Understanding the impact of different cheese-making strategies on Mozzarella cheese properties

Thèse

Vincent Banville

Doctorat en sciences et technologie des aliments
Philosophiae doctor (Ph.D.)

Québec, Canada

Understanding the impact of different cheese-making strategies on Mozzarella cheese properties

Thèse

Vincent Banville

Sous la direction de :

Yves Pouliot, directeur de recherche
Michel Britten, codirecteur de recherche

Résumé

Le fromage Mozzarella entre dans la composition de plusieurs mets populaires d'Amérique du Nord. L'aptitude de ce fromage à être râpé et ses propriétés caractéristiques de cuisson en font un ingrédient idéal. Ces qualités sont attribuées principalement aux propriétés physiques particulières de ce fromage sous certaines conditions de cisaillement et de température. Le but de ce projet était d'évaluer l'impact de différentes stratégies couramment mises en œuvre dans l'industrie fromagère sur la composition, la microstructure et les propriétés physiques du fromage. Diverses stratégies ont été étudiées : les conditions de filage du caillé lors du procédé de « pasta filata », l'addition de protéines sériques dénaturées, le contrôle de la minéralisation et le vieillissement du fromage. Les résultats ont démontré que le contrôle de l'intensité mécanique et thermique fournie lors du filage permettait respectivement de réduire les pertes de solides et d'améliorer la répartition de la phase aqueuse dans la matrice fromagère. L'aptitude au râpage du fromage peut être optimisée en combinant l'utilisation de plusieurs stratégies dont la réduction du calcium colloïdal, un temps de vieillissement adéquat et un râpage à basse température. Par ailleurs, des changements aux facteurs mentionnés précédemment sont apportés lors de l'ajout de protéines sériques dénaturées, ces dernières ayant un impact sur la composition et la structure du fromage. Des modèles prédictifs de l'aptitude au râpage ont été développés en sélectionnant uniquement les descripteurs de composition et de texture pertinents. La perception sensorielle du fromage cuit sur pizza et les propriétés physiques du fromage fondu ont été considérablement influencées par l'évolution physico-chimique du fromage au cours du vieillissement. L'utilisation d'une nouvelle approche pour la caractérisation des propriétés rhéologiques du fromage fondu sous fortes contraintes a permis d'établir de bonnes relations avec les descripteurs sensoriels de texture. Ce travail a permis de valider l'hypothèse que l'utilisation d'une ou plusieurs stratégies simples et accessibles pouvait être mise de l'avant afin d'optimiser les propriétés physiques du fromage Mozzarella. Cela contribue à une meilleure compréhension des facteurs pouvant être contrôlés afin de développer des fromages avec des attributs spécifiques, lorsqu'utilisés comme ingrédient.

Abstract

Mozzarella cheese is expected to perform various key attributes when used as a food ingredient. The shreddability and the melting properties of cheese during and after baking are mainly governed by the physical properties of cheese when subjected to external factors such as shear and temperature. Therefore, the goal of this project was to evaluate the impact of cheese-making strategies commonly used in the dairy processing industry on the cheese composition, microstructure, and physical properties. Various strategies were studied: pasta filata process conditions, addition of denatured whey protein (WP-D) to milk, control of curd mineralization, and cheese aging. Results showed that controlling the mechanical and thermal intensity during the pasta filata process can lead to reduced cheese solid losses and a better distribution of water within cheese microstructure, respectively. The ability of cheese to be shredded can be increased using a combination of multiple factors such as lowering colloidal calcium phosphate associated with casein, proper aging, and by reducing cheese temperature before shredding. However, an optimisation of the previous factors should be done if WP-D is added because of its impact on cheese composition and structure. Predictive models to assess cheese shreddability were built using only few relevant compositional and textural descriptors. Sensory perception of baked cheese texture and physical properties of melted cheese were dramatically influenced by the physico-chemical evolution of cheese during aging. Melted cheese texture was satisfactorily related to different sensory attributes using a novel approach to determine the rheological properties under the large stress experienced during mastication. This work validated the hypothesis that simple cheese-making strategies, alone or combined, can be used to optimize the cheese physical properties. This contributes to a better understanding of the factors that can be controlled to improve or develop cheese ingredient with specific attributes.

Table of contents

Résumé .... **iii**
Abstract .... **iv**
Table of contents .... **v**
Table list .... **ix**
Figure list .... **xi**
Equation List .... **xiv**
Abbreviations .... **xv**
Acknowledgement .... **xviii**
Foreword .... **xx**
1 Chapter 1 Introduction **1**
2 Chapter 2 Literature review **3**
2.1 Mozzarella Cheese 3
2.1.1 Standard of Identity 3
2.1.2 Cheese-Making Technology 4
2.2 Mozzarella: Components and Structure 6
2.2.1 Protein Phase 6
2.2.2 Aqueous Phase 10
2.2.3 Fat Phase 13
2.3 Key Attributes for the use of Mozzarella as Ingredient 15
2.3.1 Machinability 15
2.3.2 Heat-Induced Attributes 16
2.4 Impact of Cheese-making Technology on Functional Attributes 20
2.4.1 Impact of Cheese Milk Standardization 20
2.4.2 Impact of Acidification and Draining 21
2.4.3 Impact of the Pasta Filata Process 22
2.4.4 Impact of Sodium Chloride 23
2.4.5 Impact of Aging and Freezing 24
2.5 Evaluation of Cheese Structure 26
2.5.1 Microscopy 26
2.5.2 Laser Light Scattering 27
2.5.3 Dynamic Rheology 27
2.6 Evaluation of Cheese Physical Properties 31
2.6.1 Properties of Unmelted Cheese 31
2.6.2 Properties of Melted Cheese 33
2.7 Evaluation of Sensory texture of Cheese 37
2.8 Relationships between Factors affecting Cheese Characteristics 38
3 Chapter 3 Hypothesis, goal and objectives **41**
3.1 Hypothesis 41

3.2 Goal 41
3.3 Objectives 42

4 Chapter 4 Impact of thermo-mechanical treatments on composition, microstructure and rheological properties of pasta filata–type cheese 43

4.1 Résumé 43
4.2 Abstract 44
4.3 Introduction 45
4.4 Material and Methods 46
4.4.1 Curd Production 46
4.4.2 Thermo-Mechanical Treatment 47
4.4.3 Cheese Composition Analysis 47
4.4.4 Microstructural Analysis 48
4.4.5 Rheological analysis 50
4.4.6 System Analysis 50
4.4.7 Statistical Analysis 51
4.5 Results 51
4.5.1 Impact of Mixing Conditions on Cheese Composition 51
4.5.2 Impact of Mixing Conditions on Solid Loss 55
4.5.3 Impact of Mixing Conditions on Cheese Microstructure 56
4.5.4 Impact of Mixing Conditions on Rheological Properties 59
4.6 Discussion 61
4.6.1 Relationship between Mixing Conditions and System Variables 61
4.6.2 Relationship between System Variables and Cheese Characteristics 62
4.7 Conclusions 67
4.8 Acknowledgements 67

5 Chapter 5 Physical properties of Pizza Mozzarella cheese manufactured under different cheese-making conditions 68

5.1 Résumé 68
5.2 Abstract 69
5.3 Introduction 70
5.4 Material and Methods 71
5.4.1 Milk Standardization 71
5.4.2 Manufacture of Pizza Mozzarella Cheese 72
5.4.3 Chemical Analysis of Cheese 72
5.4.4 Physical Properties of Cheese 73
5.4.5 Experimental Design and Statistical Analysis 75
5.5 Results and Discussion 75
5.5.1 Cheese Composition 75
5.5.2 Shredding Experiment 78
5.5.3 Melting Experiment 87
5.6 Conclusions 90
5.7 Acknowledgements 91

6 Chapter 6 Shreddability of pizza Mozzarella cheese predicted using physicochemical properties 92
6.1 Résumé 92
6.2 Abstract 93
6.3 Introduction 94
6.4 Material and methods 96
6.4.1 Pizza Mozzarella Cheeses 96
6.4.2 Rheological Analysis 96
6.4.3 Predictive Modeling of Cheese Shreddability 98
6.4.4 Statistical Analyses 99
6.5 Results 99
6.5.1 Composition of Cheese 99
6.5.2 Rheological Properties of Cheeses 101
6.5.3 Predictive Models for Shreddability of Pizza Mozzarella Cheese 107
6.6 Discussion 110
6.6.1 Factors Influencing Rheological Descriptors of Cheeses 110
6.6.2 Prediction of Shreddability using Compositional and Rheological Descriptors 113
6.6.3 Relationship between Cheese Shreddability and Compositional and Rheological Descriptors 114
6.7 Conclusions 117
6.8 Acknowledgements 117
7 Chapter 7 Relationship between baked-cheese sensory properties and melted-cheese physical characteristics 118
7.1 Résumé 118
7.2 Abstract 119
7.3 Introduction 120
7.4 Material and methods 122
7.4.1 Cheese Sampling and Aging 122
7.4.2 Cheese Composition Analyses 122
7.4.3 Cheese-Baking Method 123
7.4.4 Baked-Cheese Properties 123
7.4.5 Cheese-Melting Method 126
7.4.6 Melted-Cheese Properties 126
7.4.7 Statistical Analysis 130
7.5 Results 130
7.5.1 Cheese Composition and Aging 130
7.5.2 Baked-Cheese Properties 131
7.5.3 Melted-Cheese Properties 133
7.6 Discussion 139
7.6.1 Impact of Baking on Cheese 139
7.6.2 Relationships Between Baked-Cheese Properties 140
7.6.3 Relationship between Melted-Cheese Rheological Parameters 142

7.6.4 Relationship between Melted-Cheese Rheological Parameters and Baked-Cheese Sensory Terms ... 143
7.7 Conclusions ... 146
7.8 Acknowledgements ... 146
8 Chapter 8 General Conclusions ... 147
8.1 Achievements and Original Contributions ... 147
8.2 Significance of the Results ... 150
8.3 Remaining Questions and Perspectives ... 153
References ... 156

Table list

Table 1.1 Some applications and attributes of Mozzarella cheese 2

Table 2.1 Regulatory specifications for Low-moisture Mozzarella cheeses 3

Table 2.2 Properties of caseins in milk conditions 6

Table 2.3 Half dissociation pH (pKa) of some water soluble compounds 11

Table 2.4 Key attributes to assess Mozzarella cheese machinability 16

Table 2.5 Key attributes to assess heat-induced properties of Mozzarella 19

Table 4.1 *F*-values and *P*-values (in parentheses) of the effects of mixing conditions on the elastic modulus at 1 Hz (G'_1) and the frequency dependence of G' (G'_{fd}). 60

Table 4.2 Models used for the prediction of some cheese characteristics using system variables. 63

Table 5.1 Means, *F*-values, and *P*-values for the analysis of the effect of denatured whey protein (WP-D) addition and renneting pH on cheese composition. 76

Table 5.2 *F*-values and *P*-values (in parentheses) for the analysis of effect of denatured whey protein (WP-D) addition and renneting pH on cheese colloidal calcium phosphate (CCP) and water-soluble nitrogen as a percentage of total nitrogen (WSN) during aging. 77

Table 5.3 *F*-values and *P*-values (in parentheses) for the analysis of the effect of denatured whey protein (WP-D) addition, renneting pH, aging, and temperature on cheese shredding properties.79

Table 6.1 Values of *F* and values of *P* (in parentheses) for the effects of added denatured whey protein, renneting pH, aging, and testing temperature on the rheological descriptors of cheeses. 100

Table 6.2 Basic statistics of shredding, compositional, and rheological descriptors of cheeses. 108

Table 6.3 Predictive models for the production of fines, production of long shreds, and adhesion to the blade during shredding. 109

Table 7.1 Sensory terms used for the texture evaluation of baked cheeses and scores attributed to reference food. 125

Table 7.2 Mean compositions and standard deviations of regular and stabilized cheeses. 131

Table 7.3 Sensory scores and statistical analysis of baked regular and stabilized cheeses at different aging times 133

Table 7.4 Values of *F* and *P* (in parentheses) for the effects of cheese type and aging on the complex modulus (G*) and the phase angle (δ) measured at different temperatures during the cooling of melted cheeses. 134

Table 7.5 Values of *F* and *P* (in parentheses) for the effects of cheese type and aging on the total creep compliance (J_{tot}), the fatigue, and the recovery percentage (R%) of melted cheeses at 60 °C. The Multiple Stress Creep Recovery test was done at two stresses: 50 Pa and 2000 Pa. 137

Table 7.6 Pearson correlation coefficients (*r*) between sensory terms and expressible liquids measured on baked cheeses (*n = 24*). 141

Table 7.7 Pearson correlation coefficients (*r*) between the rheological parameters of melted cheeses: the complex modulus (G*), the phase angle (δ), the total creep compliance (J_{tot}), the fatigue and the recovery percentage difference (R% diff.). Correlation coefficients obtained with partially dehydrated cheeses are in parentheses (*n* = 24). 143

Table 7.8 Pearson correlation coefficients (*r*) between the sensory terms of baked cheeses and the rheological parameters of melted cheeses: the complex modulus (G*), the phase angle (δ), the total creep compliance (J_{tot}), the fatigue and the recovery percentage difference (R% diff.).

Correlations coefficients obtained with partially dehydrated cheeses are in parentheses (*n* = 24). 144

Table 8.1 Summary the main factors studied and their key impact on cheese properties. 151

Figure list

Figure 2.1 Generic manufacturing diagram for the production of cultured Mozzarella cheese. Adapted from Chen et al. (2009). 4

Figure 2.2 Schematic representation of a casein gel network (adapted from Dalgleish and Corredig, 2012). Calcium phosphate nanoclusters (grey spheres) are cross-linked with 3 to 5 α- or β-CNs (orange) via their centers of phosphorylation. Some β-CNs (blue) are bound to other CNs by hydrophobic interactions and thus can be released from the protein network at cool temperature. *Para*-κ-CN (green) that remained attached via hydrophobic interactions after the scission of the caseinomacropeptide. Denser regions of CN aggregates, stabilized by hydrophobic interactions and electrostatic attractions (between carboxylate residues and divalent metal ions), are formed after the solubilization of some calcium phosphate clusters during acidification. The rearrangement and contraction of the CN network over time forces the water out in serum channels (void space). Not drawn to scale. 9

Figure 2.3 Equilibria of some salts interacting with calcium. 11

Figure 2.4 Schematic representation of different organization structure of fat in cheese. Adapted from Lopez et al. (2006). 14

Figure 2.5 Stress-strain curves of materials exhibiting strain-hardening or strain-weakening behaviors outside the nonlinear viscoelastic region. Source: Foegeding et al., 2011. 33

Figure 2.6 Scheme of the relationships between factors affecting Mozzarella characteristics. 38

Figure 4.1 Initial curd composition ($g\ 100\ g^{-1}$ curd) (a) and impact of thermo-mechanical treatments on the composition of mixed curd (b, c, d, e) presented as the percentage difference (Δ%) in cheese components (non-expressible serum [NES], expressible serum [ES], protein, fat, and other solids) before and after treatment. The main effects of mixing temperature (b), mixing time (c), and mixing speed (d) are presented. Significant interactions between mixing time and mixing speed are also presented for fat and protein contents (e). The effect (or interaction) is significant ($P \le 0.01$) when identified by an asterisk (*) or non-significant (NS) when the probability of F is $P > 0.01$. 53

Figure 4.2 Expressible serum in cheeses mixed at different temperature (a) or for different lengths of time (b), measured during a short aging period. The lines were added to guide the reader's eye. 54

Figure 4.3 Impact of mixing temperature (a) and of mixing time and speed (b) on the fat loss coefficient (L_{fat}). Different letters above the bars indicate that the means of effects differ significantly. 55

Figure 4.4 Scanning electron micrographs of cheeses produced under mild (a, b, c; temperature/time/speed = 60 °C/ 3 min/6 rpm) and severe (d, e, f; temperature/time/speed = 70 °C/ 6 min/12 rpm) mixing conditions. Bars: 50 µm (a, d), 20 µm (b, e) and 2 µm (c, f). 56

Figure 4.5 Confocal laser scanning micrographs of cheeses produced under different combinations of mixing temperature, time and speed: (a) 60 °C, 3 min, and 6 rpm, (b) 60 °C, 3 min, and 12 rpm, (c) 70 °C, 3 min, and 6 rpm, (d) 70 °C, 3 min, and 12 rpm, (e) 60 °C, 6 min, and 6 rpm, (f) 60 °C, 6 min, and 12 rpm, (g) 70 °C, 6 min, and 6 rpm, (h) 70 °C, 6 min, and 12 rpm. Protein and fat appear in red and green respectively. Bar: 50 µm. 57

Figure 4.6 Particle size distribution of fat globules in cheese curd (–□–) and in cheeses produced under mild (–○–; temperature/time/speed = 60 °C/ 3 min/6 rpm) and severe (–Δ–; temperature/time/speed = 70 °C/6 min/12 rpm) mixing conditions. 59

Figure 4.7 Effect of mixing speed (6 rpm: straight line; 9 rpm: dashed line; and 12 rpm: dotted line) on the elastic modulus (G′) of cheese at different frequencies. Mixing time was 3 min (a) or 6 min (b). 60

Figure 4.8 Mixing treatments applied to cheese curds (*n* = 54), resulting from the combination of mixing temperature, time, and speed, and represented as a function of output system variables: final curd temperature (T_f), heat load index (HL), and specific mechanical energy (SME). 61

Figure 5.1 Effect of aging on colloidal calcium phosphate (CCP; filled symbols) and the proportion of water-soluble nitrogen to total nitrogen (WSN; open symbols) in pizza Mozzarella cheeses made with milk renneted at pH values of 6.4 (▲) or 6.5 (■). Error bars represent the standard error of means. 78

Figure 5.2 Effect of aging on the percentage of fines measured after the shredding of Mozzarella cheeses made from milk with (0.25% WP-D; dashed lines) or without (0% WP-D; solid lines) denatured whey protein (WP-D) and renneted at pH values of 6.4 (▲) or 6.5 (■). Error bars represent the standard error of means. 81

Figure 5.3 Effect of cheese temperature during shredding on the percentage of long shreds in pizza Mozzarella cheeses aged for 8 (×), 22 (□), or 36 d (◆). Error bars represent the standard error of means. 81

Figure 5.4 Effect of cheese temperature during shredding on the adhesion to the blade of pizza Mozzarella cheeses made from milk with (0.25% WP-D; dashed lines) or without (0% WP-D; solid lines) denatured whey protein (WP-D) and renneted at pH values of 6.4 (▲) or 6.5 (■). Error bars represent the standard error of means. 82

Figure 5.5 Typical curve of force (F) versus volume deformation (V) of a cheese shred cake during the matting experiment. The cohesion strength is the initial slope of the curve measured at 0.5 N. The work of breaking is the force required to perform the test. 85

Figure 5.6 Matting behaviour of the pizza Mozzarella cheese shred cakes represented by the cohesion strength and the work of breaking. Results are presented for cheese made from milk with (0.25% WP-D; ▲) or without (0% WP-D; ■) denatured whey protein (WP-D). The temperature during cake preparation was 4 (hatched symbols), 13 (filled symbols), or 22 °C (open symbols). All cakes were tested at 4 °C. Error bars represent standard error of mean. 86

Figure 5.7 Effect of denatured whey protein (WP-D) in milk (a), renneting pH (R; b), cheese aging (c), and premelting treatment (d) on the melting profiles of pizza Mozzarella cheeses assessed by the complex modulus (G*; solid lines) and the phase angle (δ; dashed lines). 88

Figure 6.1 Effects of added denatured whey protein (WP-D), renneting pH (RpH; R6.4 = renneting at pH 6.4; R6.5 = renneting at pH 6.5), aging, and testing temperature on 3 cheese texture descriptors, (a) hardness, (b) adhesiveness, and (c) cohesiveness, as determined using texture profile analysis. Error bars represent the standard errors of the means. 102

Figure 6.2 Effects of added denatured whey protein (WP-D), renneting pH (RpH; R6.4 = renneting at pH 6.4; R6.5 = renneting at pH 6.5), aging, and testing temperature on 2 cheese texture descriptors, (a) Young's modulus and (b) flow modulus, as determined using the single lubricated compression test. Error bars represent the standard errors of the means. 103

Figure 6.3 Effects of added denatured whey protein (WP-D), renneting pH (RpH; R6.4 = renneting at pH 6.4; R6.5 = renneting at pH 6.5), aging, and testing temperature on 2 cheese texture descriptors, (a) cutting resistance and (b) fracture toughness, as determined using the wire-cutting test. Error bars represent the standard errors of the means. 104

Figure 6.4 Effect of added denatured whey protein (WP-D), renneting pH (RpH; R6.4 = renneting at pH 6.4; R6.5 = renneting at pH 6.5), aging, and testing temperature on 4 cheese texture descriptors, (a) complex modulus measured at 1 Hz (G^*_1), (b) frequency dependence of the complex modulus (G^*_{fd}), (c) phase angle measured at 1 Hz (δ_1), and (d) frequency dependence of the phase angle (δ_{fd}), as determined using the frequency sweep test. Error bars represent the standard errors of the means. 106

Figure 6.5 Principal component (PC) biplot of the rheological, compositional, and shredding descriptors of cheeses: LS% = percentage of long shreds; Adh = adhesiveness; WSN = water-soluble nitrogen; G^*_1 = complex modulus measured at 1 Hz; Y_m = Young's modulus; F_t =

fracture toughness; H = hardness; C_r = cutting resistance; F_m = flow modulus; M = moisture; F% = percentage of fines; Coh = cohesiveness; δ_{fd} = frequency dependence of the phase angle; CCP = colloidal calcium phophate; G^*_{fd} = frequency dependence of the complex modulus; AdhB = adhesion to the blade; δ_1 = phase angle measured at 1 Hz. 115

Figure 7.1 Schematic representation of two creep/recovery cycles in the Multiple Creep Stress Recovery test (not to scale). The cheese compliance (J) was plotted as a function of time. A total of 20 successive cycles were performed: cycle numbers (*i*) from 1 to 10 were loaded at a constant stress of 50 Pa, whereas cycle numbers from 11 to 20 were loaded at 2000 Pa. The initial compliance (J_0), maximum compliance (J_{max}) and recovered compliance (J_r) were analyzed for each cycle. 128

Figure 7.2 Effect of aging time on the percentage of water-soluble nitrogen in total nitrogen content (WSN) for regular (■) and stabilized (▲) cheeses. Error bars represent the standard error of the means. 131

Figure 7.3 Effects of cheese type and aging on the percentage of serum (dark bars) and oil (light bars) phases expressed from baked cheeses after centrifugation. Cheese types: regular cheese (RC) and stabilized cheese (SC). Aging time: 10, 25, 40 and 55 d. Error bars represent the standard error of the means. 132

Figure 7.4 Effects of cheese type (a and b) and aging (c and d) on the complex modulus (G*; a and c) and the phase angle (δ; b and d) of melted cheeses during cooling from 90 °C to 20 °C. Cheese types: regular cheese (RC) and stabilized cheese (SC). Aging time: 10, 25, 40 and 55 d. 135

Figure 7.5 Effects of cheese type (a, c and e) and aging (b, d and e) on the total creep compliance (J_{tot}; a and b), the fatigue (c and d) and the recovery percentage (R%; e) of melted cheeses at 60 °C. Multiple creep/recovery cycles were done at two stresses: 50 Pa (dark bars) and 2000 Pa (light bars). Cheese types: regular cheese (RC) and stabilized cheese (SC). Aging time: 10, 25, 40 and 55 d. Error bars represent the standard error of the means. 138

Figure 8.1 Fields of interests of the core chapters of the thesis related to the main events occurring during the life span of Mozzarella cheese ingredient. 148

Equation List

Equation 2.1 Amplitude of deformation as a time function 28

Equation 2.2 Amplitude of stress as a time function 28

Equation 2.3 Elastic modulus calculation 28

Equation 2.4 Viscous modulus calculation 28

Equation 2.5 Relationship between the viscoelastic moduli G*, G′ and G″ 29

Equation 2.6 Relationship between the viscoelastic moduli and the phase angle 29

Equation 2.7 Model of the mechanical spectrum of cheese 30

Equation 4.1 Solid loss calculation 48

Equation 4.2 Average volume-weighted diameter calculation of fat 50

Equation 4.3 Mechanical spectrum of the elastic component in cheese 50

Equation 4.4 Specific mechanical energy calculation 51

Equation 4.5 Heat load calculation 51

Equation 4.1 Retention coefficient calculation 73

Equation 6.1 Wire-cutting test parameters calculation 97

Equation 6.2 Mechanical spectrum of the complex modulus 98

Equation 6.3 Mechanical spectrum of the phase angle 98

Equation 7.1 Weight loss calculation of cheese during baking 123

Equation 7.2 Mean total creep compliance calculation at 50 and 2000 Pa 129

Equation 7.3 Fatigue calculation at 50 and 2000 Pa 129

Equation 7.4 Mean recovery percentage (R%) calculation at 50 or 2000 Pa 129

Equation 7.5 Calculation of the recovery percentage difference between the two loads applied (R% diff.) 129

Abbreviations

α-CN	Alpha-casein
β-CN	Beta-casein
κ-CN	kappa-casein
μ	Friction coefficient
σ_y	Yield stress
Adh	Adhesiveness
AdhB	Adhesion to the blade
CCP	Colloidal calcium phosphate
CLSM	Confocal laser scanning microscopy
CMP	Caseinomacropeptide
CN	Casein
Coh	Cohesiveness
C_r	Cutting resistance
EDTA	Ethylenediaminetetraacetic acid
ES	Expressible serum
F%	Percentage of fines
F_c	Cutting force
F_m	Flow modulus
F_t	Fracture toughness
H	Hardness
HL	Heat load
J	Compliance
L	Loss coefficient
LAOS	Large amplitude oscillatory shear
LS%	Percentage of long shreds
LMMC	Low-moisture Mozzarella cheese
LVR	Linear viscoelastic region
M	Moisture
M	Mass

MFGM	Milk fat globule membrane
MSCR	Multiple stress creep recovery
MPC	Milk protein concentrate
NES	Non-expressible serum
PC	Principal component
PCA	Principal component analysis
R	Recovery
RC	Regular cheese
RpH	Renneting pH
SAOS	Small amplitude oscillatory shear
SC	Stabilized cheese
SEM	Scanning electron microscopy
SDS	Sodium dodecyl sulphate
SME	Specific mechanical energy
T_f	Final curd temperature
TPA	Texture profile analysis
WP	Whey protein
WPC	Whey protein concentrate
WP-D	Denatured whey protein
WSN	Water soluble nitrogen
$\mathrm{Y_m}$	Young's modulus

À Sylvie et André qui m’ont tout légué.
Mais en particulier, chacun, un peu de leur personnalité.

