from other genotypes and originating from different countries.

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CONCLUSION GÉNÉRALE ET PERSPECTIVES

Malgré le grand nombre d'études dans le domaine de l'agroalimentaire pour déterminer la structure des constituants des produits laitiers et leurs caractéristiques, peu d'investigations ont porté sur le lait de chamelle et ses propriétés technologiques en comparaison avec d'autres types de lait. L'optimisation des propriétés technologiques des produits laitiers est considérée comme un enjeu majeur pour l'industrie agroalimentaire, en général, et l'industrie laitière, en particulier. La bonne connaissance de la composition des matières premières est indispensable, mais pas suffisante pour prédire l'aptitude du lait à sa transformation en produits dérivés.

Le principal procédé industriel appliqué au lait est un traitement thermique qui a pour objectifs d'améliorer sa qualité et prolonger sa durée de vie. Cependant, les traitements thermiques provoquent de nombreux changements indésirables qui peuvent affecter les propriétés nutritionnelles, organoleptiques et technologiques des produits finis.

Outre le traitement thermique appliqué au lait, la coagulation constitue la première étape de la plupart des produits laitiers transformés (yaourt, fromage, etc.) et conditionne la qualité finale du produit fini.

L'optimisation des propriétés organoleptiques et nutritionnelles des produits laitiers reste, à ce jour, un enjeu majeur dans l'industrie laitière. Ainsi, l'obtention d'un produit laitier de bonne qualité repose principalement sur une meilleure connaissance de l'évolution de sa structure à l'échelle moléculaire et des relations structure-texture. Ces propriétés jouent un rôle majeur dans l'acceptation du produit par le consommateur. C'est pourquoi, les industriels sont toujours à la recherche de produits ayant des propriétés organoleptiques plébiscités par le consommateur. Or, en industrie agroalimentaire, en général, et en industrie laitière, en particulier, l'étude des relations structure-texture reste encore parcellaire.

Un des objectifs de cette thèse portait sur la détermination des propriétés rhéologiques de gels obtenus par voies acide et enzymatique du lait de chamelle cru et ceux chauffés à 50 et 70 °C pendant 10 minutes et enrichis en minéraux (calcium, phosphate) en comparaison avec le lait de vache. Les résultats obtenus indiquaient que l'ajout de calcium au lait de chamelle et de vache réduisait le temps de coagulation et augmentait à la fois la vitesse de gélification et la fermeté de gels. Par contre, un effet négatif a été observé suite à l'enrichissement de ces deux laits avec le phosphate. Les résultats ont également montré que le chauffage du lait de chamelle à 50 et 70 °C avant l'ajout de la chymosine conduisait à la formation d'un gel de fermeté inférieure à celui issu du lait cru.

De ce fait, nos travaux ont démontré l'intérêt de l'enrichissement du lait de chamelle avec le calcium et le rôle majeur de cet élément dans le processus de coagulation et la formation du caillé. Ces résultats se révèlent intéressants pour l'industrie laitière du lait de chamelle puisque :

i) une diminution du temps de prise se traduit par une réduction des coûts de fabrication ; et ii) l'obtention d'un caillé ferme est, d'une part, révélateur de sa richesse en protéines et en lipides et augmente le rendement laitier, d'autre part.

Une autre partie de ce travail de thèse consistait à étudier la potentialité de la spectroscopie de fluorescence frontale couplée aux analyses statistiques multivariées pour suivre le développement du réseau de caséines au cours de la coagulation enzymatique du lait de chamelle par rapport au lait de vache. L'ACCPS appliquée, d'une part, aux données rhéologiques (G', G" et tan δ) et, d'autre part, aux spectres de fluorescence du tryptophane et de la vitamine A a permis de discriminer les laits en fonction du traitement thermique appliqué au lait de départ et de la nature / concentration du minéral ajouté. Cependant, une meilleure discrimination des laits a été obtenue en appliquant l'ACCPS aux spectres du tryptophane et de la vitamine A. Nos travaux ont montré que les interactions mises en œuvre concernent les globules gras et le réseau protéique provoquant des modifications à la fois dans les allures des spectres de la vitamine A et du tryptophane, ce qui en accord avec les travaux antérieurs d'Herbert et al. (2000) et Karoui et al. (2003a).

Enfin, notre étude a porté sur le potentiel de la spectroscopie de fluorescence frontale à suivre les modifications structurales suite au chauffage du lait de chamelle entre 55 - 75 °C à différents temps (0,5, 1, 5, 10 et 30 minutes). Une augmentation du maximum de l'intensité de fluorescence des PFRM (480 nm) et une diminution de l'intensité de fluorescence des maximas du NADH (480 nm) et de la vitamine A (320 nm) ont été observées avec l'augmentation de l'intensité du traitement thermique appliqué au lait. L'ACCPS réalisée sur l'ensemble des jeux de données a permis d'obtenir une discrimination claire des échantillons de lait de chamelle en fonction de la température et du temps. Ces résultats obtenus montrent qu'une approche globale et intégrée basée sur l'utilisation de l'ACCPS permettait de prendre en compte l'ensemble de l'information fournie par différents tableaux de données.

Les travaux menés durant cette thèse s'inscrivent dans l'optique de validation de nouvelles techniques permettant de mieux comprendre les changements au niveau moléculaire des constituants du lait de chamelle au cours de la coagulation et le traitement thermique.

Les méthodes utilisées ont montré leurs intérêts pour caractériser : i) le lait en fonction de l'intensité du traitement thermique ; et ii) les gels obtenus au cours de la coagulation acide / enzymatique. Il ressort de ces travaux que les spectres de fluorescences sont bien adaptés à la détermination des changements au niveau moléculaire et peuvent être considérés comme une empreinte digitale permettant d'identifier un lait en fonction du traitement technologique appliqué.

Les résultats encourageants obtenus dans le cadre de cette thèse annoncent des perspectives dans le domaine des sciences des aliments, en général, et des produits laitiers, en particulier, pour continuer les recherches sur le lait de chamelle qui conduiront à améliorer ses propriétés à la transformation. Des études supplémentaires, de nature fondamentale, sont toutefois requises pour élucider le mécanisme exact des réactions se produisant dans le lait de chamelle lors du traitement thermique et la coagulation enzymatique et acide. Lors de ce projet de thèse, nous avons utilisé les performances d'une seule méthode de mesure spectrale, basée sur la spectroscopie de fluorescence. Le suivi de la coagulation et du traitement thermique appliqué au lait de chamelle pourrait être envisagé aux moyens d'autres techniques spectroscopiques comme la spectroscopie dans le moyen infrarouge, le visible, la microscopie dans le moyen infrarouge, la microscopie électronique à balayage, la microscopie à force atomique, etc. Ces techniques pourraient fournir des informations complémentaires pour étudier les modifications susceptibles d'intervenir dans le gel au niveau moléculaire et microscopique. Ces méthodes nécessitent l'utilisation de différentes régions du spectre électromagnétique mettant par conséquent en jeu différents types de transitions atomiques et moléculaires. L'acquisition de ces différentes informations permettrait de voir s'il existerait ou non des différences majeures au niveau de la microstructure / structure de gels. Ces différentes connaissances pourraient s'avérer particulièrement utiles pour optimiser les conditions de fabrication de produits laitiers à base de lait de chamelle et développer de nouveaux produits aux propriétés recherchées.

Sur le plan technologique, l'enrichissement du lait de chamelle par du lait de vache et / ou avec différentes teneurs en caséines-α et κ permettrait de déterminer le rôle de celles-ci dans la formation du réseau et la rigidité de gels.

Et à visée plus appliquée, l'enrichissement en calcium du lait de chamelle peut être exploité pour fabriquer de nouveaux produits et / ou améliorer ceux qui existent déjà, et investiguer l'incidence de cet ajout sur les propriétés rhéologiques, nutritionnelles et sensorielles, du produit que l'on obtiendrait.