“Everything flows”

–Heraclitus, 501 B.C.

Acknowledgement

This thesis is the synthesis of more than 5 years of stimulating and fulfilling research work and a major achievement in my life. This thesis would not been possible if many “ingredients” such as financial support, colleagues, and close supporters did not contribute to this ambitious project. I wish here to acknowledge their support in French, my first language and the one we have used in so many grateful exchanges.

J’aimerais tout d’abord remercier les organismes subventionnaires qui ont contribué financièrement à rendre ce projet réalisable : le fond de Recherche Québécois –Nature et technologie, et le Conseil Canadien de Recherche en Science Nature et Génie. J’aimerais aussi remercier la coopérative laitière Agropur pour son implication financière, mais aussi pour son appui dans de multiples facettes de ce projet.

Dr Yves Pouliot est l’une des rares personnes que je connais qui ont un désir aussi réel de faire avancer la recherche scientifique tout en ayant à cœur les aspirations des étudiants qu’il supervise. Yves a ouvert la porte menant à mes projets ambitieux. Il a aussi été le guide qui m’a permis d’arriver au bout de cette quête. Merci, Yves, pour ton support indéfectible.

Les discussions de 15 min avec mon codirecteur, Dr Michel Britten, sont parmi les moments que j’ai le plus apprécié au cours de mes études. Bien entendu, 15 min était le temps que je demandais à Michel de m’accorder afin de parler de mon projet. C’était, en réalité, des discussions de plus de 2 heures. J’ai énormément appris de ces moments d’échanges. Michel a cette capacité de favoriser un capharnaüm intellectuel qui enrichit notre façon d’entrevoir les choses. Je n’aurais jamais cru possible d’avoir un mentor aussi dévoué et attentionné. Merci pour tout ce temps consacré à me faire grandir.

J’aimerais aussi remercier du fond du cœur mes mentors de la famille Agropur. Premièrement, un immense merci à Dr Michel Pouliot. Tu m’as donné ma chance et cru en moi dès le début. Tu es un exemple à suivre pour ton rôle majeur dans la défense d’une recherche de qualité en milieu industriel. J’aimerais aussi remercier Dr

Pierre Morin pour m'avoir si bien accueilli et pour le temps investi dans la réussite de mes études. Ton parcours a grandement influencé mes aspirations. Merci d'avoir ouvert la route. Un autre éternel remerciement à M. Nelson Power. Ton incroyable expérience et ton sens exceptionnel de l'écoute et de vulgarisation ont beaucoup enrichi ma façon de concevoir la production fromagère. Je regrette seulement ne pas avoir croisé ton chemin plus longtemps.

Bien entendu, je remercie aussi Dr Jean-Christophe Vuillemard d'avoir accepté la révision de ce manuscrit. Merci, Jean-Christophe, pour l'intérêt porté à mon travail et à tes commentaires et suggestions toujours très pertinents. J'aimerais aussi remercier Dr Mark Johnson d'avoir accepté d'évaluer cette thèse. Vos travaux ont été une source d'inspiration pour ce projet.

L'appui de plusieurs personnes a été indispensable pour moi. Premièrement à l'Université Laval, j'aimerais remercier sincèrement Dr Sylvie Turgeon qui m'a toujours épaulé et qui m'a transmis son amour pour la recherche**.** Également, Diane Gagnon et Anne-Françoise Alain pour avoir rendu cette expérience d'autant plus agréable. J'ai eu la chance d'avoir un appui inconditionnel de Nathalie Rémillard, Sophie Lamothe, Hélène Giroux, Annie Caron et Sophie Turcot au CRDA. Selon moi, il n'y a pas de meilleures conditions pour réaliser des travaux de recherche qu'en vous sachant dans les parages. Merci pour votre professionnalisme, votre rigueur, votre empathie et votre amitié. Finalement, je ne peux passer sous silence le bonheur que j'ai eu à côtoyer mes stagiaires : Myriam Chabot, Patrick Lefebvre et Stéphanie Vignola.

Merci à tous mes amis et proches de m'avoir soutenu lors de ces dernières années. Sans vous, les périodes difficiles auraient été insurmontables et les moments de joie n'auraient pas eu la même saveur. Je vous ai volé un peu de votre énergie pour la réalisation de ce projet de thèse. Je suis content à l'idée de vous en rendre un peu durant les années qui viennent. Un merci spécial à Tommy et Guillaume, mes frères d'âme, à ma sœur Rachel et à mes parents Sylvie et André. Finalement, je me dois de terminer avec celle qui m'a soutenu depuis que je l'ai connue, au début de mes études. Myriam, ta compagnie me rend heureux.

Foreword

This thesis is presented in the form of 8 chapters. The first chapter is a general introduction to the research project. Then, an overview of the current scientific knowledge about the topics covered in this thesis, and which are related to Mozzarella cheese, is presented in chapter 2. This chapter presents the composition, structure and physical properties of Mozzarella cheese, and some of the important factors that are known to impact the Mozzarella cheese characteristics.

Chapter 3 gathers the context of the research study, its general hypothesis, and the goal and objectives that were sought.

Chapters 4, 5, 6 and 7 present the research work undertaken during this thesis. They are presented in the form of inserted manuscripts that are submitted or already published in scientific journals.

In chapter 4, a simplified model system was developed to study the impact of the thermo-mechanical treatment applied to the curd during the making of pasta filata–type cheese. The content of this chapter is currently submitted to the *International Dairy Journal*. This chapter was written by this thesis author who also planned, executed and analysed the research work presented. An intern, Stéphanie Vignola, assisted the first author for laboratory experiments. M^{me} Denise Chabot, a co-author from Agri-food Canada, advised and assisted this thesis author during the microscopic experiments. Mr Nelson Power, a co-author from Agropur Dairy Cooperative, kindly advised this work and revised the manuscript. The writing of the manuscript was supervised by Dr Yves Pouliot and Dr Michel Britten who are also co-authors. Finally, the contribution of Dr Britten to the experimentation planning and to the results analysis must be acknowledged.

Chapters 5 and 6 present an extensive research work that focused on the shredding properties of Mozzarella cheese. Content of chapter 5 and chapter 6 were published essentially in this form in *Journal of Dairy Science* in August 2013 (doi: 10.3168/jds.2012-6314) and July 2014 (doi: 10.3168/jds.2014-8040) respectively. A summary of these two chapters was also presented in poster form at the 2012 IDF Cheese Ripening and Technology Symposium, Madison, Wisconsin. The two chapters were planned, executed and written by this thesis author. An intern, Myriam Chabot, assisted the first author for laboratory experiments. Dr Pierre Morin, a co-author from Agropur Dairy Cooperative, kindly advised this work and revised the manuscripts. The writing of these chapters was supervised by Dr Michel Britten and Dr Yves Pouliot and therefore, are co-authors of these chapters. The contribution of Dr Britten and Dr Pouliot to the planning of this work must be acknowledged.

Chapter 7 was published essentially in this form in *Journal of Texture Studies* in October 2015 (doi: 10.111/jtxs.12132). This chapter, which focused on the physical properties of baked cheese, was also presented in poster form at the 2013 Colloque STELA Symposium, Montréal, Canada. The work presented in this chapter was planned, executed and written by this thesis author. The planning and work related to the sensory evaluation was assisted by M^{me} Jacinthe Fortin and M^{me} Nancy Graveline. Nelson Power, co-author from Agropur Dairy Cooperative, kindly advised this work and revised the manuscript. Again, the writing of this chapter was supervised by Dr Michel Britten and Dr Yves Pouliot who also advised the planning of experiments.

Finally, chapter 8 concludes this thesis with a general discussion, the main achievements and the perspective that are envisaged for further researches in this field of study.

Chapter 1 Introduction

Cheese has become omnipresent in the worldwide culinary scene of our modern society. This rich dairy concentrate of protein and fat is appreciated not only for its wide range of flavors, but also as a complementary ingredient that brings colors and textures to a dish. In North America, many popular dishes are deeply associated with cheese: sandwich, burger, pasta, and of course pizza. The versatility of cheese as a food ingredient was overviewed brilliantly by Lucey (2008), and since then, the number of applications has been continuously growing. Cheese manufactured for the food service industry or the tertiary sector represents the most profitable products sold by the majors' dairy company in Canada and is estimated to 38% of the cheese market share in North America (Euromonitor, 2014). Among the cheese varieties, Mozzarella is a consumer' favorites and is appreciated notably for its melting properties. In this competitive market, dairy processor must innovate in the way their cheese are made and how they perform in specific food applications in order to meet consumer's expectations.

Mozzarella cheese can be required to perform key attributes, and on a various level depending on the ingredient application. Table 1.1 presents some of these attributes. One can notice that they are often related to the physical behavior (i.e., physical properties) of cheese under a given external conditions (e.g., temperature, shear). On a future perspective, it is likely that new physical properties can be developed, and that some of the actual attributes defined in Table 1.1 can be better controlled at the benefits of a wider range of food diversity for the consumer.

Physical properties of cheese are closely related to its (1) composition, (2) the cheese-making technology used, and (3) the microstructure of the final cheese (i.e., how the components are arranged together). At this moment, it remains a challenge for the cheese producers to achieve key specific attributes because the current scientific knowledge does not allow a clear prediction of the physical properties that would result of variations of their cheese-making art. Furthermore, there's a need for better characterisation of the physical properties of cheese in order to monitor, control and deliver the attributes that are in line with the consumers demands.

Table 1.1 **Some applications and attributes of Mozzarella cheese**

Food applications	Attributes required
Sandwich / burger	Ability to be sliced without losing integrity Soften but not flow excessively during baking
Cheese stick	Ability to be cut into cubes without sticking together Hold its original shape when fried Stretches into long strains when taking a bite Easy to chew
Pasta 'gratin' such lasagna	Ease of distributing shreds on topping Browns evenly without burning under high heat
Pizza topping	Ability to be shredded in long and thin shreds, or diced Good water holding after a freezing / thawing utilisation Releases of free oil during baking Browns in localized spots on the pizza coverage called blisters Melts and flow evenly to cover other ingredients Stretches into strings when lifting a pizza slice

The goal of this work was to better understand the impact of some common cheese-making strategies on the physical properties of Mozzarella cheese and how they can be related to some key attributes required for its utilisation as an ingredient.

Chapter 2 Literature review

2.1 Mozzarella Cheese

2.1.1 Standard of Identity

Traditional Mozzarella is a fresh cheese made in the southern regions of Italy. Italian verb *mozzare* means 'to tear' which refer to the manufacturing process specially used for stretched curd cheese, or pasta filata cheese. Italian immigrants implemented the production of fresh Mozzarella cheese in United States during the early twentieth century (Rankin et al., 2006). To overcome the short shelf-life of the fresh product, a lower moisture version of pasta filata cheese, and with firmer texture, was developed which is now known as Low-moisture Mozzarella cheese (LMMC). This type of cheese encompasses different cheese varieties with defined standard of identities. In Canada, the cheese varieties are regulated by their moisture and fat content (% [w/w]). Table 2.1 summarizes the varieties specifications that can be categorized as LMMC.

Table 2.1 **Regulatory specifications for Low-moisture Mozzarella cheeses**

Variety of cheese	Maximum % moisture	Minimum % fat
Mozzarella	52.0	20.0
Part Skim Mozzarella	52.0	15.0
Part Skim Pizza	48.0	20.0
Part Skim Pizza Mozzarella	61.0	11.0
Pizza	48.0	20.0
Pizza Mozzarella	58.0	15.0

Source: Food and Drug Regulations (2015)

Since 2007, the Canadian regulation also prescribes the minimum percentage of casein (CN), reported on total cheese protein, which is derived from fresh liquid milk rather than other milk products (Food and Drug Regulations, 2015). This percentage is fixed to 63 % for Part Skim Pizza Mozzarella and Pizza Mozzarella, and 83 % for the others varieties presented in Table 2.1. LMMC varieties are characterized by a clean mild favor with a slightly acidic fresh milk notes (Chen et al., 2009). The color can range from light cream to almost white and the body should be semi-firm and with a slightly rubbery texture. Unlike traditional Mozzarella, LMMC requires to be aged for a short period in order to perform adequate textural characteristics. The generic term ‘Mozzarella’ is used in this thesis to describe pasta filata LMCCs unless a given variety is specified.

2.1.2 Cheese-Making Technology

The primary objective of the cheese-making technology is to concentrate milk caseins and triglycerides into a tridimensional and bi-continuous structure composed of a hydrated protein phase with dispersed domains of fat. Figure 2.1 summarizes the main steps for the manufacture of Mozzarella cheese.

***Figure 2.1* Generic manufacturing diagram for the production of cultured Mozzarella cheese. Adapted from Chen et al. (2009).**

Mozzarella cheeses produced in Canada are made exclusively from pasteurized cow’s milk. Cheese milk is standardized to an appropriate fat-to-protein ratio, depending on cheese variety (see Table 2.1), by skim milk addition and / or milk protein concentrates (MPCs) to whole milk. Denatured whey protein aggregates may be added to milk to increase cheese yield (Hinrichs, 2001; Mead and Roupas, 2001; Schenkel et al., 2011).

Calcium chloride ($CaCl_2$) may be added to restore milk calcium equilibrium which is affected by handling and preparation (Mietton et al., 2004). Appropriate calcium concentration is required to optimise the coagulation properties of renneted CNs (Gustavsson et al., 2014; Horne and Lucey, 2014; Ong et al., 2015). Milk acidification may be initiated by (1) inoculation with lactic acid producing bacteria (cultured Mozzarella), (2) direct addition of organic acids (direct acidified Mozzarella), or (3) a combination thereof. Direct acidification procedures were studied extensively by dairy scientists (Dave et al., 2003; McMahon et al., 2005; Shakeel-Ur-Rehman and Farkye, 2006; Mizuno et al., 2009), but the use of culture remain the primary acidification approach used by the cheese industry (Kindstedt, 2004). Cultured Mozzarella is usually made using thermophilic strains such as *S. thermophilus* and *Lb. delbrueckii subsp. bulgaricus* (Kindstedt and Fox, 1993). Curd setting is initiated by the addition of coagulant such as calf rennet, fermentation-produced chymosin or fungal proteases that are chosen based on the enzymatic activity, specificity and thermal stability (Kindstedt and Fox, 1993; Sheehan et al., 2004). Recently, the suitability of fermentation-produced camel chymosin for the manufacture of Mozzarella was demonstrated by Moynihan et al. (2014). The curd is then cut when appropriate rheological and microstructural gel properties are attained (Castillo, 2010). Cutting size, thus the surface-to-volume ratio of curd grains, is controlled to optimize the whey expelling as the gel contracts. The contraction kinetic of milk gel is increased during cooking and more whey is expelled (Giroux et al., 2014). The whey is then separated from the curd grains. The grains are then allowed to acidify to pH ~5.2 under continuous stirring or by letting it fuse together (Rankin et al., 2006). The curd is then kneaded and stretched in hot water or in hot brine using a cooker-stretcher. In this step called the pasta filata process, individual curd pieces are transformed into a continuous and flowable cheese mass and then molded. The blocks are then brined to a typical salt concentration of ~1.5% and packed in air-tight plastic film for aging at low refrigerated temperature (< 8 °C).

2.2 Mozzarella: Components and Structure

2.2.1 Protein Phase

2.2.1.1 Structure of Casein Aggregates

Caseins (CNs) constitute approximately 80% of proteins in bovine milk where they are found as colloidal particles called micelles. Some properties of the four CN variants, α_{s1}-, α_{s2}-, β- and κ-CN, are summarized in Table 2.2.

Table 2.2 **Properties of caseins in milk conditions**

	Casein variant			
	α_{s1}-CN	α_{s2}-CN	β-CN	κ-CN
Molecular mass (kDa)	23.6	25.2	24.0	19.0
Concentration in milk (g L^{-1})	12-15	3-4	9-11	2-4
Total amino acid residues	199	207	209	169
Positively charged residues (%)	13	16	10	10
Negatively charged residues (%)	20	19	13	17
phosphorylated serine residues	8	11	5	1-2
(center of phosphorylation)	(2)	(3)	(1)	(0)
Calcium sensitivity	moderate	high	low	none
Hydrophobic character	moderate	low	high	moderate
pI (with phosphorylation)	4.42	4.9	4.7	~5.5

Source: Farrell et al. (2004) and Huppertz (2013)

Many models have been proposed to describe the assembly and internal structure of the CN micelle but the subject is still debated among the scientific community (De Kruif et al., 2012). Most recent models proposed that phosphorylated caseins are bound to CCP nanoclusters dispersed in the internal structure of the micelle. The protein tails sticking out of the nanoclusters would interact with other proteins through low energy interactions such as hydrophobic interactions, hydrogen bonding, ion bonding, electrostatic interactions and Van der Waals attraction (De Kruif et al., 2012). It is well recognised however that the micelle's surface is rich in κ-CNs which may act as a size-limiting factor during micelle formation due to its incapacity to interact with calcium nanoclusters (Horne, 1998; 2006). The "hairy tails" of κ-CNs (i.e., caseinomacropeptide [CMP];

residues 106-169) is sticking out of approximately 5-10 nm at the micelle surface and provides steric stabilization against the aggregation with other micelles in milk (Dalgleish and Corredig, 2012).

The protein phase of cheese is mostly composed of a network of CNs (i.e., the *para*casein network) formed by the aggregation and the rearrangement of destabilized CN micelles from milk. Two types of gelling mechanisms are recognized for the production of cheese: the acid-induced coagulation or the rennet-induced coagulation (Lucey, 2002). During Mozzarella manufacture the rennet is usually added at pH > 6.4. The rennet-induced milk clotting is usually regarded as two distinctive stages that overlap to some extend during cheese-making: (1) the enzymatic proteolysis stage (or primary phase) and (2) the aggregation and reorganization of destabilized micelles (or secondary phase) (Lucey, 2002).

During the primary phase, the enzymatic reaction of chymosin cleaves the Phe_{105}-Met_{106} bond of the κ-CNs amino-acid chain thus releasing the C-terminal region (CMP) in the aqueous phase of milk. As the CMP provided steric stabilisation, its removal from the micelle surface reduces the colloidal stability of micelles (Horne and Banks, 2004). The rate of the enzymatic reaction is dependant of environment factors affecting the coagulant enzyme such as the temperature, pH and calcium concentration (Dejmek and Walstra, 2004). When ~85% of κ- CNs is hydrolysed, the steric stabilisation energy is becoming insufficient to prevent the CNs aggregation and hence lead to the secondary phase of coagulation (Dalgleish and Corredig, 2012). The degree of κ- CNs breakdown before the aggregation phase occurs is notably dependent on temperature, ionic strength and pH of the milk (Dalgleish and Corredig, 2012; Horne and Lucey, 2014). Increasing the ionic calcium concentration up to 60 mM increases the reactivity of CNs to aggregation, thus starting at lower percentage of κ- CNs hydrolysis (Bringe and Kinsella, 1986). When decreasing the milk pH, the reduction of charges induces the collapse of the κ- CN C-terminal region at the micelle surface which reduces the steric stabilisation of micelles (De Kruif, 1999).

The gelation of milk is initiated during the secondary phase where the micelles can approach one another closely enough to interact together via hydrophobic interactions and calcium bridging between calcium sensitive patches of CNs. At first, CNs aggregate via their surfaces, and therefore, the internal structure and CCP of CNs do not influence aggregation, but become increasingly important thereafter (Dalgleish and Corredig, 2012). Chains of CNs start to form and as they become more closely packed, a gel structure is created that can be modeled using the fractal aggregation theory (Mellma et al., 2000; Horne and Banks, 2004). Intra-micellar rearrangement, individual micelle shift and strand rearrangements progress during the gel curing (Mellema et al., 2000). During this time, bonds are formed and broken in the dynamic rearrangements of the gel, but globally the number of interactions increases as observed by a greater firmness and contraction properties (Dejmek and Walstra, 2004 ; Giroux et al., 2014).

A schematic representation of the CN network structure is shown in Figure 2.2. Lucey (2002), proposed to use the Horne's dual binding model (Horne, 1998) to discuss the dynamic of interactions between CN molecules that structures the *para*casein network in cheese. According to Horne's model, individual caseins are bound together mainly through (1) hydrophobic interactions between non-polar regions of opposing CNs and by (2) colloidal calcium phosphate (CCP) bridging involving the negatively charged phosphoseryl residues of opposed CNs and calcium phosphate nanoclusters (Lucey, 2002). The global interaction energy between CNs is a function of the balance between the attractive interactions (i.e., hydrophobic, electrostatic and CCP bridging) and electrostatic repulsions (Lucey et al., 2003). Hence, the nature and strength of interactions within the protein network are strongly dependent of the surrounding environment conditions such as the pH, ionic strength (e.g., sodium, calcium and phosphorus ions concentration) and temperature (McMahon and Oommen, 2013).

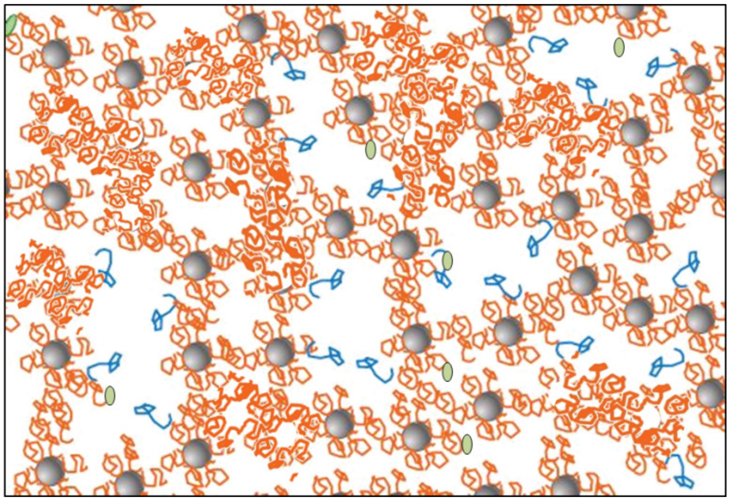

Figure 2.2 **Schematic representation of a casein gel network (adapted from Dalgleish and Corredig, 2012). Calcium phosphate nanoclusters (grey spheres) are cross-linked with 3 to 5 α- or β-CNs (orange) via their centers of phosphorylation. Some β-CNs (blue) are bound to other CNs by hydrophobic interactions and thus can be released from the protein network at cool temperature. *Para*-κ-CN (green) that remained attached via hydrophobic interactions after the scission of the caseinomacropeptide. Denser regions of CN aggregates, stabilized by hydrophobic interactions and electrostatic attractions (between carboxylate residues and divalent metal ions), are formed after the solubilization of some calcium phosphate clusters during acidification. The rearrangement and contraction of the CN network over time forces the water out in serum channels (void space). Not drawn to scale.**

2.2.1.2 Structure of Protein in Mozzarella

The thermo-mechanical treatment imposed during the pasta filata process converts the casein aggregates of fresh curd into amorphous and fibre-like protein structures having roughly parallel orientation (Taneya et al., 1992; McMahon, 1999). The melted curd has a smooth and plastic appearance. To sustain the stretching process, the CNs must interact sufficiently with each other to maintain the network integrity, but the interactions must also release some of the stress imposed (viscous behavior) and become pliable (Lucey et al., 2003). It is reasonable to believe that hydrophobic interactions play an important role for the reorganisation of protein structures during the pasta filata process as they become stronger with temperature increases (Bryant and McClements, 1998). The molecular mechanisms behind the structuration of the protein network during the pasta filata process have not been studied in detail.

2.2.2 Aqueous Phase

2.2.2.1 Components in Aqueous Phase

Water in cheese has a critical solvation role for numerous components such as the nitrogen fraction (proteins, enzymes, peptides or amino acids), carbohydrates, organic acids and salts. Moreover, water allows the diffusion of substrates and the growth and survival of bacteria (Floury et al., 2010). The protein phase properties are greatly affected by the composition of the aqueous phase; and particularly by its pH and the presence of salts such as calcium and sodium (Guinee, 2004).

The acidification of Mozzarella is largely caused by the bacterial conversion of lactose into lactic acid. However, the concentration of hydrogen ions in the serum phase (i.e., the pH) is also conditioned by the buffering capacity of proteins and other substrates such as inorganic phosphate, citrate, lactate, carbonate and acetate ions. The measure of pH is related to the degree of dissociation of these compounds and therefore, their behavior regarding water affinity (hydrogen bonding) and electrostatic attraction or repulsion with other charged molecules. Table 2.3 reports the half dissociation pH (pKa) of some compounds contributing to the buffering capacity of the serum phase.

In complex system such as cheese, the mineral environment and the ionic strength directly influence the dissociation equilibrium of buffering compounds. Calcium in cheese is present in soluble forms; as free ion or ion pairs, or in insoluble forms; mainly as CCP located within the *para*casein structure (Deeth and Lewis, 2015). The partition of calcium between the soluble and insoluble forms is subjected to the salts equilibria schematised in Figure 2.3. These equilibriums strongly depend on ionic strength and pH of the aqueous phase. An increase of H+ or citrate promotes the solubilization of calcium while CCP can be formed if calcium in the serum phase is in excess (Deeth and Lewis, 2015). It is worth noting that an increase of ionic calcium or magnesium concentration decreases the dissociated forms of phosphate, citrate and carbonate anions at given pH (Salaün et al., 2005).