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ANNEXES

ANNEXE 1 : Communications écrites à des congrès

Effect of mineral salts addition of rennet-induced coagulation of camel milk on the rheological properties and molecular structure determined by front face fluorescence spectroscopy

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Abstract

Milk coagulation is the primary step in the development of texture of most dairy products. The present work aims to monitor the rennet-induced gelation at 36 °C during 45 minutes of camel and cow's milk without/with the addition of minerals using dynamic oscillatory rheology and front face fluorescence spectroscopy.

Rheological measurements showed that the final value of storage modulus and loss modulus of camel milk gel were less than those of cow milk gel. The addition of $CaCl_2$ at 10 and 20 mM to the cow's and camel milks reduce the gelation time, accelerate the enzymatic activity and increase the firmness of the gel. Opposite trend was observed following the addition of $Na_2HPO_4\,(H_2O)_2$ at 10 and 20 mM.

Regarding the fluorescence spectra obtained after excitation at 290 nm (emission: 305-420 nm for tryptophan) and emission at 410 nm (excitation: 252-390 nm for vitamin A), a decrease in the fluorescence intensity respectively at 375 nm and 307 and 322 nm is observed during coagulation, regardless of the type and concentration of salt. The principal component analysis (PCA) applied to the spectral data sets allows detection of structural changes in casein micelles during coagulation and discrimination between: i) cow's and camel milk, regardless of the added concentration of minerals; and ii) cow's/camel milk according to the nature of salt and its concentration. Finally, a high correlation was observed between the data obtained at the macroscopic level and those at the molecular level suggesting the use of fluorescence spectroscopy to derive rheology parameters.

Effect of added minerals on rennet-induced coagulation of camel milk as investigated by rheology and front-face fluorescence spectroscopy

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Introduction: Milk coagulation is the first key in the development of texture of most fermented dairy products, in which liquid milk is transformed into a soft semi-solid coagulum by specific enzymes and/or lactic acid bacteria. Camel milk represents one of the most interesting milk having an interesting role in human nutrition, particularly in the arid and semi-arid zones. However, up to date, camel milk is very little valorized in the food industry due to its poor ability to be transformed into other milk products. The present work aims to monitor the rennet-induced coagulation of camel milk without/with the addition of minerals (calcium, phosphate) at 36 °C during 45 minutes using dynamic oscillatory rheology and front face fluorescence spectroscopy.

Materials and methods: Fresh camel milk samples were collected from an experimental station located in the center of Tunisia. Once arrival to the laboratory, the milk was kept at -18 °C up to analyses. Before performing analyses, milk samples were thawed during 12 h at 4 °C and then kept at room temperature (~ 18 °C) for 10 minutes. Milk samples were then placed in a water-bath equilibrated previously at 36 °C during 5 min. Calcium chloride ($CaCl_2$) and di-Sodium hydrogen phosphate dehydrate ($Na_2HPO_4(H_2O)_2$) were separately added to camel milk at two concentrations (10 and 20 mM) with stirring for 2 min. A volume of 6.25 µl of the rennet FAR-M®Sticks («Chr. Hansen A/S BoegeAllé, Hoersholm Denmark») prepared from 1 g of powder FAR-M® Sticks diluted in 20 g of distilled water was added to 25 ml of milk. Rheological and spectral measurements were performed using a controlled-strain rheometer and Fluoromax-4 spectrofluorometer, respectively. For each sample, elastic (G') and viscous (G") modulus were acquired. In addition, the emission spectra of tryptophan (305–420 nm) after excitation set at 290 nm, and excitation spectra of vitamin A (252-390 nm) after emission set at 410 nm were scanned.