Table 2.3 **Half dissociation pH (pKa) of some water soluble compounds**

Compound (acid form)	pKa[a]
Amino acids of proteins	
Arginine	-
Lysine	10.5
Histidine	6.5
Aspartic acid	4.1
Glutamic acid	4.6
Phosphoserine	6
Salt and organic acids	
Phosphoric acid	3–5.8–6.6
Citric acid	3–4.1–4.8
Lactic acid	3.9
Carbonic acid	6.4–10
Acetic acid	4.8

[a] Determined in milk ionic conditions (from Salaün et al., 2005)

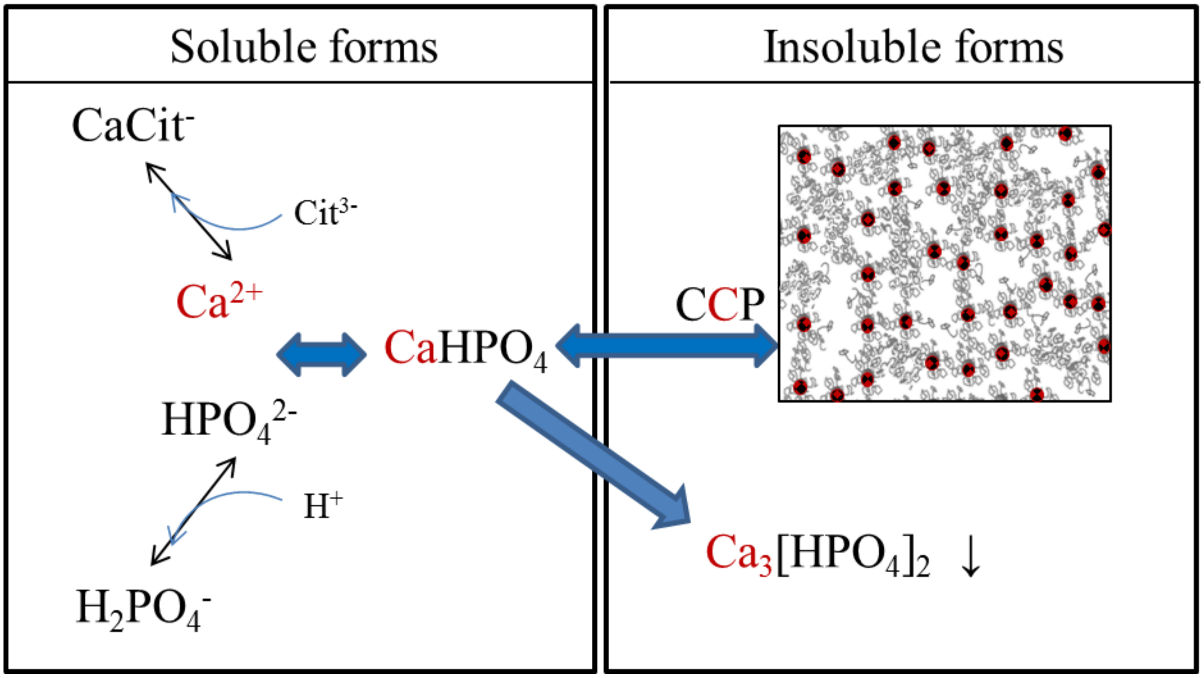

Figure 2.3 **Equilibria of some salts interacting with calcium.**

CCP has a major impact on the protein phase properties as it strongly bridges 3 to 5 different CNs together (De Kruif et al., 2012; Deeth and Lewis, 2015). Colloidal calcium can be quantified indirectly by measuring the concentration of the soluble calcium in the cheese aqueous phase or by evaluating the buffering capacity using acid-base titration (Morris et al. 1988; Hassan et al., 2004).

In milk, the CCP is dissolved completely at pH ~5.1 which can be assessed by the occurrence of a milk buffering capacity peak in milk titration curves (Lucey et al., 1993). However, significant amount of CCP were found in cheese (pH ~5.2) (Lucey and Fox, 1993; Hassan et al., 2004). By defining a standard curve and using the acid-base titration method, Rémillard and Britten (2011) estimated the amount of colloidal calcium of Mozzarella to about 21 mg g^{-1} of protein. The presence of residual CCP at pH ~5.2 was explained by the building-up concentration of soluble calcium in the curd grains during the post-cutting acidification which retarded the dissolution equilibrium of CCP and thus its loss in cheese whey (Johnson and Lucey, 2006).

The sodium ions in the serum phase contribute to the flavor, but also affect the water activity of cheese which in turn controls biochemical changes such as microbial grow and enzyme activity. Sodium concentration also affects the protein solubility through its ionic interaction with caseins (Guinee, 2004).

2.2.2.2 Water Distribution in Mozzarella

Water in cheese can be classified as bound or bulk water, which is referring to the spatial relationship between the water and the other solids constituents. Bound water is chemisorbed and thus intimately associated with solid components such as proteins. Bound water is not available as solvent, is not freezable and has slower rotational and translational properties then pure water (Vogt et al., 2015). McMahon et al. (1999) estimated the bound water in Mozzarella cheese to about 0.71 g g^{-1} of protein. In contrast, bulk water is more loosely associated with the protein phase and thus largely retains its solvent capacity and have physical properties much similar to pure water (Fife, 2003). Bulk water may be either closely entrapped within the tridimensional structure of *para*caseins (entrapped water) or more loosely imbedded by the cheese microstructure (free water). Guo and Kindstedt (1995) proposed to quantify the free water (or serum) by the centrifugation of shredded cheese. Despite the fact that the amount of expressible serum is dependent of many external factors (e.g., temperature, shred size, centrifugation force) numerous authors used this method for indirect estimation of the water holding capacity of proteins. Another method to extract bulk serum is to apply hydrostatic pressure on grated cheese-sand mixture (Morris et al., 1988; Hassan et al. 2004). However, this approach is time-demanding and requires a greater amount of sample compared to the centrifugation method.

The amount of expressible serum of newly manufactured pasta filata cheeses is about one-third of the total moisture content (Kindstedt and Guo, 1998). McMahon et al. (1999) clearly demonstrated that the swelling of the protein matrix during a short aging period explained the decreases of expressible serum to almost none after 1 or 2 weeks.

This suggest that the water holding capacity of proteins are impaired by the cheese manufacture, and probably by the thermal and mechanical treatment imparted during the pasta filata process. However, to the best of knowledge, the impact of the pasta filata process conditions on protein hydration capacity was never investigated. It is generally accepted that the equilibrium between the serum phase and the protein matrix can be restored to some extend during aging (McMahon et al., 1999; Monteiro et al., 2011). The factors affecting the gradual hydration of the protein phase during cold storage has been studied and have been related to sodium and calcium concentration and the releases of soluble proteins in the aqueous phase (Guo and Kindstedt, 1995; Paulson et al., 1998; Monteiro et al., 2011).

2.2.3 Fat Phase

Milk fat consists of about 98% of triacylglycerol molecules packed in globules that are stabilized by a milk fat globule membrane (MFGM) with amphiphilic properties (Gresti et al., 1993). The nature and position of fatty acids attached to glycerol molecules regulate the polymorphism of fat crystallization and the proportion of solid-to-liquid fat between -5 and 40 °C (Vithanage et al., 2009). Because of its rather hydrophobic nature, the presence of fat physically prevents the aggregation of the casein particles, thus creating a partially disrupted protein network in cheese (Everett, 2007). Figure 2.4 shows some different fat suprastructures that may be formed during the processing of milk into Mozzarella cheese. Among other cheese-making process steps, the thermal and mechanical treatment imposed during the pasta filata process can promote the aggregation and coalescence of fat globules and the formation of free fat pools (Auty et al., 2001; Rowney et al., 2003). Changes in fat suprastructure may have practical consequences on cheese properties. Depending on the level of interactions between the MFGM and the protein phase, entrapped fat has been considered as an inert filler (weak interaction) or as a copolymer (strong interaction) within the *para*casein matrix (Everett and Auty, 2008). Theoretically, a copolymer-type fat phase would be expected to contribute more to the global structure of cheese in comparison to inert filler (Hickey et al., 2015). The nature and extend of fat-protein interactions are however under debate, and therefore so is its impact on cheese properties (Everett, 2007; Corredig et al., 2011). Lopez et al. (2007) argued that the size and shape of fat globules and the integrity of the MFGM contributes to the texture of cheese. The work of Barden et al. (2015) recently brought further understanding on the relationship between the physical properties of cheese and the fat-protein interactions. It is however well established that the global cheese structure is influenced by its relative fat volume fraction and by the state of fat as influenced by temperature (Rogers et al., 2010; Schenkel et al., 2013a).

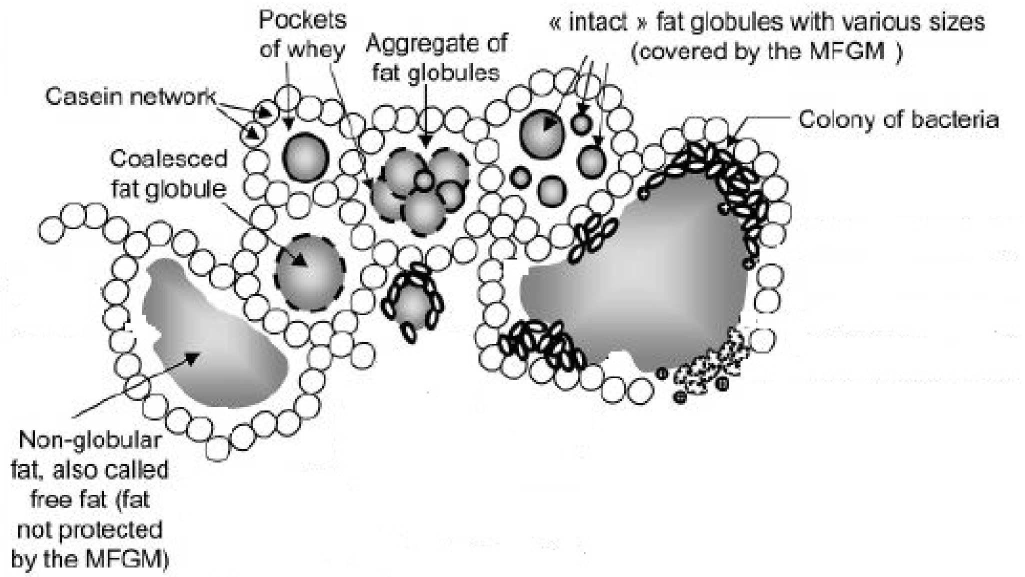

Figure 2.4 **Schematic representation of different organization structure of fat in cheese. Adapted from Lopez et al. (2006).**

2.3 Key Attributes for the use of Mozzarella as Ingredient

Mozzarella cheese is likely to be subjected to two major processes for its preparation and consumption: (1) a size reduction (e.g., portioning, shredding, dicing, slicing) and (2) heating (e.g., cooking, baking, frying). For each of these processes, the cheese is expected to perform key attributes related to a given menu application. The attributes performance is not only dependent of external processing conditions, but is also closely related to the physicochemical properties and structure of the cheese itself (Guinee, 2002; Guinee and Kilcawley, 2004).

2.3.1 Machinability

Machinability is a broad term for the ability of cheese to be shredded/diced/cut/sliced (Lucey, 2008). In order to do so, the cheese matrix must fracture by the mean of an external force (unidirectional or centrifugal) transferred to the cheese using a wire or a blade. The size reduction improves the handling and heating characteristics of cheese mainly because of the greater surface area created (Apostolopoulos and Marshall, 1994; Ni and Gunasekaran, 2004). Manufacturers and consumers are both performing size reduction process of cheese which highlights the importance of controlling cheese machinability under various conditions. Table 2.4 presents the key attributes used to assess cheese machinability.

The success of the size reduction process can be evaluated by the size and shape of the final product and compared to a predefined set of standards (Childs et al., 2007). Moreover, the process should not damage the cheese surface or the integrity of the cut pieces. Defect-oriented attributes such as fines production, the fouling of industrial equipment, the wetting of the cheese surface and the matting of pieces during storage must be controlled to prevent product downgrading, processing problems and economical losses. Storage and handling defects are usually prevented using anti-caking agents (Sing et al., 2015). Very few studies investigated the relationship between the cheese machinability and its physical properties (Chen, 2003; Childs et al., 2007; Perri, 2012). Other studies rather reported empirical observations about rheological properties and compositional characteristics related to machinability (Kindstedt, 1995; Guinee, 2002; Lucey, 2008). Childs et al., 2007 measured a large amount of fines during the shredding of commercial Mozzarella compared to other cheese varieties such as Monterey Jack and Process cheese. Against intuitive expectations, Mozzarella with higher fat content produced finer and less sticky grated pieces compared to a reduced fat Mozzarella, even though the adhesive energy (i.e., energy required to separates 2 materials that are not bound permanently) was higher with increasing fat content. At the moment, there is a lack of adequate understanding for the control of size reduction attributes and of practical solutions to improve the process efficiency (Gunasekaran and Ak, 2002).

Table 2.4 **Key attributes to assess Mozzarella cheese machinability**

Attribute	Description	Related references
Pieces uniformity		
Size and shape of pieces	Homogeneity of size and shape of the cheese pieces and consistence regarding the quality standard required.	Apostolopoulos and Marshall, 1994; Chen, 2003; Ni and Gunasekaran, 2004; Perrie, 2012.
Fine production	Production of small cheese fragments during the process.	Kindstedt, 1995; Childs et al., 2007.
Pieces integrity		
Ragged or fractured piece surface/edges	Unwanted fracture within the cheese pieces. Irregular cut surface.	Goh et al., 2005; Perrie, 2012.
Stickiness/adhesion	Industrial equipment clogged with cheese adhering/spread to its surface.	Kindstedt, 1995; Childs et al., 2007.
Storage and handling		
Wet cheese surface	Release of free moisture from the cut surface of pieces.	Kindstedt, 1995; Singh et al., 2015.
Matting of pieces	Clumping of cheese pieces during storage and reducing of the free flowing of pieces.	Kindstedt, 1995; Ni and Gunasekaran, 2004; Serrano et al., 2005.

2.3.2 Heat-Induced Attributes

Mozzarella cheese is heated/baked prior the consumption of various prepared food (e.g., pizza, lasagna, sandwiches, cheese sticks). Heating conditions such as baking temperature and duration varies greatly depending on the food application. Moreover, heating conditions varies according to the type of the heating medium (air or oil) and heating flow dynamic (convective or conductive-oriented) induced by the heating devices (Paquet and Kaláb, 1988). Table 2.5 summarizes the key attributes used to describe the heat-induced characteristics of Mozzarella cheese.

Heating is known to induce dramatic changes on the microstructure and physical properties of Mozzarella (Paquet and Kaláb, 1988). The transition from a semi-solid material at cool temperature to a more definite liquid-like one with temperature elevation is commonly referred as the melting of cheese. This change of physical properties is driven by (1) the liquefaction of fat and (2) changes in the CN-CN interactions within the cheese.

From a physical stand point, the fat phase is the only cheese constituent that truly melts under typical heating conditions. As temperature reach the 20-40 °C range, the proportion of liquid fat increase dramatically (Lopez et al., 2006). Liquid fat has lower viscosity and higher density than solid fat; thus the contribution of the fat phase to the solid behavior of cheese is lowered (Rogers et al., 2010). Simultaneously, the hydrophobic interactions within the protein matrix gradually become stronger (Bryant and McClements, 1998). Consequently, the CN's molecules contract on themselves and some water is expelled from the protein matrix as temperature reach the 35-45 °C range (Vogt et al., 2015). As temperature further increase toward ~60 °C, the number of contact points between CN's globally decrease as the hydrophobic interactions intensity peaks at their strongest. Lucey et al. (2003) postulated that the global reduction of interactions between CN molecules is promoted by the lower contribution of electrostatic repulsions and hydrogen bonding at higher temperature; and by the simultaneous increase of hydrophobic interactions that reduce the contact area between CN molecules as they contract. Hence, the softening of cheese induced by the global reduction of protein interactions and the melting of fat. Eventually, the CN's molecules can move more freely and exhibit a flow behavior. Moreover, the liquid fat acts as a lubricant allowing the protein aggregates to flow past each other under relatively low force (e.g., gravity) (Lefevere et al., 2000). The ability of cheese to stretch into long strings when pulled requires that casein continuously interacts together, but also that these interactions partially releases some of the applied stress into viscous flow (Lucey et al., 2003).

As the cheese temperature is kept increasing past ~60 °C, others changes may occurs. The gradual loss of cheese matrix structure promotes the release of oil and moisture which increase the global fraction occupied by protein. The free oil released at the cheese surface, a phenomenon called oiling-off, helps to prevent extensive dehydration of the cheese surface, but can become a defect if excessive (Rudan and Barbano, 1998; Everett et al., 2004). The inner pressure that build-up in the protein matrix due to moisture vaporisation induces localized bubble-like deformations of the cheese surface (Rudan and Barbano, 1998). These areas are more subjected to direct heat and surface dehydration, and may brown into so-called ‘blisters’ because of the Maillard reaction (Rankin et al., 2006). The formation of strong heat-induced protein interactions in cheese have also been reported at temperatures >70 °C (Udayarajan, 2007; Kim et al., 2011).

Many of the numerous studies on Mozzarella cheese published the last decades have been focusing on the meltability, stretchability, oiling off and browning attributes upon heating. However, cooked cheese must generally cool down between 65 and 50 °C prior consumption (Metzger and Barbano, 1999; Chen et al., 2009), and, in some cases; it can be reheated on another occasion. Opposingly to heating attributes, the cooling behavior of cheese, or its sensory texture after baking received very little attention in published studies.

Table 2.5 **Key attributes to assess heat-induced properties of Mozzarella**

Attributes	Description	Related references
Meltability		
Softening	Tendency of cheese to soften on heating.	Gunasekaran et al., 1998; Choi et al., 2008; Schenkel et al., 2014.
Flowing	Tendency of heated cheese to spread spontaneously, when unrestricted, during heating.	Wang et al., 1998; Muthukamarappan et al., 1999; Wadhawani et al., 2001.
Melt characteristics		
Stretchability	Tendency of heated cheese to form strings and/or sheets when extended uniaxially.	Guinee and O'Callaghan. 1997; Ma et al., 2012.
Oiling-off	Tendency of heated cheese to exude oil at surface.	Kindstedt, 1990; Wang and Sun, 2004.
Blistering/tenting	Aspect of the baked cheese surface: apparition of small bubbles with darker color (blisters), or a darker skin formed on a larger area (tenting).	Ma et al., 2013b.
Browning	Change of color of cheese during baking due to a Maillard reaction between reducing sugars (e.g., lactose) and proteins (especially amino acids).	Wang and Sun, 2003; Ma et al., 2013b.
Melt sensory texture	Mouth evaluation of melted cheese: Tendency of cheese to resist breakdown during mastication, sensory firmness, liquid release during chewing, energy required to masticates a sample before swallowing (chewiness).	Metzger and Barbano, 1999; Chen et al., 2009.

2.4 Impact of Cheese-making Technology on Functional Attributes

2.4.1 Impact of Cheese Milk Standardization

The fat-to-protein ratio of cheese milk has a critical impact on the properties of the cheese manufactured. The relative contribution of fat to the cheese firmness is important at low temperature but quickly decreases around room temperature (Yang et al., 2011). Mozzarella with high fat-to-protein ratio tends to be softer than cheeses with a higher proportion of protein (Masi and Addeo, 1986; Rudan et al. 1999). This factor has to be considered because too-soft or too-firm cheeses are usually difficult to shred or slice (Kindstedt, 1995). As discussed previously, fat also plays an important role during the melting of cheese (Guinee et al., 2000a; Lefevere et al., 2000). Many studies reported poor melting attributes of reduced-fat Mozzarella cheese (Rudan et al. 1999; Zizu and Shah, 2007; Wadhwani et al., 2011). Mozzarella with low fat-to-protein ratio released little free oil during baking which in turn increase browning and formation of blisters (Rudan and Barbano, 1998; Wadhwani et al., 2011). Lower fat content also means that the protein phase is less disrupted and that protein-protein interactions are favored during cheese-making. Consequently, this affected the amount of free water of young cheeses, and the swelling of the protein phase during aging (McMahon et al, 1999; Pastorino et al., 2002).

The standardization of the cheese milk with milk protein concentrate (MPC) and/or with denatured whey protein concentrate (WPC) is common for the making of Canadian Mozzarella. Both ingredients increased the cheese yield but contrarily to MPC, WPC addition has significant impacts on cheese characteristics (Rehman et al., 2003). There are evidences that WPC addition can adversely affect the coagulation of milk and decrease the fat retention in cheese (Punidadas et al., 1999; Mead and Roupas, 2001; Schenkel et al., 2013b). Increased moisture was also observed depending on the WPC concentration added (Mead and Roupas, 2001; Zisu and Shah, 2005; Schenkel et al., 2013b). The impact of WPC on the melting attributes of cheese is not clear. Some studies report negative (Mead and Roupas, 2001; Schenkel et al., 2011) or no impact (Punidadas et al., 1999); while others observed an increased meltability of enriched reduced fat-cheese (Zisu and Shah, 2005; Ismail et al., 2011; Schenkel et al., 2013b). This confusion may arise from the confounding effects on cheese composition (e.g., relative casein concentration decrease, loss of fat, increase of moisture) induced by WPC addition.

2.4.2 Impact of Acidification and Draining

The simultaneous management of the acidification rate and of curd moisture during cheese-making is a key factor for the control of cheese properties. Both events are closely dependent, but the art of cheese-making is to take actions that favor one or another at the appropriate time in order to customize or adjust cheese texture and composition to the needs of the end user.

The acidification induces the solubilization of the CN-associated CCP into the aqueous phase of milk (Lucey and Fox, 1993; Hassan et al., 2004). It is now well accepted that the concentration of CCP is a primary factor that affects the cheese properties, especially when heated (Johnson and Lucey, 2006; Choi et al., 2008). As discussed previously, CCP serves as crosslinking agent of the CN matrix, hence a reduction of CCP decreases the global casein interactions. Many studies reported an increase of meltability of cheese when the CCP concentration was reduced (Joshi et al., 2002; 2004a; O’Mahony et al., 2006; Choi et al., 2008). Therefore, there is a need to control the total calcium which in turn will affect the CCP concentration in the final cheese. To this end, the period at which the calcium is solubilized during cheese-making is important (Johnson and Lucey, 2006). The CCP can be readily solubilized prior the gelation of milk. At this point, the calcium that will be lost during whey drainage is directly proportional to the CCP dissolution. After gelation and cutting however, the soluble calcium concentration in the entrapped serum pockets of curd grains increases as the result of acidification, but the diffusion of this calcium outside the gelled matrix is retarded. Consequently, the loss of calcium when the whey is drained is reduced. After drainage, the whey expulsion is greatly reduced and the pseudo-equilibrium between the calcium forms is affected by the accumulation of soluble calcium that remains inside the curd grains. By the end of manufacture, the total calcium concentration is fixed, but the equilibrium between CCP and soluble calcium can be modified. Indeed, Ge et al. (2002) showed that the proportion of CCP-to-soluble calcium can be reversibly controlled by the final cheese pH.

Reducing the micellar calcium content can also promotes the hydration and swelling of the protein matrix during the early aging period of Mozzarella (Guinee et al., 2002; McMahon et al., 2005; O' Mahony et al., 2006), and thus reduce the time needed to develop adequate melting properties (Cortez et al., 2008; Mizino et al., 2009). The impact of calcium changes on the texture of unmelted cheese has been investigated (Keller et al., 1974; Lucey et al., 2005; Choi et al., 2008) but the relationship with its ability to be sliced or shreded remains to be clarified (Chen, 2003).

Practically, controlling the calcium content by modifing the renneting, draining or final pH during cheese-making may have consequences on the curd moisture at different points of manufacture (Yun et al., 1995; Sheehan and Guinee, 2004; Lee et al., 2005). Higher moisture content usually results in a softer cheese because the relative content in casein and fat, which are structuring components, is lowerered (Keller et al. 1974; McMahon et al., 2005). Research studies proposed that the amount of water interacting with proteins, rather than the total moisture content, can have an impact on cheese meltability (Kindsted and Guo, 1997; McMahon et al., 1999). The mobility of protein during melting would be increased if protein-water interactions are favored against protein-protein interactions (Everett, 2007). It was shown that increasing the total moisture content did not have a major impact on the melting properties of Mozzarella (Pastorino et al., 2003a; McMahon et al., 2005).

2.4.3 Impact of the Pasta Filata Process

The singular ability of Mozzarella cheese to melt, stretch and release free oil during baking has been in part attributed to the curd plasticization occurring during the pasta filata process (Kindstedt and Guo, 1997). It is recognized that a pH between 5.3 and 5.1 must be reached for successfully melt and knit cultured Mozzarella curd (Rowney et al., 2003). Industrial pasta filata processing equipment vary greatly: batch or continuous process, single or twin auger systems designed with different materials, flight effects, barrel geometries and heating systems. Moreover, in-process control parameters such as the barrel temperature, the curd feeding rate and the auger speed can be adjusted. Hence, the thermo-mechanical treatments imposed may largely differ, but the curd temperature at the end of the process is usually between 55 to 65 °C (Mulvaney et al., 1997; Bahler and Hinrichs, 2013).

Processing the curd at high temperature (typically between 50 and 65 °C) induces the loss of solids and moisture into the stretching brine (Renda et al., 1997; Yu and Gunasekaran, 2005a; Gernigon et al., 2009). Some proteolytic enzymes can be inactivated depending on the process conditions, but it is recognized that some residual chymosin activity and starter culture activity persist during cheese aging (Creamer, 1976; Feeney et al., 2002; Costabel et al., 2007; Moynihan et al., 2014). Systematic studies of the impact of pasta filata processing conditions on cheese characteristics are scarce. Some researchers measured the specific mechanical energy (SME) imparted and the final curd temperature to characterize the processing conditions. Based on that approach, Yu and Gunasekaran (2005a) and Mulvaney et al. (1997) showed that dramatics changes in the cheese microstructure may be caused by the application of different thermal and mechanical treatments, but its impact on physical and melting properties remains unclear. One limitation of these studies is that the residence time in the cooker-stretcher is closely related to the auger's speed, which in turn, also affects the final curd temperature. Rowney et al. (2003) used a simplified experimentation model to investigates the impact of stretching extend and rate on curd melted at different temperatures. They found that increasing stretching temperature increased free oil releases. The stretching conditions had no impact on that oiling off but modified the milk fat suprastructure. It is clear that the current literature does not provide sufficient data that would allow the tailoring of Mozzarella cheese properties by controlling the thermo-mechanical conditions during the pasta filata process.

2.4.4 Impact of Sodium Chloride

Mozzarella cheese is usually brine-salted although salt may be added prior the pasta filata process or into the stretching water (Paulson et al., 1998; Guinee et al., 2000b). The osmotic pressure difference between the cheese and the brine induces a mass transport of solutes and water. Water content decreases from the surface to the center as the sodium concentration gradient diffuses towards the inside the cheese. For each quantity of sodium gained, the moisture mass loss is about two times higher (Guinee, 2004). Other solutes such as calcium are also diffused out of cheese during brining. The sodium intake was found to be reduced with increasing concentration of calcium in the brine solution (Luo et al., 2013). This was attributed to a lower loss of calcium, thus preventing the excessive swelling of the protein phase at the cheese surface and apparition of soft rind defects.