Results:

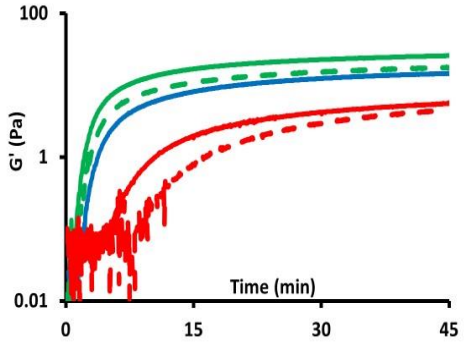

Figure 1: Evolution of storage modulus (G') of camel milk gels as a function of time for raw milk (—), and raw milk added with : 10 mM $CaCl_2$ (—), 20 mM $CaCl_2$ (---),10 mM $Na_2HPO_4(H_2O)_2$ (—) and 20 mM $Na_2HPO_4(H_2O)_2$ (---).

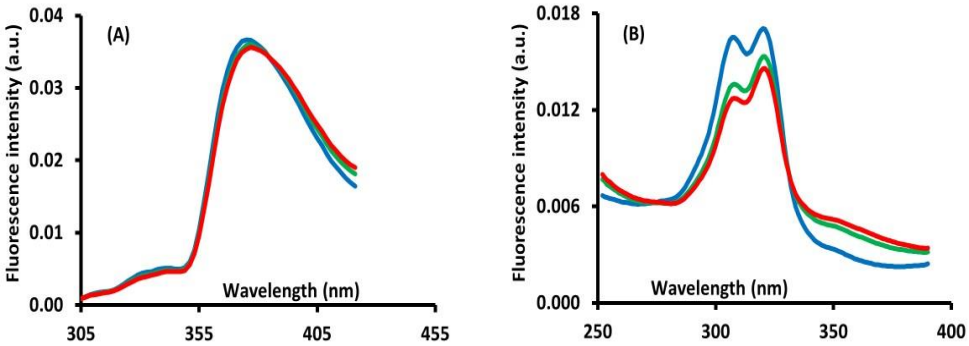

Figure 2: Tryptophan emission spectra (A) and vitamin A excitation spectra (B) acquired during the gelation of camel milk at 0 (—), 30 (—) and 45 (—) minutes.

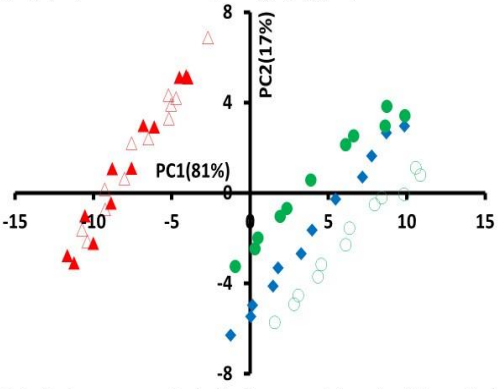

Figure 3: Principal component similarity map determined by principal components 1 (PC1) and 2 (PC2) of tryptophan spectra recorded on camel milk during the gelation of raw milk (♦), and raw milk added with : 10 mM $CaCl_2$ (o), 20 mM $CaCl_2$ (●), 10 mM $Na_2HPO_4(H_2O)_2$ (Δ) and 20 mM $Na_2HPO_4(H_2O)_2$ (▲).

- An increase in the firmness of the camel milk gels is observed with 10 mM and 20 mM added $CaCl_2$ and an opposite trend is obtained with $Na_2HPO_4(2H_2O)_2$ added at 10 mM and 20 mM (Fig. 1).
- A decrease in the fluorescence intensity of tryptophan (Fig. 2A) and vitamin A (Fig. 2B) respectively at 375 and 322 nm is observed during camel milk coagulation, regardless of the type and concentration of minerals.
- Principal component similarity map determined by principal components 1 (PC1) and 2 (PC2) of tryptophan spectra allowed:

i) Monitoring the structural changes occurring in casein micelles during coagulation.

i) Discrimination between camel milks added with $CaCl_2$ from those with $Na_2HPO_4(H_2O)_2$, regardless of the concentration (Fig. 3).

Conclusion: The present study showed that the addition of $CaCl_2$ at 10 and 20 mM to the camel milks reduced the gelation time, accelerated the enzymatic activity and increased the firmness of the gel, while opposite trend was observed following the addition of $Na_2HPO_4(H2O)_2$. Front-face fluorescence spectroscopy showed high capacity for monitoring changes occurring in caseins micelles during the gelation. In addition, the similarity map showed a clear discrimination between added $CaCl_2$ and $Na_2HPO_4(H_2O)_2$ to camel milk, regardless of the considered concentration (10, 20 mM). Finally, a high correlation was observed between the data obtained at the macroscopic level and those at the molecular level suggesting the use of front fluorescence spectroscopy as a rapid tool for monitoring camel milk coagulation.

Fluorescence Spectroscopy Allows to Discriminate between Camel Milk According to Mild Heat Treatment

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Abstract

Camel milk is considered one of the main components for human nutrition in several countries of the world, since it: i) contains all essential nutrients; and ii) has a great biological value due to the higher levels of antimicrobial components such as lysozyme, lactoferrin, immunoglobulins, and so on. Camel milk is mostly consumed in the fresh state. Its preservation can be achieved following the application of heat treatments (e.g. pasteurization, sterilization) that could have influences on both nutritional and functional properties of milk proteins. In the present study, the potentiality of front-face fluorescence spectroscopy coupled with chemometric tools was studied to characterize changes in camel milk following thermal treatments in the 55-75 °C temperature range. Nicotinamide adenine dinucleotide (NADH), fluorescent Maillard reaction products (FMRP) and vitamin A fluorescence spectra were recorded on camel milk. Using the principal component analysis (PCA), the vitamin A spectra allowed to discriminate partially between samples according to heat treatment and time. The best results was obtained by using common components and specific weights analysis (CCSWA) applied to the 3 spectral data sets, since a clear differentiation between camel milks according to temperature and time of heat treatment was obtained. It could be concluded that front-face fluorescence spectroscopy coupled with chemometric tools has the potential as a rapid and non-destructive analytical technique for the characterization at the molecular level of changes occurred in camel milk by low thermal treatment.

Monitoring mild heat treatment of camel milk by front-face fluorescence spectroscopy

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Introduction: Camel milk represents one of the main food resources insuring the nutritional requirements of inhabitants in many parts of the world, especially in arid and semi-arid areas. Thermal processing of milk is considered as an essential step of milk production since it improve the quality of milk and prolong its shelf life by either partial or total destruction of microorganisms. However, thermal treatment has a direct influence on the functional properties of milk proteins and cause changes in their physical, chemical and sensorial properties, such as the denaturation of whey proteins, hydrolysis of proteins and lipids, destruction of some vitamins, etc. The present work aims to explore the potential of front-face fluorescence spectroscopy combined with multivariate statistical methods to characterize changes occurring in milk by the application of mild heat treatment in the 55-75°C range from 0.5 min up to 30 min.

Materials and methods: Camel milk samples were collected from an experimental station located in the centre of Tunisia. Once arrived to the laboratory, milk samples were kept at -18 °C until analysis. Series of milk samples were prepared by heating milk in a water-bath preset at 55, 60, 65, 70 and 75 °C for 0.5, 1, 5, 10 and 30 min at each temperature considered. Then, the heated milk samples were cooled in ice water bath for 5 min and left at room temperature for 15 min. Raw milk samples were also studied.
Fluorescence spectra were recorded using a fluroMax-4 spectrofluorometer (Jobin Yvon, Horiba, NJ, USA). For each sample, the emission spectra of nicotinamide adenine dinucleotide «NADH» (360–600 nm) and fluorescent Maillard reaction products «FMRP» (380–680 nm) were recorded after excitation set respectively at 340 and 360 nm. The excitation spectra of vitamin A (252–390 nm) were scanned with the emission wavelength set at 410 nm.