Higher sodium concentration usually leads to firmer and less cohesive cheese body (Guinee and Fox, 2002; Pastorino et al., 2003b). The effect of sodium on cheese texture may be attributed to its effect on composition (moisture-to-protein ratio), the protein hydration, solubility and conformation, and age-related effect on pH and proteolysis (Guinee, 2004). Salting increased melting properties and the melt viscosity of Mozzarella cheese (Paulson et al., 1998; Joshi et al., 2002; Everett et al., 2004), but some studies reported limited impact at different salt concentrations (Pastorino et al., 2003b; Ma et al., 2013). Increase of meltability was explained by the impact of sodium on hydration of protein, combined with the proteolysis action occurring during aging. Clearly, the presence of sodium chloride increases the protein hydration and the water-binding capacity of protein in Mozzarella up to a concentration of ~1.4 % compared to unsalted cheese (Guo et al., 1997; Rowney et al., 2004). A salting-in effect of sodium ions on protein would explain these changes. Unlike some authors hypothesized, the substitution of calcium CN-associated with sodium ions is unlikely (Pastorino et al., 2003b; Floury et al., 2009). Interestingly, the partial substitution of NaCl by KCl had only a very slight effect on the melting characteristics of Mozzarella cheese (Ayyash and Shah, 2011; Thibaudeau et al., 2015). Less free oil was released from heated Mozzarella cheese with higher salt content, but apparently this effect was more pronounced on aged cheese (> 8 d.) (Rowney et al., 2004; Everett et al., 2004). Again, this was attributed to a better swelling of the protein matrix as the salt concentration did not influenced the proteolysis rate in these studies. Ma et al. (2013) observed a negative relationship between salt content and the size of blisters formed upon Mozzarella baking, but this should be validated as the effect of other factors could have impacted this result. Clearly, any modulation of salt concentration may have multiple impacts on cheese properties.

2.4.5 Impact of Aging and Freezing

It is generally accepted that Mozzarella cheese must undergo a short aging period in order to develop satisfactory machinability and heat-induced attributes. It has been argued that this period corresponds to the time needed for the reabsorption of free serum imbedded in the protein phase (Kindstedt, 1995; McMahon et al., 1999). Most studies reported that no serum could be expressed by the centrifugation of shredded Mozzarella after ~1-2 weeks post-manufacture (Guo and Kindstedt, 1995; McMahon et al., 1999; Kuo et al., 2001). Another important age-related change is the gradual solubilization of calcium and phosphorus initially present as CCP into the serum phase (Hassan et al., 2004). The production of organic acids by bacteria, and thus the release of H+ ions, might be responsible for the solubilization of inorganic phosphate which plays an important buffering role (Upreti and Metzger, 2007), but other factors were suggested (Lucey, 2008). As CCP is solubilized, the number of effective bonds between proteins is reduced. This loosening of the protein matrix may increase the protein surface that could interact with water and thus allow a better hydration of protein with aging (Lucey et al., 2003). For Cheddar and Mozzarella cheeses, a softer body and increasing melting properties were observed during early aging. These changes have been attributed to the solubilization of CCP rather than proteolysis action (Lucey et al., 2005; O'Mahony et al., 2005; Choi et al., 2008). As aging progresses however, the impact of proteolysis on cheese properties is becoming increasingly important (Kindstedt and Fox, 1993; Kindstedt et al, 1995). The relative contribution of the different proteolytic agents involved in CNs breakdown is still object to debate but certainly may be affected by the cheese-making technology used. Residual coagulant that is retained in cheese matrix and has not been denatured during the pasta filata process contributes to the proteolysis action during aging (Sheehan et al., 2004; Costabel et al., 2007; Bansal et al., 2009). The proteolytic and peptidolytic activities of starter bacteria and the milk-indigenous proteases such as plasmin have also been suggested as key proteolytic agents (Feeney et al., 2002; Ayyash and Shah, 2011). Mozzarella becomes softer, less fracturable and stickier with age which usually decreases its machinability (Kindstedt, 1995; Watkinson; Lucey, 2008). When heated, aged Mozzarella has lower viscosity and better flow, but can develops a 'soupy' texture if extended proteolysis occurred (Kindstedt, 1995; Sheehan et al., 2004). Stretching, oiling off and blister formation performances are usually increased during aging but may also become adversely affected if extended proteolysis occurs (Lucey, 2008). Changes of physical properties are caused by the partial breakdown of the *para*casein matrix into smaller peptides that lost their capacity to participate in cheese structuration and texture (Lucey et al., 2003). The extent of proteolysis during aging has been related to the amount of CCP that crosslinks the protein matrix and to the pH (Fox, 1970; Watkinson et al., 2001; Feeney et al., 2002; Zisu and Shah, 2005). Indeed, low CCP concentration would allow a greater accessibility of cleavage sites by the proteolytic agents. For all these reasons, aging conditions can be controlled in order to achieve desired attributes of ingredient Mozzarella. Machinability and heat-induced attributes are usually at their best during a relatively narrow window of aging time. Obviously, Mozzarella manufacturers seek to reduce the time before reaching adequate performance and maintain these optimal properties as long as possible.

Freezing shredded or diced Mozzarella is a convenient way to limit the physicochemical and biochemical reactions occurring during aging. Quick freezing methods are preferred because the crystallization of water into bigger ice crystals, that may damage the cheese structure, is reduced (Reid and Yan, 2004; Kuo and Gunasekaran, 2009). However, limited impacts of freezing (-20 °C) and storing Mozzarella aged between ~1 to 4 weeks on its microstructure and texture properties were reported (Bertola et al., 1996; Kuo and Gunasekaran, 2009).

2.5 Evaluation of Cheese Structure

2.5.1 Microscopy

Scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) have been used extensively for the visual examination of Mozzarella cheese microstructure. SEM provided high resolution topographical views of the protein fibers featuring in pasta filata cheese (Taneya et al., 1992; Oberg et al., 2003; Yu and Gunasekaran, 2005a). SEM was also used to evaluate structural impact of freezing (Kuo and Gunasekaran, 2009) and to visualize the swelling of the protein phase during aging (McMahon et al., 1999). However, the sample preparation requires many steps such as fixing, dehydrating, defatting, fracturing and coating which may induce artefacts. CLSM is increasingly used to investigate cheese microstructure, mostly because the fat suprastructure can be readily visualized and that the sample preparation is relatively simple. Auty et al. (2001) proposed different fluorescent probes in order to visualize the protein and fat phase changes during the manufacture of Mozzarella cheese. The internal cheese matrix can be scanned at various depths without structure damaging allowing a 3- dimensional analysis of microstructure (Ko and Gunasekaran, 2007; El-Bakry and Sheehan, 2014). CLSM protocols were also developed for the dynamic study of microstructure during large deformation fracture (Abhyankar et al., 2014) or melting (Auty et al., 1999). The fat size distribution in pasta filata cheese has been estimated via image analysis of micrographs (Colin-Cruz et al., 2012;

Ma et al., 2013). However, the representativeness of the area of interest and changes induced by the heat generated from the light source must be considered as possible bias.

2.5.2 Laser Light Scattering

The laser light scattering technique can be used to estimate the size distribution of various particles (>0.1 µm) or emulsified phase in food products (Pechak and Smith, 2007). Fat globules size was measured directly from diluted milk based on the work of Michalski et al. (2001) (Lopez et al., 2007; Michalski et al., 2007; Schenkel et al., 2013). However, the estimation of fat size distribution trapped in a gelled matrix (e.g., cheese) requires the dissociation of the protein phase and the stabilization of fat suprastructure originally present in the product. EDTA has been used as dissociating agent for process cheese (Ye and Hewitt, 2009) and urea was added for Camembert and Emmental cheeses (Michalski et al., 2003; Lopez et al., 2007). The addition of SDS has been suggested to prevent fat aggregation during laser light scattering measurement (Lopez et al., 2007). The laser light scattering can be a complementary technique to the visual assessment (i.e., microscopy) of fat suprastructure, but this method has never been used for pasta filata cheese varieties such as Mozzarella.

2.5.3 Dynamic Rheology

Rheology aims at measuring the properties of materials that control their flow and deformation behavior when subjected to external forces (Gunasekaran and Ak, 2002). Upon the application a force, an ideal solid (truly elastic material) will deform while storing the energy imparted whereas an ideal liquid (truly viscous material) will flow and dissipates the energy. Cheeses, including Mozzarella, typically display an intermediate rheological behavior, and therefore can be classified as a semi-soft viscoelastic material (Foegeding et al., 2011). The fundamental study of the cheese behavior in different condition of stress (a surface area-independent value related to a force), strain (a size-independent value related to deformation) or time allow the understanding of the strength (quantitative) and behavior (qualitative) of interactions that hold the cheese components together.

Small amplitude oscillatory shear (SAOS) is the primary rheological approach to investigate the cheese microstructure (Gunasekaran and Ak, 2002). The stress and strain amplitude at which the sample is subjected remains within the linear viscoelastic region

(LVR). In this region, the material displays a linear relationship between stress and strain which allows the characterisation of its viscoelastic behavior without any significant structural damage (Gunasekaran and Ak, 2002). Modern rheometers can either apply sinusoidal stress of constant amplitude (stress-controlled mode) or sinusoidal strain of constant amplitude (strain-controlled mode) (Läuger and Stettin, 2010). Hence, the time (*t*) function of a strain of constant amplitude (γ°) applied to a sample at a given oscillatory frequency (ω) would be:

Equation 2.1 Amplitude of strain as a time function

$$\gamma(t) = \gamma \circ \ \sin(\omega t)$$

and the stress (σ) response of the material is also sinusoidal but with an phase angle decay (δ) such as:

Equation 2.2 Amplitude of stress as a time function

$$\sigma(t) = \ \sigma \circ \sin(\omega t + \delta)$$

The torque response (in strain-controlled mode) or the angular displacement (in stress-controlled mode) is recorded during experimentation and the software converts the raw data into stress or strain according to the testing conditions (e.g., geometry and size of the measurement device, sample gap). From these data, the dynamic moduli G' and G'' can be calculated as follow:

Equation 2.3 Elastic modulus calculation

$$G'(\omega) = \frac{\sigma \circ}{\gamma \circ} \cos(\delta)$$

Equation 2.4 Viscous modulus calculation

$$G''(\omega) = \frac{\sigma \circ}{\gamma \circ} \sin(\delta)$$

where the G′ represents the apparent elastic component of the sample and is a measure of the stored energy in the sample from the imparted stress or strain, while the G″ represents the apparent viscous component of the sample and is a measure of the energy lost due to viscous dissipation (Gunasekaran and Ak, 2002). G′ and G″ are related to the magnitude of the complex modulus (G*) and the phase angle (δ) as follow:

Equation 2.5 Relationship between the viscoelastic moduli G*, G′ and G″

$$|G*| = \sqrt{G'^2 + G''^2}$$

Equation 2.6 Relationship between the viscoelastic moduli and the phase angle

$$\tan\delta(\omega) = \frac{G''(\omega)}{G'(\omega)}$$

The G* represent the overall response to the imputed stress or strain while the δ (varying from 0 to 90 °) is an indicator of the relative amount of the energy stored or dissipated. The material has a definite solid-like behavior when δ<<45° while a δ>>45° indicate a dominant liquid-like behavior. The material is equally solid and liquid at a δ=45° and thus this point is called the crossover modulus in reference to the changing of the material behavior when crossing this point (Gunasekaran and Ak, 2002).

In practice, dynamic rheological experiments are performed by holding all controlled rheological parameters (i.e., stress or strain, frequency, time, temperature) constant but one: the sweep parameter (Melito, 2012). The dynamic moduli (i.e., G′, G″, G* or δ) can be determined regardless of the type of sweep experiment performed. However, different insight on cheese properties can be obtained by choosing the appropriate sweep experiment (Gunasekaran and Ak, 2002).

Stress or strain sweep tests are typically used for the determination of the LVR limit of the cheese at a given frequency and temperature (Gunasekaran and Ak, 2002). The strain limit of LVR was determined for Mozzarella cheese by several authors (Subramanian and Gunasekaran, 1997; Muliawan and Hatzikiriakos, 2007), but one must carry its own experimentation in order to select conditions within the LVR. This is because the LVR range is dependent on many factors such as temperature and the intrinsic cheese properties (Gunasekaran and Ak, 2002).

The frequency sweep experiment allows the study of the mechanical spectrum of cheese: a representation of the viscoelastic moduli generally measured over three Logs of frequencies (Tunick, 2011). Hence, the response of the cheese structure to different observation time (typically for a complete sinusoidal strain or stress cycle) can be investigated. Lower frequencies correspond to longer observation time while higher frequencies correspond to short cycles. During the observation time of a cycle, interactions between particles within the sample may be formed or broken, either spontaneously or because of the imposed energy (Lucey et al., 2003; Tunick, 2011). These changes affect the cheese matrix structure and consequently its rheological properties. In past studies, the cheese mechanical spectrum was fitted to an exponential equation such as (Subramanian et al., 2003; Subramanian et al., 2006; Tunick, 2011):

Equation 2.7 Model of the mechanical spectrum of cheese

$$G' \text{ or } G''(\omega) = a\,\omega^{n}$$

where a is a constant and n is the frequency dependence index of the measured moduli. The frequency dependence index indicates the characteristic of the matrix network: a value near to zero indicates a strong (chemically) cross-linked structure with more covalent interactions; whereas increasingly positive values (but usually <1) indicate weaker structure with a greater proportion of non-covalent interactions (Lucey et al., 2003; Tunick, 2011).

Time sweep experiments are useful to assess the building-up or the breakdown of structure. As an example, this test can be used to characterize the evolution of the physical properties of renneted milk gel (Castillo, 2010). However, time sweep experiment have limited application for Mozzarella because the biochemical changes that impact cheese rheological properties usually occur at a slower rate than appropriate test duration (e.g., proteolysis during aging).

Temperature sweep experiments are very useful to assess the temperature dependence of the dynamic moduli. For Mozzarella cheese, the temperature of testing usually range between refrigerated to 60 °C or above (Ma et al., 2011; Vogt et al., 2015). The temperature is increased gradually while rheological properties are measured (Guinee et al., 1999; Tunick, 2010, Schenkel et al., 2014) but the temperature can also be controlled in a stepwise manner (Ma et al., 2011; Gunasekaran et al., 2002). Many interpretation of the temperature-moduli curve were proposed including the measurement of activation energy and the application of time-temperature superposition principle (Stenz et al., 2006; Udyarajan et al., 2007; Tunick, 2010).

2.6 Evaluation of Cheese Physical Properties

2.6.1 Properties of Unmelted Cheese

The ability of cheese to be sliced, shredded or diced is intimately related to its rheological properties. Kindstedt (1995) observed that cheese with soft or pasty body cannot withstand the shredding process because it tends to stick and spread on the cutting blade. However, firm body and brittle cheese may impair the shreds integrity and lead to extensive fines production. In addition to the SAOS rheological tests discussed in the previous section, others methods can be used to assess unmelted cheese physical properties and therefore potentially used for the prediction of cheese machinability.

Creep-recovery testing is another fundamental and non-destructive methods (measured within the LVR range) to evaluate the viscoelastic behavior of cheeses (Olivares et al, 2009). First, the sample is loaded with a constant and instantaneous stress for a set period of time, then the stress is removed and the cheese structure is allowed to relax and recover from deformation. The strain experienced by the cheese is measured as function of time and the data can be interpreted using mechanical models with meaningful viscoelastic parameters (Gunasekaran and Ak, 2002; Subramanian et al., 2003). The relevance of viscoelastic modulus (from creep or SOAS data) to predict the cheese machinability was investigated by Childs et al. (2007). In that study, the creep data measured were only poorly correlated to cheese shreddability; possibly because the fundamental mechanics of cutting intuitively involves fracture, large deformation and surface friction properties of cheese (Goh et al., 2005).

The large-strain rheological behavior of cheese is usually tested using compressive or torsional methods. For compressive methods, a uniaxial force is imparted to a cylindrical shaped sample which induces its deformation. Depending on the objective of testing, the sample is allowed to fracture or not upon the stress imparted (Charalambides et al., 2001). This method is frequently used in the dairy processing industry because the sample preparation and test protocol are simple. Based on that principle, the texture profile analysis (TPA), a two compressive test, can be used to characterize the cheese with an array of textural descriptors (Bourne, 1978). Some of them, such as hardness, brittleness and adhesiveness, have been found to be partly related to the cheese shred quality (Chen, 2003; Childs et al., 2007; Lucey, 2008). Some of the disadvantages of the compression methods include: the uneven strain gradient imposed throughout the sample, the poor control of sample temperature, and the lack of physical basis of some TPA parameters (Gunasekaran and Ak, 2002; Brenner and Nishinari, 2014). Moreover, the use of the compression method to assess facture behavior has been questioned (Truong and Daubert, 2000).

Kamyab et al. (1998) proposed to study the fracture properties of cheese by the wire cutting method. The cutting force was found to be linearly related to the diameter of the cutting wire. From these data, the fracture toughness, the energy per unit area necessary to create new cracked surface, can be calculated. The wire cutting method allows simple test preparation with consistent results when compared with other fracture methods such as the notched bending test and uniaxial compression (Kamyab et al., 1998). Other large-strain facture methods include the vane test and the tortional fracture of a capstan shaped sample (Luyten et al., 1992; Truong and Daubert 2001; Truong et al., 2002; Patarin et al., 2014). For the last method, preparation can be time-consuming and the sample must have particular properties to withstand the sample preparation process (Truong and Daubert, 2000). One drawback of all large-strain methods discussed above is that the results are dependent of the strain rate applied during testing because the cheese properties are measured outside of the LVR limit. Hence, a comprehensive understanding of the stain-stress relationship measured using large-strain methods would requires testing under several strain rate conditions (Goh et al., 2003). Nevertheless, large-strain tests provide additional information on the cheese behavior that is not measured by small-strain tests (Foegeding et al., 2011). This is illustrated in Figure 2.5 as the stress-strain relationship of two different products may diverge when measured outside the LVR.

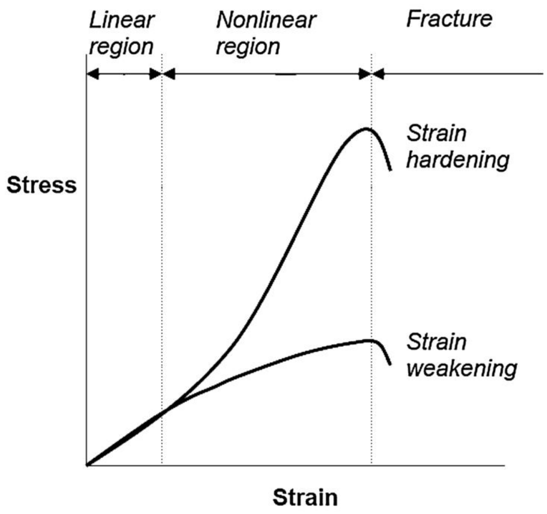

Figure 2.5 **Stress-strain curves of materials exhibiting strain-hardening or strain-weakening behaviors outside the nonlinear viscoelastic region. Source: Foegeding et al., 2011.**

Recently, there was a growing interest on measuring and interpreting large-strain behavior of cheese with reliable and consistent methods that provide meaningful rheological parameters (Melito and Daubert, 2011). The large-strain oscillatory shear (LAOS) protocol of Ewoldt et al. (2008) was validated for food systems (Melito et al., 2012) and applied to natural cheeses (Melito et al., 2013). Moreover, the application of mechanistic models on large-strain data from large-stress creep measurements was investigated (Barrangou et al., 2006; Melito et al., 2013; De Souza Mendes and Thompson, 2013). Applications of fundamental studies about large-strain behavior in cheese system remain limited to this day. Other techniques used in the material sciences field, such as the multiple stress creep recovery (MSCR) test has the potential to bring significant insights to the large-strain rheological behavior of material, but have not yet been applied to food products (ASTM, 2010; Delgadillo et al., 2012; Shirodkar et al., 2012).

2.6.2 Properties of Melted Cheese

2.6.2.1 Methods based on Empirical or Semi-Empirical Approach

Most cheese manufacturers evaluate the properties of heated Mozzarella cheese using highly empirical methods. As a large share of the Mozzarella production is destined to pizza restaurant chains, the quality of the product is generally evaluated after a pizza baking test under conditions (e.g., type of oven, temperature, time, type and amount of ingredients) that match the end user demands (Chen et al., 2009). Then, the heat induced characteristics of cheeses are evaluated, usually rather subjectively, and compared to a set of standards. Some common evaluation criteria's are:

(1) visual aspect:
  - Degree of shred fusion, coverage of melted cheese, opacity, color, size and coverage of blisters, presence of free oil.

(2) Stretching characteristics (evaluated by pulling a cheese strand with a fork):
  - Length and thickness of the pulled strand, force to stretch.

(3) Taste and texture of the cheese:
  - Global appreciation of the flavor, chewiness (ease of chewing to prepare a cheese piece to be swallowed), extend of liquid release when chewing.

As much as the baking tests have the advantage of providing direct assessment on how the product will behave on a pizza, it also suffers from serious disadvantages. Indeed, the consistency of the results can be fairly low as the evaluation criteria only relies on the perception of one or, at best, few assessors. Additionally, the properties of cheese are difficult to evaluate objectively because the conditions of testing involve numerous factors that are not strictly controlled. Hence, the development of simple, reliable and relevant methods for the evaluation physical properties of heated cheese has been the focus of many researchers for the past few decades.

Empirical methods related to the Schreiber test are a simple way to determine the melting behavior of a cheese sample. In the case of the Schreiber test, a cheese disk is heated in a Petri dish at baking temperature for a set period of time. The melted cheese surface area is measured and reported to the original cheese surface in order to calculate a meltability index (Cais-Sokilinska and Pikul, 2009). In fact, this method mostly describes the flowing behavior of cheese during baking. The results obtained are dependent of the shape and size of the sample, the melting temperature and time, and the type of heating used (convection; conduction; microwave) (Gunasekaran and Ak, 2002). Many variations were proposed for these tests such as covering the sample during melting, various melting containers or baking temperatures, and the use of a computer vision technology to perform data analysis (Muthukamarappan et al., 1999; Wang and Sun, 2002a; 2002b; 2004; Altan et al., 2005).

Nevertheless, this method and its modified versions suffer from a great experimental variability (Stenz et al., 2006).

From a mechanical point of view, the ease of flow is related to the external stress applied (e.g., for melted cheese: gravity or pulling a strand with a fork). Kindstedt et al., (1989) proposed to measure the apparent viscosity of melted Mozzarella using a T-bar spindle coupled to a Brookfield viscometer. Because of the helical motion of the probe, the phase separation (liquid fat or serum) at the cheese-probe interface was avoided during measurement. However, the melt must be complete and homogeneous which can be questioned for young Mozzarella cheese (Savage and Mullan, 2000). Many methods were also developed to evaluate the stretching properties of the melted cheese (Guinee and O'Callaghan, 1997; Fife et al., 2002; Hicsasmaz et al., 2004). Most of them measure the force required to pull a strand of cheese and the distance at which the strand breaks. To avoid the cooling or drying of the strand during testing, Ma et al. (2012) immersed the testing device into a temperature-controlled oil bath. Different parameters can be interpreted from the force-displacement curve such as the yield load, the unstable deformation gradient and the inversion point (Fife et al., 2002; Ma et al., 2012).

2.6.2.2 Methods based on a Fundamental Approach

The flowing behavior of melt can also be assessed by lubricated squeeze flow rheometry which imposes biaxial deformation that well represents the melting phenomenon (Gunasekaran and Ak, 2002). From this principle, a team from the University of Wisconsin-Madison developed an apparatus, the UW Meltmeter, which limits the temperature gradient within the sample and the loss of moisture during testing (Wang et al., 1998). When a constant stress is imposed instead of a deformation, the method (Meltprofiler) can be considered as a creep test from which viscoelasticity indexes can be calculated (Kuo et al., 2000). Moreover, the softening point and the flowing rate can be calculated from the resulting melting profile (Ko and Gunasekaran, 2008; 2014). Despite the evident advantages of this approach, it is not widely used by other research groups.

In contrast to the previous approaches, the temperature sweep test performed under SAOS conditions has been used extensively to measure the melted cheese properties. Usually, the viscoelastic moduli (i.e., G', G'', G^*, or δ) are reported at different heating temperatures, allowing the representation of a 'melting profile'. However, the rheological behavior of cheese during cooling was only reported in a few studies (Guggisberg et al., 2007; Udyarajan et al., 2007). From the data obtained, one can evaluate both the softening of the cheese matrix (i.e., decrease of G′, G″, and G*) and its evolution toward a more liquid-like material (i.e., increase of δ) as the temperature increase (Guinee and al., 1999; Lucey et al., 2003). The cross over point (i.e., temperature at which G′=G″) is used to identify the gel melting transition (Gunasekaran and Ak, 2000; Guggisberg et al., 2007), but the relevancy of this parameter to assess a dynamic process such as the melting of cheese can be questioned. Mechanistic models were also proposed to interpret the data from the temperature sweep experiment (Subramanian and Gunasekaran, 1997; Muliawan and Hatzikiriakos, 2007). One of the key limitations about the SAOS parameters is the poor information available regarding their relationship with the baking behavior in 'real life' conditions. Nevertheless, Stenz et al. (2006) previously reported satisfactory correlation ($r > 0.8$) between some SAOS parameters and Schreiber test results, but more research is needed to better understand the relations between SAOS data and baked cheese attributes (Reparet and Noël, 2003).

The studies reporting the non-linear viscoelasticity of melted cheese are scarce. The early works from Tariq et al. (1998) and Wang et al. (2001) evaluated the impact of temperature (up to 60 °C) on Mozzarella properties at different strain amplitudes using a sliding plate rheometer, but data interpretation was limited due to the lack of appropriate constitutive model at the time. Recent works from Muliawan and Hatzikiriakos (2008) and Bähler and Hinrichs (2013) used a capillary rheometer in order to evaluate viscoelastic properties of Mozzarella cheese in conditions that simulated the pasta filata process. Others used a mixing unit coupled with a transducer to measure the torque necessary for the development of imitation cheese mass at elevated temperature (Noronha et al., 2008; Solar et al., 2009; El-Bakry et al., 2010; Inayat et al., 2014). However, the shearing field, or how the shear stress is locally imposed to the cheese, was not easily acknowledgeable using this last approach (Solar et al., 2009). A better understanding the rheological behavior of cheese under large deformation is required since melted cheese is subjected to many processes that impart deformation well over its LVR limit (e.g., pasta filata process, stretching and mastication).

2.7 Evaluation of Sensory texture of Cheese

Food texture was defined by Lawless and Heymann (1998) as all the rheological and structural attributes perceptible by mean of mechanical, tactile, and where appropriate, visual and auditory receptors. The ultimate measurement of food texture is the sensory perception experienced by human (Foegeding et al., 2011). However, the human sensory response to external stimulus is very complex and is sometimes difficult to express in simple sensory terms or quantified in intensity. Recent studies involving multidisciplinary expertise such as psychophysics, food rheology and oral processing science have brought significant contributions to the understanding of sensory perception of texture (Foegeding et al., 2011; Chen and Stokes, 2012; Pascua et al., 2013). Depending on the final objectives, sensory analysis can be used for collecting like or dislike appreciation (consumer panel), analytical and objective measurements of sensory attribute intensity (descriptive sensory analysis), or detect difference and compare samples (e.g., triangular test, grading, competition) (Drake, 2009). A consumer panel session usually require between 50 and 100 panelists, while around 8 to 10 panelists trained for at least 50 h is recommended for descriptive sensory analysis (Heymann et al., 2012). Descriptive sensory analysis methodology should include control mechanisms for the environmental, physiological and psychological conditions during evaluation, the development of sensory language adapted to the product evaluated and the training of the panellist to assess the intensity of these sensory terms based on a standard references. Relevant sensory lexicon for the texture evaluation of unmelted cheese has been suggested (Truong et al., 2002; Brown et al., 2003; Pascua et al., 2013). The quality of shredded or sliced cheese quality can be evaluated visually (Apostolopoulos and Marshall, 1994; Chen, 2003; Perrie, 2012) and by hand or mouth sensory terms (Chen, 2003). Baking characteristics of low-fat Mozzarella (e.g., blister formation, color) were assessed visually (Rudan and Barbano, 1998; Zisu and Shah, 2007; Wadhwani and al., 2011), but without any evaluation of mouth sensory texture. Recently, Chen et al. (2009) proposed a specific sensory lexicon for the mouth texture evaluation of baked cheese. To the best of knowledge however, no formal descriptive sensory analysis of baked cheese texture has been published to this day.

2.8 Relationships between Factors affecting Cheese Characteristics

An overview of the relationships between the main factors that affect the characteristics of Mozzarella cheese is depicted in Figure 2.6.

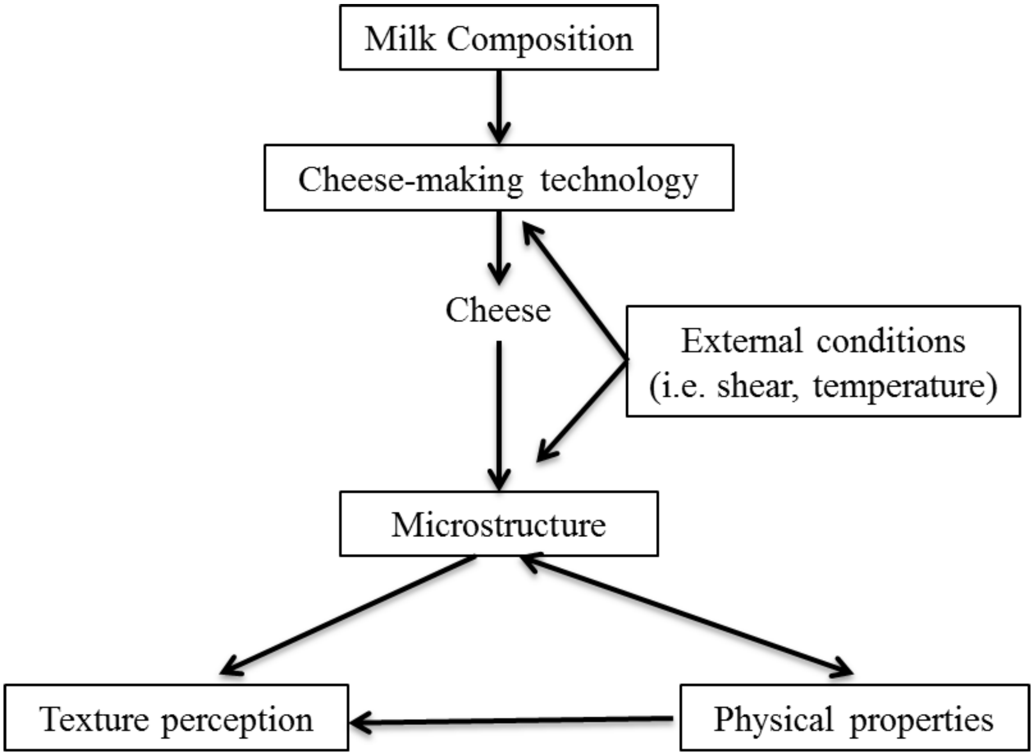

***Figure 2.6* Scheme of the relationships between factors affecting Mozzarella characteristics.**

The production of quality Mozzarella cheese is strongly dependent of the cheese milk constituent and on the cheese-making technology used, including aging conditions. Indeed, the cheese components and how they are arranged together determines the cheese's microstructure. Despite the fact that many studies investigated the effect of manufacturing conditions on cheese composition and structure, the dairy industry does not usually adapt their cheese-making strategies in order to develop cheeses with tailored microstructure. In the case of Mozzarella cheese, this approach would require a deeper understanding of the impact of the manufacturing conditions such as those used during the pasta filata process.

Intuitively, the microstructure has, in turn, a primary impact on the texture perception and the physical properties of food systems (Lilliford, 2011). As mentioned previously, pasta filata cheeses have a peculiar microstructure due to the thermo-mechanical treatment imposed. The water and fat distribution in the cheese matrix, in addition to the fibrous arrangement of *para*casein network has practical consequences on cheese physical properties. One of the consequences is that Mozzarella can exhibit a strong anisotropic behavior when texture measurements are performed either perpendicularly or parallel to the oriented microstructure (Bast et al., 2015). The impact of different fat suprastructures on stretching, elasticity and oiling off was demonstrated on Emmental and Mozzarella cheeses (Rowney et al., 2003; Michalski et al., 2007). Moreover, Mosca et al. (2012) showed that the spatial distribution of fat in gel food models affected the texture perception. Recent works reviewed the impact of structure modifications on the mastication behavior and on the texture perception, but specific works on cheese are scarce (Koç et al., 2013; Pascua et al., 2013, Boisard et al., 2014).

External conditions such as increasing temperature, high shear or pressure also influence cheese characteristics. The impact of heating Mozzarella on microstructure and rheological properties were studied extensively (Paquet and Kaláb, 1988; Lucey et al., 2003; Vogt et al., 2015), but the global impact on consumer perception is not fully understood.

Studies of food models with different physical properties resulted in many adaptations of chewing mechanisms and important impacts on texture perception (Foegeding and Drake, 2007; Çakir et al., 2012a; Chen and Stokes, 2012; Yven et al., 2012). The relationships between cheese physical properties measured by rheological parameters and the sensory texture have long been of interest for researchers and the cheese industry. Indeed, correlations between mechanical tests and sensory perception would reduce the need of the timely and costly sensory evaluation analysis. Many studies investigated these relations for unmelted cheeses (Drake and al., 1999, Brown et al., 2003; Everard et al., 2005). It is noticed from these work that small deformation tests usually correlated less with sensory perception than large deformation tests. In addition, discrepancies exist between the measurement of mechanical properties and the complex textural sensation experienced by the consumer (Foegeding and Drake, 2007). This may lead to oversimplified relationships that are often meaningless. This may be the case with some TPA test parameters, or when establishing a relationship between an empirical fracturing method and the rather complex chewiness sensory term (Metzger and Barbano, 1999, Brenner and Nishinari, 2014). Hence, the development of new large deformation tests to understand the physical properties of cheese in the conditions found during mastication represents a promising avenue (Melito et al., 2013). Furthermore, the democratization of advanced statistical tools such as multivariate analysis allows the complex relations between the factors affecting cheese characteristics to be evaluated for the best interest of a deeper understanding (Drake et al., 1999; Ma et al., 2013a; Schenkel et al., 2014).

Chapter 3 Hypothesis, goal and objectives

Mozzarella cheese properties and manufacturing conditions have been the subject of more than 200 scientific research and publications over the last few decades. A great number of them proposed to establish a relationship between cheese characteristics (e.g., moisture, fat, calcium or proteolysis) and its physicochemical behavior when subjected to heat (e.g., melting, stretchability, free oil formation, browning). These studies provided clear evidences that Mozzarella cheese is subjected to profound compositional, microstructural and textural changes during heating. However, very few of these studies envisaged the consequences of these changes on the physical properties of cheese after the heat treatment. This understanding would be insightful since two post-heating situations occur frequently during the life-span of most Mozzarella cheese: after the pasta filata process and after baking. In between these two events, ingredient cheese is almost systematically subjected to a size reduction process in order to facilitate its handling and preparation. The studies interested on the factors that impact the cheese machinability have been scarce, and so are the approaches for determining the adequate window of textural properties required for an efficient size-reduction process. This understanding is required in order to optimise Mozzarella cheese properties when used as an ingredient for different menu applications.

3.1 Hypothesis

The key attributes of Mozzarella cheese required for its uses as ingredient food are primarily governed by its composition, microstructure and physical properties, and these properties can be modulated using simple and common cheese-making strategies.

3.2 Goal

The goal of this doctoral research was to develop and evaluate different methodological approaches in order to better understand, control and eventually predict the impact of some manufacturing practices on Mozzarella cheese properties.

3.3 Objectives

1. Determine the compositional, microstructural and rheological changes caused by the pasta filata process: a critical step of Mozzarella cheese manufacturing.

2. Measure the impact of addition of denatured whey protein, pH at milk renneting, aging and cheese temperature on the machinability of Mozzarella cheese.

3. Predict the machinability of Mozzarella cheese using compositional and textural descriptors.

4. Understand the relationship between the cheese physical properties and sensory perception caused by the baking of Mozzarella cheese.

Chapter 4 Impact of thermo-mechanical treatments on composition, microstructure and rheological properties of pasta filata–type cheese

4.1 Résumé

Cette étude vise à comprendre l'impact global du traitement thermomécanique appliqué lors de la fabrication de fromage pasta filata. Le caillé de fromage a été traité mécaniquement à différentes températures (60, 65, ou 70 °C), durées (3 ou 6 min) et vitesses (6, 9, ou 12 rpm) dans une unité mesurant le couple nécessaire au traitement. La température finale du caillé ainsi que l'énergie mécanique spécifique et la charge thermique requises pendant le traitement ont été reliées aux propriétés du fromage. Les différents traitements appliqués ont provoqué d'importants changements à la composition des fromages et notamment les pertes de gras qui étaient positivement reliées à l'énergie mécanique fournie ($R^2 = 0.92$). Des photographies obtenues en microscopie confocale et en microscopie électronique montrent un impact des traitements sur la microstructure de la phase protéique, grasse et aqueuse. Les caillés ayant subi une charge thermique et une température plus élevées avaient une quantité plus importante d'eau libre emprisonnée dans la matrice fromagère ($R^2 = 0.60$). Les traitements étudiés ont eu un très faible impact global sur les propriétés rhéologiques des fromages. Cette étude permet de mieux comprendre l'impact de la cuisson-extrusion sur les fromages de type pasta filata ouvrant ainsi la porte à une optimisation rationnelle des paramètres de contrôle de ce procédé.

4.2 Abstract

The impact of thermo-mechanical treatments imparted during the pasta filata process was investigated. Curd was mixed at different temperatures (60, 65, or 70 °C), for different lengths of time (3 or 6 min), and at different speeds (6, 9, or 12 rpm) in a torque rheometer. Specific mechanical energy, final curd temperature, and heat load index were measured to predict cheese properties. Considerable changes in cheese composition were observed depending on the mixing treatment, and fat loss was related to the mechanical energy imparted ($R^2 = 0.92$). Microstructural changes in fat were considerable in mixed cheese, as observed by scanning electron microscopy and confocal laser scanning microscopy. Bulk water entrapped in the cheese structure increased with curd temperature and heat load ($R^2 = 0.60$) but was not affected by the mechanical energy imparted. Only slight differences in the elastic behaviour of cheese were found. Understanding the impact of thermo-mechanical treatments is essential in order to optimize in-process controls during the manufacture of pasta filata cheese.

4.3 Introduction

Low-moisture Mozzarella is undoubtedly the most popular pasta filata cheese variety in North America, where it is in increasing demand as an ingredient for various menu applications. During the pasta filata process, the curd is gradually warmed to about 50 to 65 °C in hot brine while being physically kneaded in an auger system. Hence, both thermal energy and mechanical energy are required to transform the curd into a heterogeneous but continuous cheese mass, which is then allowed to cool after moulding. The final product is characterized by the organization of *para*casein aggregates into roughly parallel fibres interspersed with elongated fat and serum channels. The singular ability of Mozzarella cheese to melt, stretch, and release free oil during baking has been attributed in part to the curd plasticization that occurs during the pasta filata process (Kindstedt and Guo, 1997).

Systematic studies investigating the impact of the pasta filata process on cheese properties are scarce. A wide array of industrial pasta filata processing equipment is used by cheese manufacturers: batch or continuous process, and single- or twin-auger systems designed with different materials, flight effects, barrel geometries and heating systems. Moreover, in-process control parameters such as barrel temperature, curd feeding rate, and auger speed can be adjusted. Consequently, the thermo-mechanical treatments imposed may differ considerably.

The impacts of varying the process control parameters during the stretching step were reported in some studies. Increasing auger speed and barrel temperature in a twin-screw cooker-stretcher system resulted in a general decrease in cheese moisture and fat-in-dry matter (Renda et al., 1997; Yu and Gunasekaran, 2005a). Stretching conditions have been shown to affect the cheese microstructure, as observed in confocal laser scanning micrographs (Rowney et al., 2003; Ma et al., 2013a) and scanning electron micrographs (Yu and Gunasekaran, 2005a). Recently, Bähler and Hinrichs (2013) used capillary rheometer to investigate the impact of temperature on the physical properties of the curd during simulations of the pasta filata process. The impact of the process control parameters on the physical properties of cheese after the process remains to be clarified, however. Yu and Gunasekaran (2005a) and Mulvaney et al. (1997) measured greater elasticity in cheese with higher thermal and mechanical treatments, but no significant differences were observed in other studies (Renda et al., 1997; Ma et al., 2013a). Varying the control parameters in traditional cooker-stretcher systems may lead to confounding effects. Indeed, increasing auger speed to achieve greater mechanical working also reduces the curd's residence time in the cooker-stretcher and therefore also decreases the curd's final temperature. Hence, the measurement of system variables such as specific mechanical energy (SME) or final curd temperature seems more appropriate to characterize the pasta filata process, and those variables were suggested as scale-up parameters (Mulvaney et al., 1997; Yu and Gunasekaran, 2005b). Altogether, a better understanding of the impact of thermo-mechanical processes on cheese characteristics is needed in order to be able to produce pasta filata cheese with customized properties.

The primary objective of the present study was to investigate the effect of the independent control of mixing conditions (i.e., temperature, time, and speed) on the composition, microstructure, and rheological properties of cheese. A simple system model using a mixing torque rheometer was developed for the application of the thermo-mechanical treatments in controlled conditions. The secondary objective of this study was to investigate the relationship between cheese characteristics and the mechanical or thermal energy imparted during the process through the measurement of system variables.

4.4 Material and Methods

4.4.1 Curd Production

Low-moisture Mozzarella curd was produced in the pilot plant at the Agropur Research and Development Center (Saint-Hubert, QC, Canada). Fresh raw milk was standardized to a fat-to-protein ratio of 0.71 and pasteurized at 74 °C for 16 s. Cheese milk (300 kg) was cooled to about 33 °C and supplemented with calcium chloride (0.005% [w/w]) and inoculated with direct-in-vat thermophilic culture (0.008% [w/w], Easy-Set i482; Chr. Hansen, Milwaukee, WI). Coagulant (0.0045% [v/w], CHY-MAX® M 1000; Chr. Hansen) was added after 35 min of ripening, and the gel was allowed to set for 30 min. Cutting pH was 6.53. Then, the coagulum was cut and healed for 5 min before the temperature was raised (0.2 °C min^{-1}) to 39 °C under stirring. The whey was drained at pH 6.15, and the curd was cheddared to pH 5.25. The curd was then rapidly cooled to about 20 °C, milled and dry-salted (1.5% [w/w]). Random samples of cheese curd (500 g) were vacuum-sealed in a single layer occupying the maximum surface area (VAK*3.0 film; Winpak Ltd., Winnipeg, MB, Canada). Finally, the samples were quickly frozen (−20 °C) until the application of a thermo-mechanical treatment. Three independent batches of curd were produced.

4.4.2 Thermo-Mechanical Treatment

A model system was developed to simulate thermo-mechanical conditions imposed during the pasta filata process. The conditions tested were combinations of (1) mixing temperature (Tp_m; 60, 65, or 70 °C), (2) mixing time (Ti_m; 3, or 6 min), and (3) mixing speed (Sp_m; 6, 9, or 12 rpm). Mixing was performed in a Farinograph-E system (C.W. Brabender GmbH & Co., Duisburg, Germany) equipped with a Sigma mixing unit (model S 300; C.W. Brabender GmbH & Co.). An external water bath was used to control the temperature of the mixing unit jacket. The mixing torque was recorded during experimentation by the equipment software, and continuous temperature readings were provided by a probe (Oakton Temp-300 datalogger; Cole-Parmer, Montreal, QC, Canada) installed in the mixing unit.

Samples were prepared by heating the curd in a water bath maintained at 58 °C for 25 min. The curd reached about 56 °C and was immediately transferred into the mixing bowl, which contained 500 g of stretching brine (3% [w/w] NaCl, 500 ppm of calcium; pH 5.2) preheated at the experiment temperature. After treatment, the mixed curd was separated from the stretching brine, moulded in a stainless steel casing (12 × 12 × 6 cm), and pressed lightly (16 kPa) for 30 min at room temperature. The resulting formed cheese blocks were sealed and kept at 4 °C for about 24 h before testing.

4.4.3 Cheese Composition Analysis

Moisture, nitrogen, and fat contents were determined by the forced-air oven-drying, macro-Kjeldahl, and Mojonnier methods, respectively (Wehr and Frank, 2004). Protein content was estimated using a nitrogen conversion factor of 6.38. Other solids in the cheeses, mainly soluble organic molecules and minerals, were calculated by subtracting the protein and fat contents from the total solids. To assess the effect of mixing on solids loss, the loss coefficient (L) of the solid species n was calculated as follows:

Equation 4.1 Solid loss calculation

$$L_n = \frac{M_{curd} \times [n]_{curd} - M_{cheese} \times [n]_{cheese}}{M_{curd} \times [n]_{curd}}$$

where M is the mass of curd (M_{curd}) or cheese (M_{cheese}), and $[n]$ is the concentration of the solid species in curd ($[n]_{curd}$) or cheese ($[n]_{cheese}$) (% [w/w]). The sodium chloride content was determined using a Sherwood model 926 chloride analyzer (Sherwood Scientific Ltd., Cambridge, UK), and the pH was measured with a Symphony SP70P pH meter (VWR, Mississauga, ON, Canada).

The mobility of the aqueous phase in the cheese matrix was evaluated by the amount of expressible serum (ES) obtained by centrifugation (12 500*g* / 30 min.) of 50 g of freshly shredded cheese, as described by Kindstedt and Guo (1997). Non-expressible serum (NES; unit: g 100 g^{-1} cheese) represents water that is tightly bound to the cheese matrix and was calculated by difference with ES (unit: g 100 g^{-1} cheese), which represents the aqueous fraction that interacts poorly with the cheese matrix. Expressible serum was measured on the cheeses after storage at 4 °C for 1, 2, 3, 5, and 8 d.

4.4.4 Microstructural Analysis

4.4.4.1 Scanning Electron Microscopy (SEM)

In a cold room (4 °C), sample pieces of cheese (3 × 3 × 7 mm) were cut, transferred into 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2), and kept at 4 °C overnight. The samples were dehydrated in a graded ethanol series (30%, 50%, 70%, 80%, 90%, 95%, and 100% twice) for 15 min per step, and then defatted with chloroform (3 × 15 min) and transferred back to absolute ethanol. The samples were freeze-fractured in liquid nitrogen; some of the samples fractured spontaneously. Pieces of the fractured samples were thawed in absolute ethanol and dried using a critical point dryer (Biodynamic Research Corp., Rockville, MD, USA), and then they were mounted on double-sided carbon tape stacked on aluminum stubs and were coated with gold (7 nm) using a Speedivac 12E6/1258 sputter coater (Edwards High Vacuum Ltd., Crawley, UK). The cheese samples were then observed and imaged with scanning electron microscopy (SEM) using a Quanta 600 SEM microscope (FEI Company, Brno, Czech Republic) at accelerating voltages of 5 or 20 kV depending on the magnification.

4.4.4.2 Confocal Laser Scanning Microscopy (CLSM)

Sample pieces of cheese were cut as described above (section 4.4.4.1) and transferred into 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) until microscopy observations. Immediately before microscopy, the pieces of cheese were frozen on a cold stub with Tissue-Tek O.C.T. Compound (Sakura Finetek, Torrance, CA, USA) in a precooled (−16 °C) Reichert-Jung Cryocut E cryotome (Leica Microsystems, Vienna, Austria). Thin slices (14 μm) were cut and mounted on slides in an aqueous stain mixture containing 0.0025% Nile red, 0.075% Fast Green FCF, and 25% polyethylene glycol 400. During preparation, special care was taken to keep the samples, stain mixture, tools, and slides cold (< 4 °C) at all times to limit microstructural changes, especially in fat globules. Confocal laser scanning microscopy (CLSM) observations of fat and protein were performed with an LSM 510 Duo microscope (Carl Zeiss MicroImaging Inc., Göttingen, Germany) using an Alpha-Plan Apochromat 63× objective (numerical aperture of 1.46), with excitation at 532 and 633 nm and emission at 575 to 750 nm and 650 to 750 for Nile red and Fast Green FCF, respectively. The imaging was performed with ZEN 2009 software (Carl Zeiss MicroImaging Inc.) and shows the cheese fat globules in green and protein in red.

4.4.4.3 Laser Light Scattering Measurement

Fat globule size distributions in curd and mixed cheese were measured by laser light scattering, as described by Lopez et al. (2007) with some modifications. Briefly, 2.5 g of diced cheese (3-mm edges) was prepared in a cool room (4 °C), and then gently stirred for 2 h (25 °C) in 25 mL of dissociating buffer (6 M urea, 0.1 M ethylenediaminetetraacetic acid [EDTA], 35 mM sodium dodecyl sulphate [SDS], and 20 mM imidazole buffer; pH 6.6). The urea and EDTA disrupted the casein network, which allowed the release of fat globules. The SDS stabilized individual globules and dissociated milk fat globule aggregates. A Mastersizer 3000 particle size analyzer (Malvern Instruments Ltd., Malvern, UK) was used to estimates the milk fat globule sizes with a refractive index of 1.460 at 466 nm. The average volume-weighted diameter, D[4,3], was calculated by the instrument software using the following equation:

Equation 4.2 Average volume-weighted diameter calculation of fat

$$D[4,3] = \frac{\sum n_i \times d_i^4}{\sum n_i \times d_i^3}$$

where n_i is the number of fat globules found in size class diameter d_i. Analyses were performed in duplicates.

4.4.5 Rheological analysis

Small-amplitude oscillatory measurements were performed at 10 °C using a Haake MARS II rheometer (Thermo Fischer Scientific, Newington, NH) equipped with serrated parallel plates (PP35 Ti LS, 35-mm diameter; Thermo-Fisher Scientific) and a Peltier temperature controller (UTC DC30; Thermo Fisher Scientific). A cheese disc (35-mm diameter, 2.5-mm height) was prepared and loaded under a normal force of 1 N for 5 min to allow temperature equilibration and sample relaxation. The analysis was performed at 0.1% strain under which the cheese properties remained within the linear viscoelastic region. The elastic modulus (G′) was recorded during a frequency sweep experiment, and the data were fitted to a power law model as follows:

Equation 4.3 Mechanical spectrum of the elastic component in cheese

$$G' = G'_1\, \omega^{G'_{fd}}$$

where ω is the frequency (Hz), and G'_1 is the elastic modulus measured at 1 Hz. The G'_{fd} is the power index, which represents the frequency dependence of the elastic modulus between 1 and 30 Hz (Tunick, 2011).

4.4.6 System Analysis

The thermo-mechanical treatment imposed during mixing was characterized by the measurement of system variables: (1) the specific mechanical energy (SME; unit: kJ kg^{-1})

imparted during mixing, (2) the final curd temperature (T_f; unit: °C), and (3) the heat load (HL; unit: °C min), which is an index created to account for the duration and intensity of the thermal treatment. The SME was calculated according to Auger et al. (2008) as:

Equation 4.4 Specific mechanical energy calculation

$$\mathrm{SME} = \frac{2\pi N}{M\,60}\int_{t_i}^{t_f} \tau(t)\,\mathrm{d}t$$

and the HL was calculated as:

Equation 4.5 Heat load calculation

$$\mathrm{HL} = \int_{t_i}^{t_f} T(t)\,\mathrm{d}t$$

where N is the mixing speed (rpm); M is the curd mass (kg); $\tau(t)$ is the torque (N m) measured at time t between the final (t_f) and initial (t_i) mixing time (s); and $T(t)$ is the temperature (°C) at time t between the final (t_f) and initial (t_i) mixing time (min).

4.4.7 Statistical Analysis

A randomized complete factorial design was used to determine the effect of the temperature, time, and speed of mixing and their interaction on cheese characteristics. The SAS software program (version 9.3; SAS Institute Inc., Cary, NC) was used to process the variance analysis with PROC MIXED and to perform regressions with PROC REG. The experiments were replicated in three different trials. Effects were declared significant when the probability level of F was $P \leq 0.01$.

4.5 Results

4.5.1 Impact of Mixing Conditions on Cheese Composition

As expected, the thermo-mechanical treatments imposed by different mixing conditions influenced the composition of cheeses compared with the initial curd composition (Δ%). These changes are presented in Figure 4.1. The three cheese-making production resulted in curds of similar composition ($P > 0.05$): moisture, 46.0% ± 0.3% (w/w); protein, 26.5% ±

0.4% (w/w); fat, 19.1% ± 0.1% (w/w); salt-to-moisture, 3.0% ± 0.1% (w/w); and pH 5.2 (Figure 4.1a). Expressible serum represented 7.3% ± 0.7% (w/w) of the curd weight, whereas the rest of the moisture content was defined as NES.