Results:

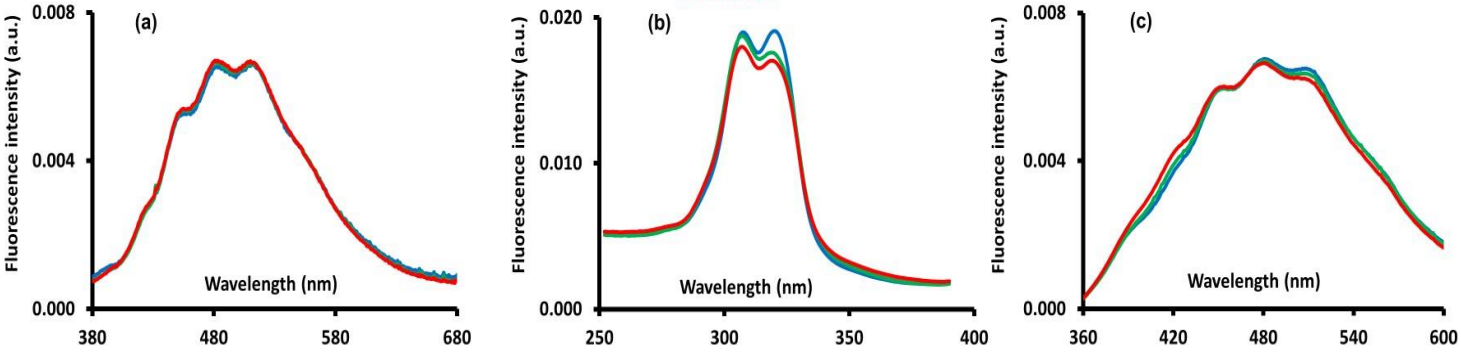

Figure 1: Normalized fluorescence spectra of FMRP (a), vitamin A (b), and NADH (c) acquired on raw camel milk (—) and those heated at 60 (—) and 75 °C (—) for 30 min.

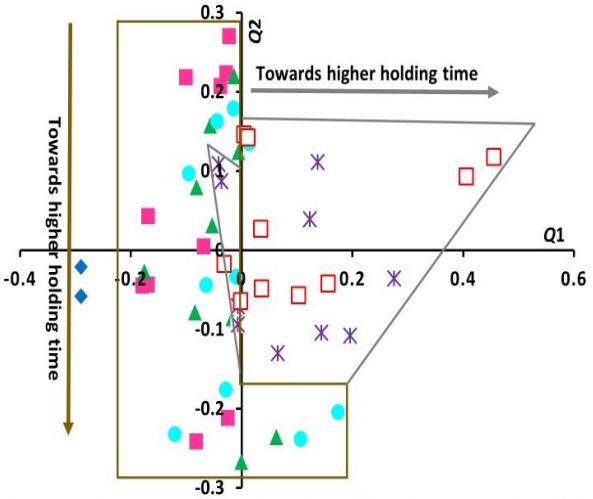

Figure 2: Common components and specific weights analysis (CCSWA) similarity map defined by the common components 1 and 2 of raw (♦), and heated camel milk at 55 (■), 60 (▲), 65 (●), 70 (✱) and 75 (□) for 0.5, 1, 5, 10, and 30 min.

- FMRP spectra exhibited three peaks located around 450, 480 and 510 nm, which increased with the increase of the heating intensity.
- The highest fluorescence intensity at 480 nm of FMRP spectra was observed for milk heated at 75 °C for 30 min, while the lowest intensity was noted for raw milk **(Fig. 1a)**.
- A decrease in the fluorescence intensity of vitamin A spectra at 307 and 320 nm was observed following heat-treatment **(Fig. 1b)**.
- The NADH spectra exhibited two maxima located ~ 480 and 510 nm that change slightly as a function of heat treatment intensity **(Fig. 1c)**.
- The CCSWA defined by the first two common components q_1 and q_2 **(Fig. 2)** allowed to:

i) Monitor changes at the molecular level of camel milk following heat treatment.
ii) Discriminate between camel milk samples according to heat treatment intensity and holding time.