The effect of the main thermo-mechanical treatments (Tp_m, Ti_m, and Sp_m) are presented in Figure 4.1b, 4.1c, and 4.1d, respectively, and the significant interaction effect of Ti_m and Sp_m on cheese protein and fat is shown in Figure 4.1e. The average total moisture of mixed cheeses was fairly similar to that of the initial curd (Δ% = +0.36), but the mixed cheeses generally retained more serum and thus had less ES than the initial curd did. However, the amount of NES and ES depended on the mixing conditions (Figure 4.1b, 4.1c and 4.1d). The amount of ES increased when Tp_m increased, whereas the amount of NES remained fairly similar (Figure 4.1b), thus indicating that free water was retained within the protein matrix at a higher Tp_m. Doubling the Ti_m increased the Δ% ES by +1.3, but NES increased by only 0.7, indicating a higher proportion of ES as Ti_m increased (Figure 4.1c). Although Sp_m did not significantly impact the amount of ES, the amount of NES was slightly higher when the curd was mixed at 12 rpm (Figure 4.1d). Figure 4.2 presents the ES of mixed cheeses measured during the short aging period normally required to achieve adequate shredding and baking properties. The amount of ES fell below 1% of the cheese mass within 8 d of manufacture, but marked differences within Tp_m (Figure 4.2a) and Ti_m (Figure 4.2b) conditions were found shortly after manufacture. Even though a similar time-dependent water-reabsorbing capacity was found within the studied condition, it should be noted that Tp_m and Ti_m can be controlled to limit the amount of free serum in cheese. High ES in Mozzarella cheese was reported to be symptomatic of poor shredding and baking properties (Guo and Kindstedt, 1995; McMahon et al., 1999).

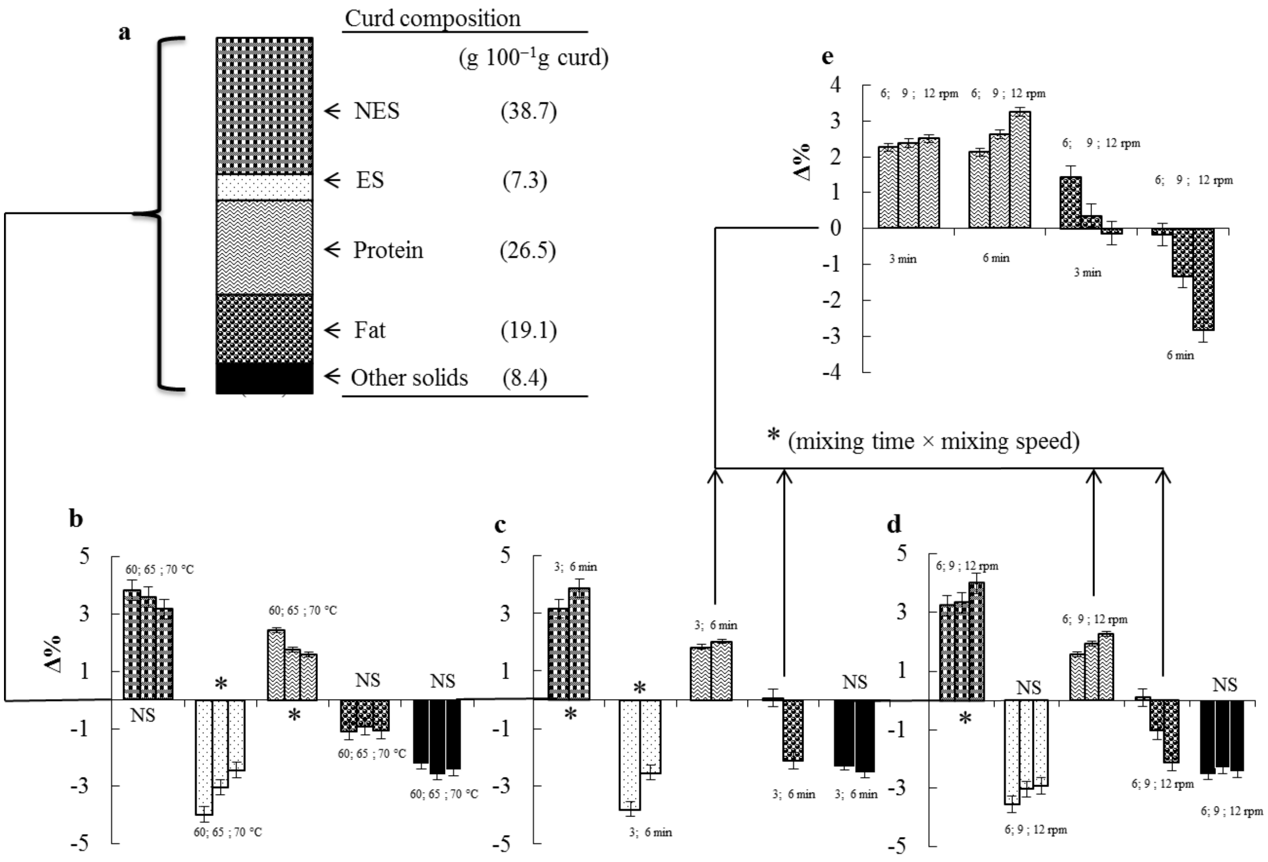

Figure 4.1 **Initial curd composition (g 100 g^{-1} curd) (a) and impact of thermo-mechanical treatments on the composition of mixed curd (b, c, d, e) presented as the percentage difference (Δ%) in cheese components (non-expressible serum [NES], expressible serum [ES], protein, fat, and other solids) before and after treatment. The main effects of mixing temperature (b), mixing time (c), and mixing speed (d) are presented. Significant interactions between mixing time and mixing speed are also presented for fat and protein contents (e). The effect (or interaction) is significant ($P \leq 0.01$) when identified by an asterisk (*) or non-significant (NS) when the probability of F is $P > 0.01$.**

Solids (protein, fat, and other solids) content in the mixed cheeses varied compared with the curd before mixing. Generally, the mixed cheeses had higher protein and lower fat contents than the initial curd did. The cheeses mixed at the lowest temperature (60 °C) had higher protein content than those mixed at 65 or 70 °C, but Tp_m did not affected the amount of fat in the cheeses (Figure 4.1b). The protein and fat contents depended on Ti_m and Sp_m ($P < 0.01$) (Figure 4.1e): higher protein content was found when Sp_m increased and when the cheeses were mixed for 6 min rather than for 3 min. Conversely, high Ti_m and Sp_m resulted in cheeses with significantly lower fat content. Other solids content (i.e., non-nitrogenous and non-fat solids) measured in the mixed cheeses was about 2.4% lower than in the initial curd but was not influenced by the mixing conditions studied ($P > 0.05$).

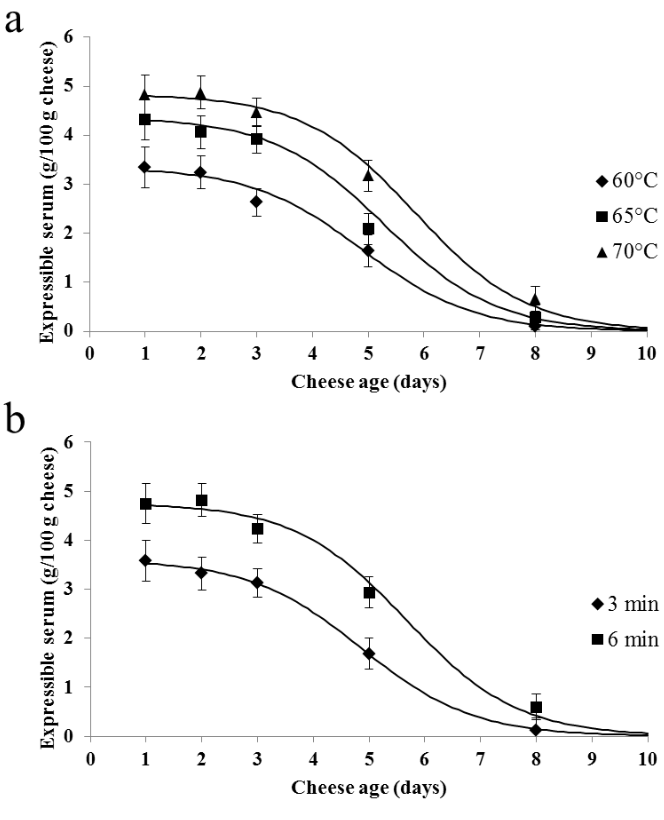

Figure 4.2 **Expressible serum in cheeses mixed at different temperature (a) or for different lengths of time (b), measured during a short aging period. The lines were added to guide the reader's eye.**

4.5.2 Impact of Mixing Conditions on Solid Loss

An average weight loss corresponding to about 10% of the initial curd weight was observed after the thermo-mechanical treatments and can be attributed mostly to the loss of solids in the stretching brine during mixing. The average loss coefficients (all mixing treatments combined) for protein, fat, and other solids were 0.016, 0.121, and 0.357, respectively. However, the solids loss did not totally explain the total curd weight loss observed, indicating that the thermo-mechanical treatments also globally induced water loss equivalent to about 4% (w/w) of total curd weight.

According to the variance analysis results, the protein loss coefficient decreased by 0.014 when Tp_m was increased from 60 to 70 °C ($P = 0.01$) whereas the loss of other solids (i.e., non-nitrogenous, non-fat solids) was not influenced by any of the mixing conditions ($P > 0.05$). Protein loss and other solids loss explained only 6% and 10%, respectively, of the mean total solids lost during mixing, whereas fat loss explained 84% this variation (data not shown). Fat loss was impacted ($P < 0.01$) by Tp_m, and the interaction effect of Ti_m and Sp_m on fat loss is presented in Figure 4.3. About 3% (w/w) more fat was lost when the curd was mixed at 60 °C compared with the warmer Tp_m (Figure 4.3a). Moreover, the combination of longer Ti_m and higher Sp_m accentuated the release of fat into the stretching brine (Figure 4.3b). Close examination of the data shows that globally, both solids and moisture were lost during mixing. Intensive mixing conditions seem to promote extensive loss of fat solids, which in turn results in cheese with higher relative moisture and protein content.

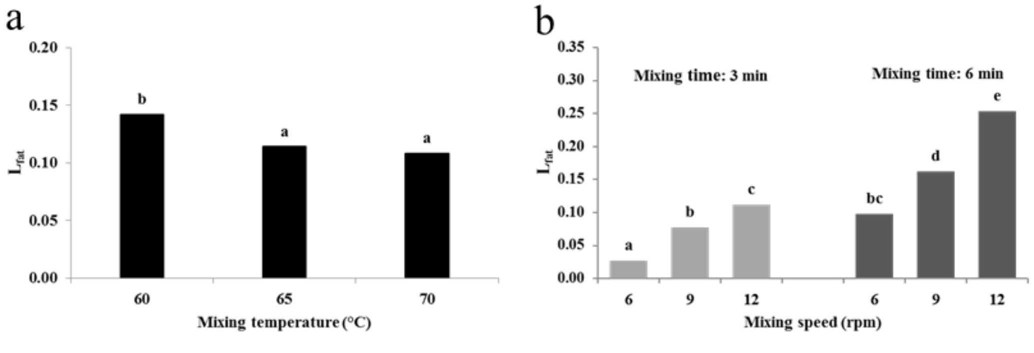

Figure 4.3 **Impact of mixing temperature (a) and of mixing time and speed (b) on the fat loss coefficient (L_{fat}). Different letters above the bars indicate that the means of effects differ significantly.**

4.5.3 Impact of Mixing Conditions on Cheese Microstructure

4.5.3.1 Microscopy

Figure 4.4 presents the SEM micrographs, at different magnifications, of cheeses prepared under the mild (60 °C, 3 min, and 6 rpm) and severe (70 °C, 6 min, and 12 rpm) mixing conditions in terms of temperature, time, and speed. The protein phase, interrupted by irregularly shaped holes representing the serum and fat phases, was clearly affected by the mixing conditions. Under the mild conditions, the fat and serum were more uniformly distributed throughout the protein phase. In comparison, the severe mixing conditions resulted in thicker and denser protein segments. The high-magnification SEM micrographs clearly show that the protein phase surface was rough and appeared shrunken as the result of the severe mixing conditions (Figure 4.4f).

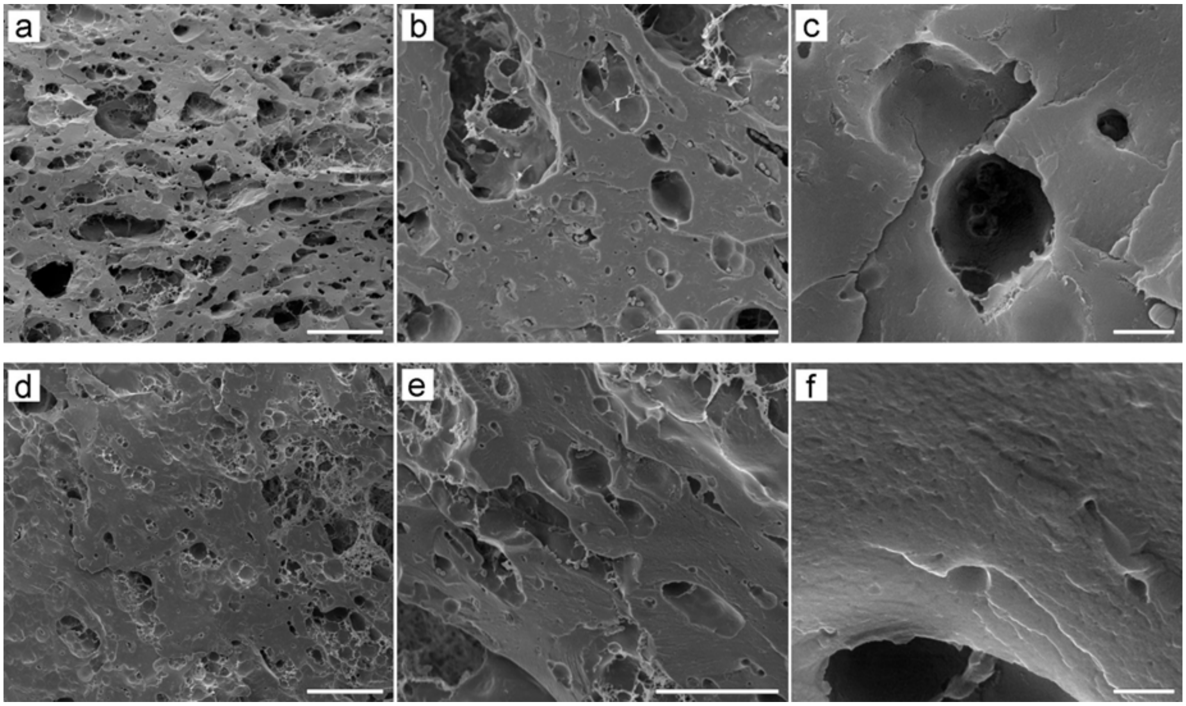

Figure 4.4 **Scanning electron micrographs of cheeses produced under mild (a, b, c; temperature/time/speed = 60 °C/ 3 min/6 rpm) and severe (d, e, f; temperature/time/speed = 70 °C/ 6 min/12 rpm) mixing conditions. Bars: 50 µm (a, d), 20 µm (b, e) and 2 µm (c, f).**

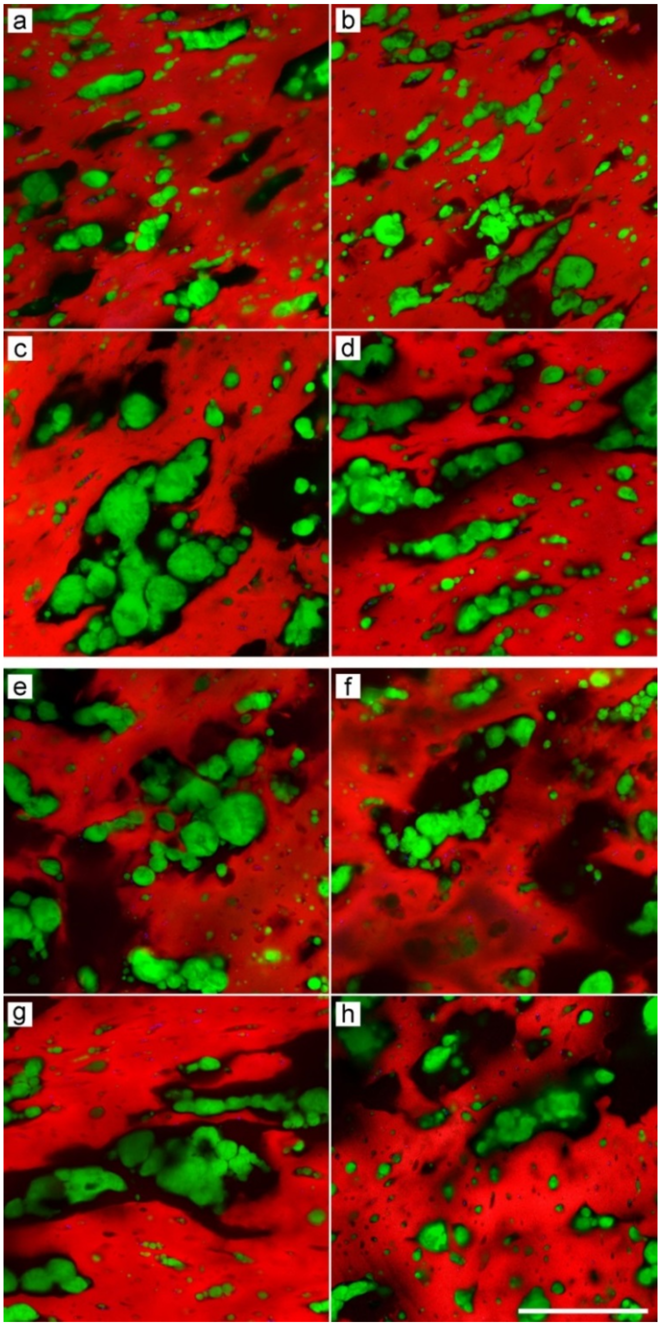

Figure 4.5 **Confocal laser scanning micrographs of cheeses produced under different combinations of mixing temperature, time and speed: (a) 60 °C, 3 min, and 6 rpm, (b) 60 °C, 3 min, and 12 rpm, (c) 70 °C, 3 min, and 6 rpm, (d) 70 °C, 3 min, and 12 rpm, (e) 60 °C, 6 min, and 6 rpm, (f) 60 °C, 6 min, and 12 rpm, (g) 70 °C, 6 min, and 6 rpm, (h) 70 °C, 6 min, and 12 rpm. Protein and fat appear in red and green respectively. Bar: 50 µm.**

The CLSM micrographs provide further insight into the suprastructure of fat in mixed cheeses (Figure 4.5). As expected, various fat globule sizes were observed. Small native globules, which are closely surrounded by the protein phase, appeared to be in greater number for the cheeses mixed at the lowest temperature and for the shortest time. Indeed, increasing Ti_m and Tp_m seemed to promote the formation of larger fat globules and, most evidently, of fat aggregates that were enclosed in large cavities throughout the protein phase. These aggregates were separated from the protein phase by aqueous serum, which is represented by darker areas in the micrographs in Figure 4.5. The spherical shape of most of the fat globules indicates that they may have been well stabilized by milk fat globule membrane constituents or other amphiphilic molecules from the serum phase (Lopez et al., 2008). However, poorly stabilized fat (also called free fat), recognizable by its non-globular shape, was also identified in the cheeses subjected to severer mixing conditions.

4.5.3.2 Laser Light Scattering Measurements

Size distributions of individualized fat globules liberated by dissociation of the casein network were measured using the laser light scattering technique. Figure 4.6 presents the representative fat droplet size distribution of cheese curd in comparison with the size distribution of cheeses mixed under the mild and severe conditions ($P \leq 0.01$). The average globule diameter, D[4,3], increased from about 16 μm in cheese curd to about 31 μm after the mixing treatments. However, no significant difference in fat globule size was detected among the mixing conditions studied ($P > 0.05$).

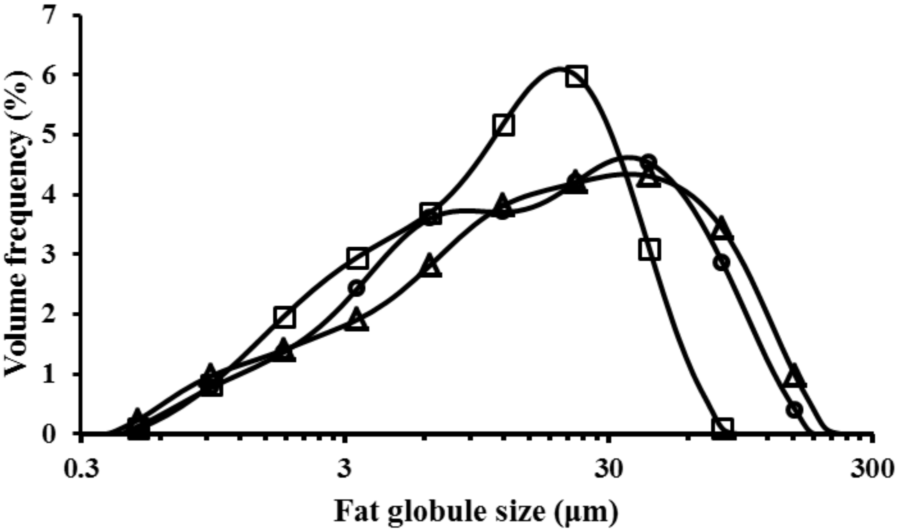

Figure 4.6 **Particle size distribution of fat globules in cheese curd (–□–) and in cheeses produced under mild (–○–; temperature/time/speed = 60 °C/ 3 min/6 rpm) and severe (–Δ–; temperature/time/speed = 70 °C/6 min/12 rpm) mixing conditions.**

4.5.4 Impact of Mixing Conditions on Rheological Properties

The mechanical spectrum of cheeses was determined by the measurement of G'_1, which is the elastic modulus measured at 1 Hz, and G'_{fd} , which is its dependence over a range of frequencies. The G'_1 indicates the global strength of the elastic interactions that structure the cheese matrix. The measurement of these interactions under different experimental time scales (i.e., different frequencies) provides insight into the behaviour and type of bonds in the cheese. Low G'_{fd} indicates a strongly cross-linked elastic network that has high relaxation properties. Conversely, a weaker network with more slowly relaxing bonds has a more liquid-like behaviour, which can be detected by a higher G'_{fd} (Lucey et al., 2003; Tunick, 2011).

Table 4.1 presents the variance analysis results for the effects of mixing conditions on the rheological parameters G'_1 and G'_{fd}. The Sp_m and Ti_m had the largest impact on G'_1 and G'_{fd} (Figure 4.7). For cheeses mixed for 3 min, lower G'_1 was measured with mixing at 9 rpm in comparison with 6 or 12 rpm, and G'_{fd} increased with Sp_m (Figure 4.7a). An opposite trend was observed for cheeses mixed for 6 min: G'_1 was the highest for cheeses mixed at 9 rpm, and G'_{fd} decreased with higher Sp_m (Figure 4.7b).

Table 4.1 ***F*****-values and** ***P*****-values (in parentheses) of the effects of mixing conditions on the elastic modulus at 1 Hz (G'_1) and the frequency dependence of G′ (G'_{fd}).**

	Rheological parameters	
	G'_1	G'_{fd}
Factors[a]		
Tp_m	2.7 (0.08)	10.1* (<0.01)
Ti_m	4.0 (0.05)	0.5 (0.48)
Sp_m	0.4 (0.69)	0.2 (0.82)
$Tp_m \times Ti_m$	6.4 (0.05)	3.9 (0.31)
$Tp_m \times Sp_m$	0.5 (0.73)	1.2 (0.32)
$Ti_m \times Sp_m$	11.0* (<0.01)	7.3* (<0.01)
$Tp_m \times Ti_m \times Sp_m$	3.7 (0.02)	0.6 (0.66)

[a] Mixing conditions: Tp_m, temperature; Ti_m, time; and Sp_m, speed
* Significant effects at $P \leq 0.01$

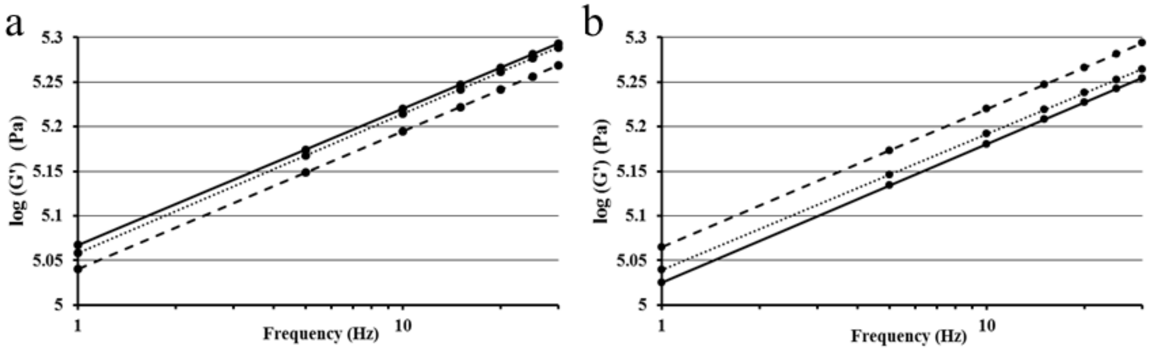

Figure 4.7 **Effect of mixing speed (6 rpm: straight line; 9 rpm: dashed line; and 12 rpm: dotted line) on the elastic modulus (G′) of cheese at different frequencies. Mixing time was 3 min (a) or 6 min (b).**

The highest Tp_m resulted in cheeses with higher G'_{fd} in comparison with the lowest Tp_m (data not shown). It must be stressed, however, that the absolute differences in the magnitude of the rheological parameters measured were unexpectedly low, as shown in

Figure 4.7. Overall, the impact of the mixing conditions studied on the rheological properties of cheeses was very slight.

4.6 Discussion

4.6.1 Relationship between Mixing Conditions and System Variables

The use of the Farinograph unit as the mixing system in this study allowed strict control of the temperature, time, and speed of mixing. This approach also allowed the system variables (SME, T_f, and HL) to be determined by *in situ* continuous torque and temperature recording. Hence, the relationship between the mixing conditions and system variables was investigated.

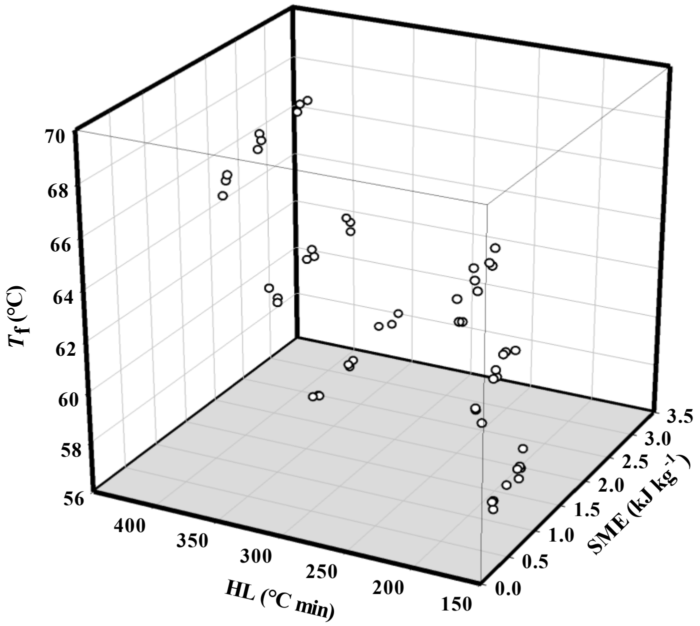

Figure 4.8 **Mixing treatments applied to cheese curds (*n* = 54), resulting from the combination of mixing temperature, time, and speed, and represented as a function of output system variables: final curd temperature (*T_f*), heat load index (HL), and specific mechanical energy (SME).**

Figure 4.8 illustrates the different thermo-mechanical treatments imposed in this study as a combination of the different system variables. It is clear that the relationships among the system variables SME, T_f, and HL were relatively low and hence that these variables can be considered to be independent from each other. To further understand the mixing system used, the impact of the mixing conditions on system variables was examined.

Total variations in SME explained by Ti_m, Sp_m, and Tp_m represented 73%, 24%, and 3 %, respectively. Hence, longer and faster mixing resulted in higher SME, whereas higher temperature slightly decreased the mixing torque because of the lower curd viscosity. It must be stressed at this point that SME encompasses the energy required for pure extensional flow, viscous shear, and rigid body motion of the curd (Auger et al., 2008). The relative importance of these motion types in this particular mixing system is not known because their calculation requires extensive mathematical simulation that was outside of the scope of this study. However, mathematical estimations of the shear stress in a system similar to the one used in the present study were performed by Solar et al. (2009). Moreover, interesting work aiming to describe the flow field in a conventional single-screw cooker-stretcher was presented by Yu and Gunasekaran (2004). Despite discrepancies between the different mixing systems, SME is generally recognized as a good engineering scale-up parameter (Mulvaney et al., 1997; Yu and Gunasekaran, 2005a; 2005b; Auger et al., 2008).

As expected, T_f was strongly related to Tp_m, which explained 91% of its variance. Indeed, T_f increased slightly with Ti_m and Sp_m (7% and 1% of variance explained) because of longer and more efficient heat transfer from the stretching brine to the curd. The thermal energy created by friction during mixing was shown to be negligible in a similar system (Solar et al., 2009).

The magnitude of HL was essentially dependent on Ti_m (99%), with a minor contribution from Tp_m (1%) and with no Sp_m effect. The low collinearity among system variables means that SME or T_f, and HL can be used to predict the impact of mechanical or thermal treatment on cheese properties.

4.6.2 Relationship between System Variables and Cheese Characteristics

System variables were considered to be potential explanatory variables for the mathematical prediction of cheese characteristics. Squared values of system variables were also included as potential explanatory variables to account for possible quadratic effects.

All system variables in the models were selected by the stepwise method and added a significant contribution to the models based on an entry level of $P < 0.10$. Table 4.2 presents the linear models that satisfactorily predicted the cheese characteristics ($R^2 \geq 0.6$) observed in this study.

Table 4.2 **Models used for the prediction of some cheese characteristics using system variables.**

			Predictive variables[a]	
Predictive model	Equation	R^2	a	b
Fat loss coefficient	$= 0.10\ a - 0.026$	0.92	SME	
Expressible serum (g 100^{-1}g cheese)	$= 1.25 \times 10^{-3}\ a + 1.073 \times 10^{-5}\ b - 1.61$	0.60	T_f^2	HL^2

[a] System variables used for the prediction of cheese characteristics: SME, specific mechanical energy (kJ kg^{-1}); T_f, final curd temperature (°C); HL, heat load index (°C min).

4.6.2.1 Solid Loss

The loss of solids into the stretching brine during the pasta filata process is a major concern for the cheese manufacturers because of the negative impacts of that loss on cheese yield and on the overall value of these solids (Gernigon et al. 2009). In our study, the fat loss coefficient was found to be very well predicted by the SME imparted during mixing (Table 4.2), whereas others solids loss and protein loss seemed unaffected by the system variables studied. Increasing the mechanical energy linearly increased curd fat loss ($R^2 = 0.92$). Renda et al. (1997) observed that cheeses with lower fat content were produced when the screw speed of the cooker-stretcher was increased. In a similar pilot system, Yu and Gunasekaran (2005a) reported that the fat in dry matter depended on both SME and T_f. Higher SME, caused by an increase in either speed or time of mixing, increases the contact surface area between the cheese and the stretching brine from which the fat can be released. Moreover, increasing SME may promote the discharge of fat from openings created by local fractures in the continuous protein phase. Hence, the resilience of the protein network when exposed to shear may also be important. The results presented in Figure 4.3 tend to support this assumption. Indeed, fat loss increases at lower Tp_m, which is believed to be caused by the decrease in network flowability. Consequently, the protein network would be more prone to “melt fracture”. This agrees with the view of Renda et al. (1997) and Bähler and Hinrichs (2013) that a critical temperature, related to the gel-sol transition point of the protein network, must be exceeded in order to plasticize curd into pasta filata cheese. In the present study, SME seemed to satisfactorily account for this temperature effect, based on the good predictability of the model obtained (Table 4.2).

4.6.2.2 Microstructure

Cheese structure can be viewed as a complex bi-continuous gel arrangement of hydrated protein networks with localized domains of fat (Vogt et al., 2015). It is well known that the pasta filata process provokes the alignment of the amorphous casein aggregates into roughly parallel fibres, which are separated by elongated fat and serum pockets (Everett, 2007). In this study, the Farinograph system did not impose systematic unidirectional extension on the cheese curd. However, the moulding of the melted curd may have favoured the extension of cheese in a direction perpendicular to the pressing load.

The impact of the thermal and mechanical treatments on cheese microstructure can be readily observed in the SEM and CLSM images (Figure 4.4 and 4.5). It is clear that a wide range of fat globule sizes were formed, indicating that coalescence occurred during the process (Figure 4.5). This is further supported by the results obtained from the laser light scattering measurements presented in Figure 4.6. Coalescence implies that smaller fat globules are eventually brought into contact through protein network openings and that sufficient shear is imparted to deform the fat globules to the point of milk fat globule membrane rupture. The deformation of milk fat globules during shear depends on (1) their size, given that large globules are more easily deformed, (2) their content of solid fat, given that liquid fat is more easily deformed, and (3) the physical properties of the protein phase, given that the viscous flow of the casein network exposes the globules to higher shear (Lopez et al., 2007). Therefore, thermal energy and mechanical energy are likely to have both contributed to milk fat coalescence, although this could not have been confirmed by the laser scanning measurement experiments (section 4.5.3.2). Similar observations were made by Rowney et al. (2003), who measured an increase in fat globule size with increasing temperature and extensional stretching. Another interesting feature that can be seen in the CLSM micrographs is the formation of globular fat aggregates into large "fat pool" areas. Increasing the temperature and duration of the treatment seemed to promote the formation not only of larger aggregates but also of non-globular fat, or so-called free fat (Figure 4.5). Fat pools enable protein−protein association on larger stretches in comparison with dispersed native fat globules, thus creating weaker areas in the cheese matrix (Everett, 2007). Large fat pools also have an important lubricating function during the melting of cheese, because they allow the protein segments to flow past each other (Everett, 2007). Moreover, the presence of free fat has been positively correlated to oiling off (i.e., release of free oil) in baked cheese (Lopez et al., 2008).

The protein phase of the mixed cheeses seemed to have been affected by the intensity of the thermo-mechanical treatments, as shown in the SEM micrographs (Figure 4.4). The protein appeared denser and more dehydrated as the temperature and speed of the mixer is increased. It was found that the T_{f} and HL system variables could satisfactorily predict the amount of cheese ES ($R^2 = 0.60$; Table 4.2). This means that higher thermal energy coupled with increased process duration reduces the ability of the protein network to closely retain its aqueous phase. This is in agreement with the presence of bulk water in cheese microstructure, as seen in Figure 4.5. According to Lucey et al. (2003), heating the cheese matrix would increase the hydrophobic interaction, which induces the size reduction of individual casein particles. Consequently, the global hydrophobicity β-CN aggregates, composed of numerous hydrophobic patches, is increased at elevated temperature (Pastorino et al., 2002). Moreover, heat-induced protein−protein crosslinks may also form via hydrophobic interactions and calcium bridging (Udayarajan, 2007). Hence, the protein network is globally contracted and expels the unbound serum into the openings formed by fat globules (McMahon et al., 1999; Pastorino et al., 2002; Colìn-Cruz et al., 2012; Vogt et al., 2015). Some studies also suggested that protein−protein interactions during heating are time-dependent (Kuo et al., 2001; Udayarajan, 2007; Kim et al., 2011). In the present study, HL was found to be a good predictor of ES when combined with final cheese temperature (Table 4.2), which would support the assumption that processing time has an effect on protein phase characteristics.

Expressible serum, which is mostly bulk water in cheese, acts as a solvent for enzymes, bacteria, and minerals. Hence, the amount of bulk water that is formed as a result of pasta filata processing conditions could have an effect on cheese ripening behaviour. It was found that the amount of ES remained constant for about 48 h after the thermo-mechanical treatment but decreased steadily afterward (Figure 4.2). Gradual hydration of the protein phase was reported by other authors and has been related to the solubilization of β-casein into the serum phase at low temperature and to the concentration of sodium and calcium in cheese (Kindstedt and Guo, 1998; McMahon et al., 1999; Guinee et al., 2002; Joshi et al., 2004b). The delay observed before the decrease in ES with aging time might be explained by the slow diffusion dynamics of the bulk serum during the first few hours after thermo-mechanical treatment because of the dense protein aggregates that formed. The gradual swelling of the protein phase during aging favoured protein−water interactions at the expense of protein−protein interactions, which promoted the loosening of the protein phase and allowed greater protein mobility upon heating (Everett, 2007). Optimal hydration of the protein phase, with solubilisation of calcium associated to caseins, is believed to be a critical factor for the expression of desirable shredding and melting properties (Kindstedt and Guo, 1998; Guinee et al., 2002).

4.6.2.3 Rheological Properties

Very slight differences in the elastic properties of the cheeses were found among the different mixing conditions applied. Consequently, poor correlation with system variables was established ($R^2 < 0.5$; data not shown). This result was unexpected, given that rheological properties are generally dependent on the network structure and composition of cheese (Lucey et al., 2003). Mulvaney et al. (1997) observed that higher temperature and lower SME conditions resulted in cheese with a higher elastic modulus. However, another study found no significant variation in texture parameters of Mozzarella cheeses prepared at different screw speeds (Renda et al., 1997). Yu and Gunasekaran (2005a) measured G′ values between 200 and 600 kPa for cheeses stretched at different SME levels (~1.2–6.5 kJ kg^{-1}) and temperatures (~49–67 °C). In the present study, G'_1 varied only between 94 and 126 kPa. It is believed that confounding and antagonist effects on cheese elastic properties may have caused this overall low variation in elastic properties. For example, mixed cheese is expected to have lower fat volume fraction after treatment, because of the loss of fat during process, and thus the G′ is expected to be higher because of the greater relative importance of the protein phase (Ustunol et al., 1995). Conversely, high thermal treatment is expected to increase the formation of bulk serum pool, which creates weaker areas and disrupts the protein network, reducing strength of the cheese matrix (Everett, 2007). Other factors such as the shape and size of fat globules, or of fat aggregates, in cheese may also have an impact on rheological properties (Yang et al., 2011).

4.7 Conclusions

This study evaluated the impact of different thermo-mechanical treatments on cheese characteristics using a Farinograph model system. This approach allowed the temperature, time, and speed of mixing, which are the main control parameters of the industrial pasta filata process, to be changed independently. The thermo-mechanical treatments greatly impacted the composition, solids loss, and microstructure of the cheeses, but with an overall limited impact on the rheological properties. Our results show that (1) the amount of mechanical energy provided was directly proportional to cheese fat loss during the treatment and (2) the amount of ES was related to the intensity of the thermal treatment. This study contributes additional knowledge about the effects of thermo-mechanical treatments that is critical for the optimization of in-process controls and pasta filata process designs.

4.8 Acknowledgements

This work was financially supported by the Natural Sciences and Engineering Research Council of Canada (Ottawa, ON, Canada), the Fonds de recherche du Québec – Nature et technologies (Québec City, QC, Canada) and Agropur Dairy Cooperative (Saint-Hubert, QC, Canada).

Chapter 5 Physical properties of Pizza Mozzarella cheese manufactured under different cheese-making conditions

5.1 Résumé

Cette étude s'intéresse à l'impact de différentes pratiques fromagères sur l'aptitude au râpage et la fonte du fromage pizza Mozzarella. Quatre fromages ont été produits en usine pilote avec ou sans ajout de protéines sériques dénaturées (0% ou 0,25%), en procédant à l'emprésurage à pH 6.4 ou 6.5. Les fromages ont été maturés pendant 8, 22 ou 36 jours. L'aptitude au râpage était évaluée à 4 °C, 13 °C ou 22 °C à l'aide des indicateurs suivants : quantité de fines, taille des râpures et adhésion du fromage à la lame de râpage. Une méthode semi-empirique a été développée pour quantifier le phénomène d'agglomération des râpures dans les conditions d'entreposage habituelles chez les transformateurs laitiers. Les caractéristiques de fonte et de refonte du fromage ont, pour leurs parts, été évaluées à l'aide de la rhéologie dynamique. Cette étude démontre que la diminution du pH d'emprésurage réduit la quantité de fines produite lors du râpage. L'ajout de protéines sériques influence aussi la quantité de fines, mais son impact varie selon le pH d'emprésurage et l'âge du fromage. L'élévation de la température de râpage diminue la taille des râpures et augmente l'agglomération du fromage et son adhésion au matériel de découpe. Les données rhéologiques obtenues indiquent qu'un pH d'emprésurage plus bas et l'extension de la période de maturation sont des pratiques fromagères favorables à l'amélioration de la fonte. Le profil de fonte d'un fromage ayant reçu un traitement thermique, puis refroidi révèle une structure plus faible qu'un fromage subissant sa première fonte à basse température (<40 °C), mais qu'il devient passablement plus dur à des températures supérieures à 50 °C. Cette étude montre qu'il est possible de moduler certaines stratégies fromagères afin d'optimiser les propriétés de râpage et de fonte du fromage Mozzarella.

5.2 Abstract

The effect of manufacturing factors on the shreddability and meltability of pizza Mozzarella cheese was studied. Four experimental cheeses were produced with 2 concentrations of denatured whey protein (0% or 0.25%) and 2 renneting pH values (6.4 or 6.5). The cheeses were aged 8, 22, or 36 d before testing. Shreddability was assessed by the presence of fines, size of the shreds, and adhesion to the blade after shredding at 4 °C, 13 °C, or 22 °C. A semi-empirical method was developed to measure the matting behavior of shreds by simulating industrial bulk packaging. Rheological measurements were performed on cheeses with and without a premelting treatment to assess melt and post-melt cheese physical properties. Lowering the pH of milk at renneting and aging the cheeses generally decreased the fines production during shredding. Adding whey protein to the cheeses also altered the fines production, but the effect varied depending on the renneting and aging conditions. The shred size distribution, adhesion to the blade, and matting behavior of the cheeses were adversely affected by increased temperature at shredding. The melting profiles obtained by rheological measurements showed that better meltability can be achieved by lowering the pH of milk at renneting or aging the cheese. The premelted cheeses were found to be softer at low temperatures (<40 °C) and harder at high temperatures (>50 °C) compared with the cheeses that had not undergone the premelting treatment. Understanding and controlling milk standardization, curd acidification, and cheese aging are essential for the production of Mozzarella cheese with desirable shreddability and meltability.

5.3 Introduction

Low-moisture part-skim pizza Mozzarella is a variety of pasta filata cheese used extensively as a topping on baked dishes in North America. Ingredient cheeses such as Mozzarella must exhibit key physical attributes in both the unmelted and melted states (Lucey, 2008).

Pizza Mozzarella cheese is almost systematically shredded, cut or diced to improve its handling and enhance its meltability (Gunasekaran and Ak, 2002). Shreddability is a broad term that includes physical attributes such as the ease of machinability, the shape and integrity of shreds, the propensity of shreds to mat, and the excessive production of fines during shredding (Childs et al., 2007). Good shredding behavior is observed in a relatively narrow range of textural properties and is not fully understood, or controlled, by cheese manufacturers. Soft cheese usually shows poor shredding characteristics because it sticks to the blade, forms gummy balls of cheese, and produces shreds that tend to mat together. On the other end of the spectrum, firm and dry Mozzarella cheese easily shatters into fines (Kindstedt, 1995). Unfortunately, few methods are available to assess the shredding behavior of cheese. The quality grading of shreds based on visual evaluation has been used by some researchers (Apostolopoulos and Marshall, 1994; Chen, 2003; Ni and Guansekaran, 2004). In an effort to assess the defects observed during the shredding process, Childs et al. (2007) empirically measured the fines production and the adhesion to the blade of cheeses during shredding. These attributes, along with the matting of shreds, are still considered the most common problems, and little is known about the factors that cause them.

Unlike shredding behavior, the physical properties of cheese in its melted state have been studied extensively. When Mozzarella cheese is baked, its quality is associated with extent of flow, stretchability, free-oil formation, blistering, and browning (Gunasekaran and Ak, 2002). Metzger and Barbano (1999) also pointed out the importance of the post-melt texture of Mozzarella cheese in regard to its quality and acceptability. However, the post-melt texture of cheese has received little attention from researchers.

More than ever, the growing demand for tailored cheese ingredients requires a better understanding of the effect of cheese-making processing variables on the physical properties and microstructure of cheese. Freshly manufactured Mozzarella cheese exhibits typical protein fiber orientation and melts poorly. The hydration of the protein matrix and the proteolysis that occur conjointly during the short aging of Mozzarella cheese dramatically change the microstructure and texture of the cheese (McMahon et al., 1999). For control of the baking characteristics, many studies also demonstrated the primary importance of the calcium content in Mozzarella cheese, but most important is the partitioning of the calcium between insoluble and soluble states (Joshi et al., 2003; Johnson and Lucey, 2006; Choi et al., 2008). The effect of the addition of milk ingredients on cheese physical properties has been studied to some degree in full-fat cheeses (Punidadas et al., 1999; Mead and Roupas, 2001) but most interest has focused on low-fat cheeses (Zisu and Shah, 2005; Ismail et al., 2011; Schenkel et al., 2011). Milk ingredients, particularly denatured whey protein (WP-D) concentrate, are widely used in the industrial production of Canadian pizza Mozzarella cheese to increase yield (Hinrichs, 2001). The aim of the present study was therefore to evaluate the effect of adding WP-D and controlling cheese mineralization through the renneting pH on the shreddability and rheological properties of pizza Mozzarella cheese.

5.4 Material and Methods

5.4.1 Milk Standardization

Cheese milk was formulated from (1) raw skim milk (2.5% [w/w] casein), (2) milk protein concentrate (~77% [w/w] casein on a dry basis; 872B, lots L610009 and L610010; Ingrédia SA, Arras, France) rehydrated at 10.1% (w/w) casein in water, (3) fresh cream (1.5% [w/w] casein; ~40% [v/v] milk fat), and (4) WP-D concentrate (~55% [w/w] protein on a dry basis; Agropur, Granby, QC, Canada) rehydrated at 10.5% (w/w) protein in water. Casein concentration and fat-to-protein ratio in standardized milk were respectively 3.0% (w/w) and 0.85 and the concentration of protein from WP-D concentrate was fixed to 0 and 0.25% (w/w). Total protein concentration in cheese milk, with and without added WP-D, were respectively 3.95 and 3.70% (w/w). The standardized milk was pasteurized at 74 °C for 16 s before cheese-making.

5.4.2 Manufacture of Pizza Mozzarella Cheese

Cheese milk (350 kg) was supplemented with calcium chloride (0.0063% [w/w]) and ripened with a direct-in-vat thermophile starter culture (0.104% [w/w], Easy-Set i420; Chr. Hansen, Milwaukee, WI) until the appropriate renneting pH was reached. Coagulant (Fromase XLG; DSM Food Specialties Inc., Eagleville, PA) was added to the milk (0.0075% [v/w]) at a renneting pH of 6.4 or 6.5. The coagulum was cut 30 min after renneting and healed for 5 min. Then, the curd-whey temperature was raised (0.2 °C min^{-1}) to 40 °C under low agitation. The whey was drained when the pH decreased 0.5 units below the renneting pH. The drained curd was cheddared (30 to 60 min) to a fixed pH value of 5.2. The acidified curd was milled and then knitted in hot water (65 °C) with a pilot-scale cooker stretcher (JN-500 CS; Johnson/Nelles Corp., Windsor, WI). The rotational speed of horizontal and vertical screws was respectively 6 and 8 rpm and residence time was approximately 5 min. The cheese reached approximately 55 °C at moulding. Cheese obtained from the beginning and the end of the cooking/stretching process was discarded. Twelve cheese blocks (26.5 × 9.5 × 9.5 cm) of approximately 2.3 kg each were molded and brined in saturated sodium chloride solution (~24% (w/w), 4 °C) for 4 h. The cheese blocks were vacuum sealed and stored at 4 °C. Two vats were available for cheese production and the 4 treatments (2 WP-D concentration × 2 renneting pH) were performed from the same batch of milk within 72 h. Cheese productions were repeated 3 times within a month.

5.4.3 Chemical Analysis of Cheese

For compositional analyses, one cheese block was cut in 4 symmetrical parts and one part was finely grated using a food processor. The moisture content was determined by the oven-drying method (Marshall, 1992). Ash was measured by incineration in a muffle furnace at 550 °C for 12 h. Water-soluble nitrogen was reported as the percentage of total nitrogen (WSN) to monitor proteolysis of the cheese during aging (Watkinson et al., 2001). The nitrogen content was determined using the macro-Kjeldahl method, and total protein was calculated using a protein conversion factor of 6.38 (Marshall, 1992). The lipid content was obtained by the Mojonnier method (Marshall, 1992). The sodium chloride content was determined using a Chloride Analyser 926 (Sherwood Scientific Ltd., Cambridge, UK) and the pH of the cheese was measured with an AR15 Accumet pH meter (Fisher Scientific, Ottawa, ON, Canada). The colloidal calcium phosphate (CCP) content in the cheese was estimated by the acid-base titration method adapted by Rémillard and Britten (2011). The retention coefficient in the cheese of the species n, the protein or fat, was calculated as follows:

Equation 4.1 Retention coefficient calculation

$$\text{Retention coefficient of } n = 100\text{-}\left(\frac{n\% \text{ in whey}}{n\% \text{ in cheese milk}}\right)\times 100$$

Milk and whey composition was measured by infrared spectrometry (MilkoScan FT-120; Foss Electric, Eden Prairie, MN).

5.4.4 Physical Properties of Cheese

5.4.4.1 Shreddability of Pizza Mozzarella Cheese.

The external layer of a cheese block was carefully trimmed and 2 identical cheese slabs were cut (25 × 9 × 4 cm) using a metal guide and a cheese wire. Four symmetrical samples (9 × 4 × 3.5 cm) were obtained from the ends of the 2 slabs. Each cheese sample was shredded under constant load (1.25 kg) in a household food processor (FP-12DCC; Cuisinart, Woodbridge, ON, Canada) equipped with a circular shredding blade (5 × 10-mm openings). Cheese residue adhering to the blade was determined gravimetrically. The shredded cheese was transferred to the top of a stacking sieve [American Society for Testing and Materials (ASTM) specification no. 4, 6, and 10; Retsh Inc., Newtown, PA] and mechanically shaken for 60 s at 278 oscillations and 150 ± 10 taps per min (Ro-Tap RX-29 sieve shaker; W.S. Tyler, Mentor, OH). The cheese fractions retained by the mesh sieves with 22.26-, 11.22-, and 4.00-mm^2 openings were classified as long, medium, and small shreds, respectively. Cheese particles passing through the mesh with 4.00-mm2 openings were classified as fines and reported as a proportion of the total weight of the shredded cheese. The fines production, the size distribution of the shreds, and the adhesion of cheese to the blade were used as indicators of shreddability.

5.4.4.2 Matting of Shreds

The preparation of shred cakes was inspired by Akbulut et al. (2011) and simulated the behavior of shreds under bulk packaging conditions. The long and medium shreds collected from the shredding experiment were stored at 4, 13, or 22 °C for 1 h. Then, 15 g of shredded cheese was placed into plastic cylinder (25-mm diameter.) that was capped on one end, and the cheese was gently compacted with a free-moving piston to a constant volume of 30 cm^3. Then, the cylinder was set vertically on its piston and stored at 4 °C for 18 h. The shred cake was unmolded onto the lower plate of a TA-XT2 texture analyser (Stable Micro Systems, Scarsdale, NY). The matting behavior was measured by the penetration of a conical stainless steel tool (20° angle) at constant speed (0.5 mm s^{-1}). The penetration force (in N) was reported against the volume deformation induced by the cone inside the shred cake.

5.4.4.3 Rheological Properties of Pizza Mozzarella Cheese

Dynamic oscillatory measurements were performed using a strain-controlled rheometer (Physica MCR 301; Anton Paar GmbH, Graz, Austria) equipped with 25-mm parallel plates (PP25; Anton Paar Gmbh). Cylindrical cheese samples (25-mm diameter and 2-mm height) were carefully cut from the center portion of the cheese slabs using a cork borer and a cheese-cutting wire. The cheese disks were tested immediately after sampling. Fine-grade sandpaper (Grade 180B) was glued to the rheometer plates to prevent slippage, and a protection hood with a solvent trap (17780; Anton Paar GmbH) covered the samples during the test. The cheese disks were compressed at 1 N normal force for a 5-min equilibration period before the temperature-sweep experiment started. The temperature was raised from 5 to 80 °C at a rate of 5 °C min^{-1} while constant strain amplitude (0.1%) was applied on the samples at an oscillation frequency of 1 Hz. The complex modulus (G^*) and the phase angle (δ) were recorded and plotted against the temperature to obtain the cheese melting profile. The softening point, the temperature recorded at $\delta = 45°$, was also extracted from the melting profile data. Full descriptions of the rheological parameters used in this study can be found elsewhere (Gunasekaran and Ak, 2002; Lucey et al., 2003; Everett and Auty, 2008). The melting profile was also determined on premelted cheeses, which were prepared as follows: cheese disks were sampled and vacuum-sealed (VAK*3.0R; Winpak, Winninped, MB, Canada). The cheeses were then immersed in hot water (90 °C) for 15 min and cooled to 4 °C. The analyses of the premelted cheeses were performed 90 min after heat treatments.