Conclusion: Throughout camel milk heat treatment, fluorescence spectra were found to be very sensitive to the changes occurring at the molecular level. Front face fluorescence spectroscopy along chemometric tools has a great potential to evaluate camel milk subjected to different heat treatment and holding time. The results showed that fluorescence spectroscopy could be used as a heating index in heat processed milk since the technique was found to be clearly dependent of the applied temperature and time. Fluorescence spectra contained molecular information that allow to derive information on the molecular structure and interaction of milk components. This technique would be used as a screening tool for the on line monitoring and controlling thermal processing of milk.

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ANNEXE 2 : Participation aux doctoriales (Poster)

Étude de la cinétique de coagulation enzymatique du lait de chamelle

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Equipe Qualité et Sécurité des Aliments (QSA)
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CONTEXTE : Pourquoi le lait de chamelle ?

- Est la principale source d'alimentation dans les zones arides et semi–arides.
- Présente des propriétés nutritionnelles et vertus thérapeutiques.

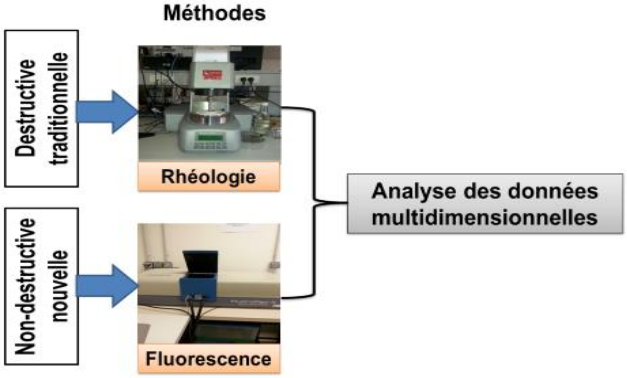
PROBLÉMATIQUES

- Le lait de chamelle reste très peu valorisé dans l'industrie agroalimentaire.
- Faible aptitude à transformer le lait de chamelle en d'autres produits laitiers :
  - ✓ Faible teneur en matière sèche totale.
  - ✓ Diamètre élevé des micelles de caséine.

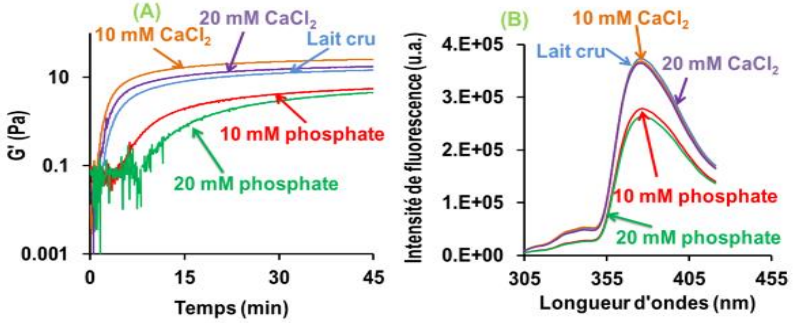
OBJECTIFS

- Étudier les propriétés gélifiantes (acide-enzymatique) du lait de chamelle en comparaison au lait de vache suite :
  - ✓ À l'ajout des minéraux ($CaCl_2$, phosphate).
  - ✓ Au traitement thermique appliqué au lait.

DEMARCHE UTILISÉE



RÉSULTATS



Évolution de la fermeté du gel (A) et des spectres d'émission du tryptophane (B) au cours de la coagulation enzymatique du lait de chamelle sans et avec ajout des minéraux.

COMPETENCES DEVELOPPÉES

- Spectroscopie de fluorescence, rhéologie, chimiométrie.
- Autonomie, travail en équipe, gestion du temps, communication scientifique, rédaction et synthèse.

PERSPECTIVES

- Étudier la cinétique de coagulation acide du lait de chamelle.
- Établir les relations entre les données rhéologiques et les mesures spectrales.

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