5.4.5 Experimental Design and Statistical Analysis

5.4.5.1 Main Treatments

Pizza Mozzarella cheeses were randomly produced using 2 renneting pH values (6.4 or 6.5) and 2 added WP-D concentrations (0 and 0.25%) according to a factorial experimental design repeated 3 times.

5.4.5.2 Shredding Experiment

The 4 cheeses were tested for compositional properties (WSN and CCP) and shredding properties (fines production, size distribution of shreds, adhesion to the blade, and matting behavior) at 8, 22 and 36 d of aging. The cheeses were also shredded at 3 different temperatures (4, 12, and 22 °C). Data were analysed according to a factorial split-split plot design, with aging time and shredding temperature in the subplots and sub-subplots, respectively.

5.4.5.3 Melting Experiement

The cheese melting profiles were monitored at 8 and 36 d of aging. The softening point of the cheeses was analysed according to a factorial split-split plot design, with aging time and premelting treatment in the subplots and sub-subplots, respectively.

5.4.5.4 Statistical Analysis

Analysis of variance was performed on the data using SAS software with PROC MIXED (version 2.0.3, 2008; SAS Institute Inc., Cary, NC). Error bars on the figures represent the standard errors of the mean.

5.5 Results and Discussion

5.5.1 Cheese Composition

The effect of WP-D addition and renneting pH on cheese composition at d 1 is summarized in Table 5.1. The pH and the salt-to-moisture ratio were similar among the cheeses ($P > 0.05$). As expected, the addition of WP-D to milk increased the moisture content of cheeses by about 2.4%. WP-D aggregates are known to bind water effectively and may have detrimental effects on whey drainage, thus increasing water retention in cheese

(Hinrichs, 2001). Protein and fat content was inversely related to moisture. The addition of WP-D significantly increased the protein retention in the cheeses. This result indicates that the WP-D aggregates were added were effectively retained in the cheese matrix.

Table 5.1 **Means, *F*-values, and *P*-values for the analysis of the effect of denatured whey protein (WP-D) addition and renneting pH on cheese composition.**

	Mean of treatments[a]					Factor effect[c] [*F*-value (*P*-value)]		
	0% WP-D		0.25% WP-D					
Item	R6.4	R6.5	R6.4	R6.5	Error[b]	WP-D	RpH	WP-D × RpH
Composition								
Moisture (%)	45.26	44.23	47.52	46.78	0.44	28.01* (<0.01)	3.23 (0.11)	0.01 (0.92)
Protein (%)	25.78	26.79	25.15	25.23	0.21	28.39* (<0.01)	7.19* (0.03)	6.17 (0.06)
Fat (%)	23.13	24.40	22.68	22.06	0.63	5.13* (0.05)	0.27 (0.62)	2.35 (0.16)
Ash (%)	2.79	2.87	2.84	3.04	0.05	4.40 (0.07)	7.07* (0.03)	1.22 (0.30)
Salt/Moisture (%)	2.22	1.99	2.28	2.38	0.11	3.47 (0.10)	0.26 (0.62)	1.97 (0.20)
pH	5.28	5.29	5.30	5.31	0.06	0.09 (0.77)	0.04 (0.85)	0.00 (0.97)
Retention coefficient								
Protein (%)	76.91	77.45	77.65	77.68	0.19	8.08* (0.03)	3.04 (0.13)	1.23 (0.31)
Fat (%)	87.81	89.92	89.30	89.76	0.87	0.59 (0.47)	2.20 (0.19)	0.93 (0.87)

[a] Means of the 4 main treatments: with (0.25% WP-D) and without (0% WP-D) denatured WP added to cheese milk renneted at pH = 6.4 or 6.5 (R6.4 and R6.5 respectively).

[b] Standard error of means

[c] *F*-values and *P*-values (in parentheses) of the effect of WP-D addition, renneting pH (RpH), and the interaction effect (WP-D × RpH) on the composition of cheese.

* $P < 0.05$.

Important physicochemical and biochemical changes occurred in the cheeses during the aging period (Table 5.2). The addition of WP-D did not significantly affect the WSN ($P = 0.08$) or CCP content ($P = 0.45$), but the renneting pH had a significant effect on those descriptors ($P = 0.02$ and $P = 0.04$, respectively). Figure 5.1 presents the evolution of CCP and WSN during aging as affected by renneting pH. Colloidal calcium content was higher when milk was renneted at pH 6.5 and remained higher during the aging period. Modifying the renneting pH is an efficient approach to control the demineralization of the cheese without changing its final pH (Johnson and Lucey, 2006; Choi et al., 2008). Insoluble calcium, linked to the caseins, is known to dissociate as milk pH decreases. Acidification before renneting allows better solubilisation of calcium compared with after renneting because soluble calcium diffuses faster in a liquid than in a gel. As a result, more calcium is removed at drainage when the renneting pH is reduced. It should, however, be recognized that in the present study the drainage pH was reduced for cheeses renneted at lower pH and the effect of renneting pH cannot be separated from the effect of drainage pH. The CCP content slightly decreased in the early stage of storage but stabilized after 22 d of aging. Solubilization of CCP during cheese aging was reported by other researchers (Lucey et al., 2005; O'Mahony et al., 2005), and these changes are known to be of primary importance for the rehydration of the *para*casein matrix and textural changes in cheese during the first 10 d or so after manufacturing (McMahon et al., 1999; Johnson and Lucey, 2006).

Table 5.2 **F-values and P-values (in parentheses) for the analysis of effect of denatured whey protein (WP-D) addition and renneting pH on cheese colloidal calcium phosphate (CCP) and water-soluble nitrogen as a percentage of total nitrogen (WSN) during aging.**

		F-value (*P*-value)			
Factors[a]	df	CCP		WSN	
Whole plot					
WP-D	1	0.67	(0.45)	4.47	(0.08)
RpH	1	6.64*	(0.04)	10.11*	(0.02)
WP-D × RpH	1	0.08	(0.79)	0.00	(0.95)
Error	8				
Subplot					
A	2	7.62*	(0.01)	37.40*	(< 0.01)
A × WP-D	2	0.10	(0.90)	0.88	(0.44)
A × RpH	2	0.59	(0.57)	0.42	(0.67)
A × WP-D × RpH	2	1.38	(0.29)	0.26	(0.77)
Error	16				

[a] Split-plot design with the WP-D addition and the renneting pH (RpH) as factorial 2 × 2 main plot. Subplot included the effect of aging of cheese (A).
$^{*}P < 0.05$.

The analysis of the WSN revealed important residual proteolytic activity in the cheeses during aging ($P < 0.01$), possibly from active coagulant enzyme, which resisted heat treatment during the pasta filata process (Costabel et al., 2007). During aging, a higher WSN value was consistently observed for the cheeses made from milk renneted at pH 6.4 (Figure 5.1). As demonstrated by other researchers, a low CCP content in cheese also promotes a lower degree of casein aggregation, which facilitates the accessibility of proteolytic enzymes that eventually release free α- and β-CN from the *para*casein matrix (Feeney et al., 2002; Zisu and Shah, 2005).

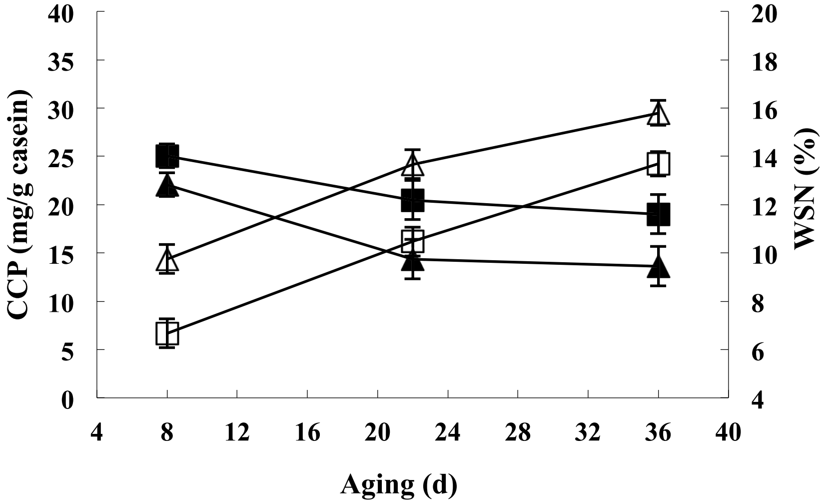

Figure 5.1 **Effect of aging on colloidal calcium phosphate (CCP; filled symbols) and the proportion of water-soluble nitrogen to total nitrogen (WSN; open symbols) in pizza Mozzarella cheeses made with milk renneted at pH values of 6.4 (▲) or 6.5 (■). Error bars represent the standard error of means.**

5.5.2 Shredding Experiment

5.5.2.1 Size Distribution of Shredded Cheese Fractions

A close examination of the partition between the 4 shred fractions showed that the percentage of fines (size < 4.00 mm^2) produced during shredding was positively correlated to the percentage of small shreds (4.00 mm^2 < size <11.22 mm^2), at a coefficient of determination of 0.72. Therefore, the percentage of fines and long shreds obtained from the shredding experiment are further examined.

***Table 5.3 F*-values and *P*-values (in parentheses) for the analysis of the effect of denatured whey protein (WP-D) addition, renneting pH, aging, and temperature on cheese shredding properties.**

		F-value (*P*-value)				
Factors[a]	df	Fines	Long shred	Adhesion	Cohesion strength[b]	Work of breaking[c]
Whole plot						
WP-D	1	5.1 (0.06)	0.5 (0.52)	0.8 (0.41)	1.3 (0.29)	14.0* (0.01)
RpH	1	50.3* (<0.01)	45.0* (<0.01)	1.8 (0.23)	0.1 (0.82)	0.0 (0.98)
WP-D × RpH	1	8.6* (0.03)	3.3 (0.12)	0.4 (0.55)	0.1 (0.79)	0.1 (0.72)
Error	8					
Subplot						
A	2	170.6* (<0.01)	34.4* (<0.01)	3.1 (0.08)	1.9 (0.19)	0.3 (0.76)
A × WP-D	2	25.7* (<0.01)	3.1 (0.08)	1.1 (0.38)	0.6 (0.57)	0.3 (0.77)
A × RpH	2	13.1* (<0.01)	2.4 (0.13)	0.4 (0.67)	0.5 (0.65)	0.1 (0.90)
A × WP-D × RpH	2	13.6* (<0.01)	6.13* (0.01)	0.84 (0.45)	0.4 (0.70)	0.3 (0.72)
Error	16					
Sub-subplot						
T	2	2.9 (0.07)	22.3* (<0.01)	18.8* (<0.01)	61.4* (<0.01)	100.4*(<0.01)
T × WP-D	2	0.5 (0.61)	0.4 (0.65)	4.6* (0.02)	1.2 (0.31)	5.4* (<0.01)
T × RpH	2	2.9 (0.07)	0.8 (0.48)	4.2* (0.02)	1.7 (0.19)	1.8 (0.17)
T × WP-D × RpH	2	2.6 (0.09)	0.4 (0.70)	0.0 (0.99)	0.2 (0.85)	1.1 (0.36)
T × A	4	0.1 (0.98)	4.68* (<0.01)	1.7 (0.17)	1.1 (0.37)	1.3 (0.29)
T × A × WP-D	4	1.6 (0.21)	0.8 (0.55)	0.7 (0.63)	0.3 (0.90)	1.8 (0.15)
T × A × RpH	4	1.2 (0.32)	1.1 (0.37)	0.3 (0.91)	0.2 (0.93)	0.3 (0.91)
T × A × WP-D × RpH	4	1.0 (0.41)	0.7 (0.60)	0.8 (0.55)	0.3 (0.85)	1.7 (0.17)
Error	48					

[a] Split-split-plot design with the WP-D addition and renneting pH (RpH) as a factorial 2 × 2 main plot. Subplot included the effect of aging of cheese (A), and sub-subplot accounted for the effect of the shredding temperature (TO.

[b] The cohesion strength corresponds to the initial force required to penetrate the cake and depends on the closeness of the shreds and their ability to form strong links (i.e., interactions between shreds).

[c] The work of breaking represents the force required to perform the matting test, before the point where all cakes fractured.

*$P < 0.05$.

5.5.2.2 Fines Production.

The effect of factors influencing the fines production of cheeses is summarized in Table 5.3. Although the effect of aging on fines production seemed to be a dominant factor, the effect varied with renneting pH during cheese-making and WP-D concentration ($P < 0.01$). The proportion of fines produced during the cheese-shredding operation is presented in Figure 5.2. For the cheeses without WP-D, lowering the renneting pH to 6.4 and aging the cheese systematically decreased the fines production during shredding. The effect of aging and the renneting pH probably influenced the mechanical properties of the *para*casein matrix. Young Mozzarella cheese typically has a dehydrated, relatively rigid and fibrous protein structure surrounded by channels of free water and fat (Auty et al., 1999; McMahon et al., 1999). These characteristics could be responsible for cheese brittleness and partly explain the high proportion of fines produced when Mozzarella cheese is shredded (Childs et al., 2007). As reported by Gunasekaran and Ak (2002), the fracture mechanism of cheese is closely related to its microstructure and, more importantly, to the protein phase and the homogeneity of the cheese. Under sufficient stress, a rigid protein matrix is expected to fracture more cleanly. At the opposite end of the spectrum, fewer and weaker bonds holding the casein matrix together allow faster rearrangement of the cheese microstructure and better adaptation to deformation (Masi and Addeo, 1986; Watkinson et al., 2001). Heterogeneity in cheese structure can also promote microcrack formation because heterogeneity causes an unequal distribution of the stress applied to the matrix (Gunasekaran and Ak, 2002). The protein elongation during the stretching of pasta filata is recognized as promoting the distinctive segregation of protein, lipid, and aqueous phases, which is especially noticeable in young cheeses (Auty et al., 1999; Everett and Auty, 2008). Strong casein interactions, via calcium phosphate cross-linking, also contribute to the matrix heterogeneity, probably through water expulsion from the protein fibers (McMahon et al., 2005). As the cheese ages, however, the *para*casein matrix progressively absorbs the free serum trapped in the interprotein channels. Microscopic experiments showed that the fibrous protein structures eventually disappear during aging and the cheese matrix becomes more homogeneous (Auty et al., 1999; McMahon et al., 1999; Kuo and Gunasekaran, 2009). In addition to the solubilisation of CCP, the proteolysis of caseins during cold storage also contributes to protein hydration (Kindstedt and Guo, 1997; Joshi et al., 2004b; McMahon et al., 2005; Cortez et al., 2008).

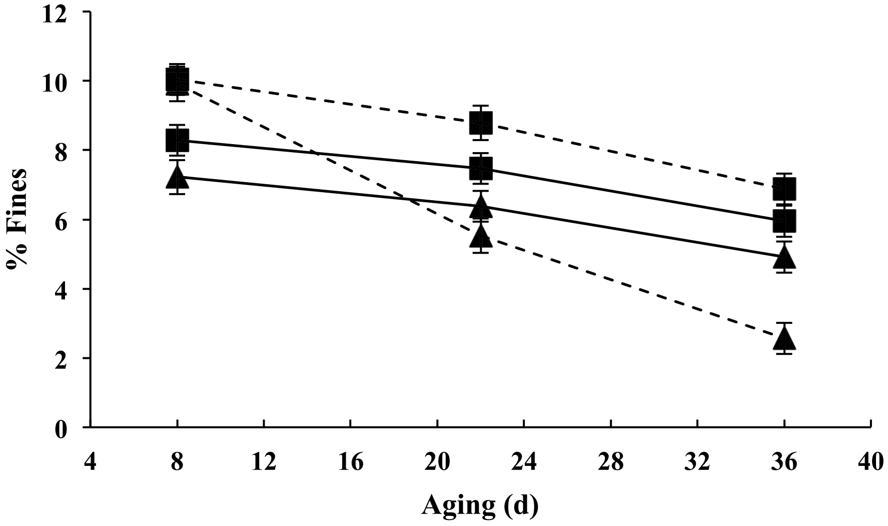

Figure 5.2 **Effect of aging on the percentage of fines measured after the shredding of Mozzarella cheeses made from milk with (0.25% WP-D; dashed lines) or without (0% WP-D; solid lines) denatured whey protein (WP-D) and renneted at pH values of 6.4 (▲) or 6.5 (■). Error bars represent the standard error of means.**

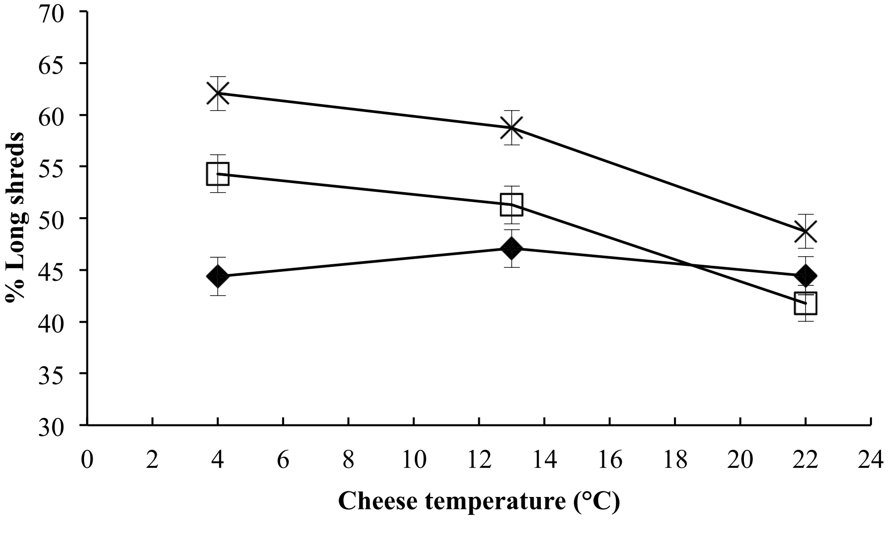

Figure 5.3 **Effect of cheese temperature during shredding on the percentage of long shreds in pizza Mozzarella cheeses aged for 8 (×), 22 (□), or 36 d (◆). Error bars represent the standard error of means.**

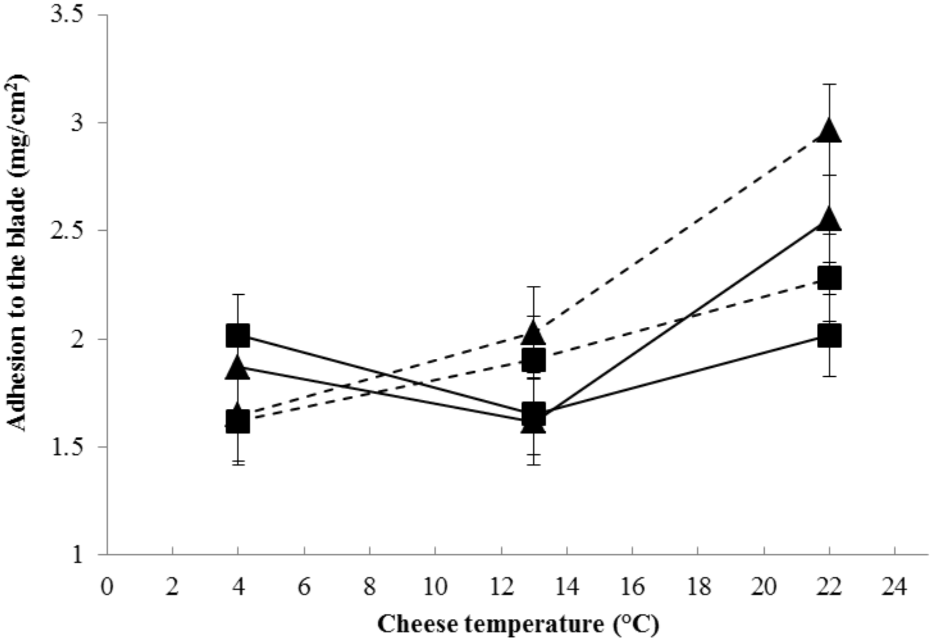

Figure 5.4 **Effect of cheese temperature during shredding on the adhesion to the blade of pizza Mozzarella cheeses made from milk with (0.25% WP-D; dashed lines) or without (0% WP-D; solid lines) denatured whey protein (WP-D) and renneted at pH values of 6.4 (▲) or 6.5 (■). Error bars represent the standard error of means.**

Figure 5.2 also shows that adding WP-D to the cheese formulation increased fines production was dramatically reduced during aging, and this effect became more important when the renneting pH was reduced. Fines production from the cheese with WP-D (renneting pH 6.4) was reduced by a factor of 4.5 between 8 and 36 d of storage. In comparison, for the cheeses without WP-D, fines production was reduced only by a factor of 1.7 over the same aging period. The effect of WP-D addition on the properties of part-skim Mozzarella cheese is not fully understood.

Some authors have suggested that WP aggregates act as inert fillers that might disrupt the *para*casein matrix of cheese (Steffl et al., 1999; Schenkel et al., 2011). The inclusion of WP-D would then induce weak spots in the matrix of young cheese. Fenelon and Guinee (1997) reported that a cheese made with commercial WP (Dairy-Lo) was more crumbly than a control cheese. Mead and Roupas (2001) noticed that the addition of WP resulted in a more fibrous Mozzarella cheese with poor functional characteristics compared with a

WP-free control cheese after the same aging period. As aging occurs, the hydration and proteolysis of the cheese *para*casein matrix facilitate the inclusion of WP-D aggregates into a reorganized and more homogeneous matrix. A low renneting pH during cheese-making would increase this effect because it has been shown to promote proteolysis (Figure 5.1). Lelièvre (1995) also suggested that a disrupted casein network, induced by lowering cheese pH, improved the integration of WP aggregates into the cheese matrix with minimal effect on microstructure. A low CCP content in cheese promoted by reducing the pH at renneting or drainage, or both, is expected to decrease casein interactions, thus loosening the protein matrix. The cheese temperature at shredding had no significant effect on fines production ($P = 0.07$), and no particular trend could be attributed to this factor. This is surprising considering that temperature has a well-known effect on cheese texture (Gunasekaran and Ak, 2002). Childs et al. (2007) reported an increase of about 71% in fines production when the shredding temperature of Mozzarella cheese increased from 12 to 20 °C. Those authors suggested that the softening of cheeses observed at higher temperatures caused more fines, but no explanation was provided. The low dependency observed between the fines production and the cheese temperature in our shredding experiment supports the hypothesis that the protein microstructure (through its fibrous arrangement) is the primary factor involved in the shattering of Mozzarella cheese into fines.

5.5.2.3 Long-Shred Production

The effect of factors influencing the long-shred production during shredding is presented in Tables 5.3. Contrarily to WP-D addition, the renneting pH significantly affected the proportion of long shreds. Reducing renneting pH from 6.5 to 6.4 increased the proportion of long shreds of 45% ± 1% to 55% ± 1% (data not shown). The production of long shreds also varied with the cheese aging time and the shredding temperature ($P < 0.01$). Figure 5.3 presents the interaction effect of aging and shredding temperature on long-shred production. For young cheese (8 d), the proportion of long shreds was shown to decrease with increasing shredding temperature, whereas for cheese aged for 36 d, the shredding temperature had no effect on the proportion of long shreds.

Chen (2003) evaluated shredded Mozzarella cheese using a sensory panel and found that shred preferences and quality were related to the length (60%), thickness (20%), and straightness (20%) of shreds. The same author reported that firmness, measured by torsion stress rheometry, was the best analytical descriptor that correlated with shred preferences. Therefore, it seems that a high proportion of long shreds is desirable when shredding Mozzarella cheese. Given that the firmness of cheese decreases with rising temperature and increasing aging time (Gunasekaran and Ak, 2002), our results suggest that firmer cheese would generally produce a larger amount of long shreds. This observation is in agreement with the assumption that firm cheeses produce shreds with better characteristics (Kindstedt, 1995; Chen, 2003; Childs et al., 2007). However, the temperature had no effect on long-shred production in older cheeses (36 d). Apparently, the appropriate microstructure for shredding cannot be restored by decreasing the shredding temperature for older cheese.

5.5.2.4 Adhesion of Cheese to Shredding Blade

The effect of factors influencing the adhesion of cheese to the blade is presented in Table 5.3. Aging the cheeses did not significantly affect the adhesion of cheese residue to the blade ($P = 0.08$). However, the trend indicates that aging increased cheese adhesion. An average difference of 18% was observed between the cheeses stored for 8 and 36 d in terms of the weights of residue adhering to the blade (data not shown). Childs et al. (2007) obtained similar results between Mozzarella cheeses aged for 7 and 28 d. The increased gumminess reported by Kindstedt (1995) in Mozzarella cheeses after 2 to 3 weeks of storage could not be clearly seen in the current study, probably because of variations in the method used for measuring adhesion to the blade. The cheeses may also have required, under the manufacturing conditions used in the current study, further aging to develop the gumminess reported by that author.

The temperature of cheese during shredding was the main factor influencing adhesion, but this effect was dependant on the presence of WP-D in cheese milk and renneting pH (Table 5.3). As shown in Figure 5.4, the weight of cheese residue was relatively low and constant at 4 and 13 °C but increased when the cheese was shredded at 22 °C. According to visual observation, the residue appeared as small and definite particles when the cheese was shredded at 4 or 13 °C. When the cheese was shredded at room temperature (22 °C), however, a spread of cheese was visible on the blade. A lower renneting pH and the addition of WP-D further increased the adhesion of the cheese shredded at 22 °C. A decrease in protein cross-linking caused by the solubilisation of CCP and a high moisture content are among the factors that promote soft and spreadable cheese (Marchesseau et al., 1997; Dimitreli and Thomareis, 2008).

5.5.2.5 Matting Behavior of Shreds

A typical curve obtained from the matting test and the descriptors used for the characterization of the shred cakes are presented in Figure 5.5. The matting test was designed to simulate the matting behavior of shreds in industrial packaging. The textural descriptors were the cohesion strength and the work of breaking of the cake. The cohesion strength corresponds to the initial force required to penetrate the cake and depends on the closeness of the shreds and their ability to form strong links (i.e., interactions between shreds). The work of breaking represents the force required to perform the matting test, before the point where all cakes fractured. Low work of breaking is a desirable attribute because it reflects the ease of separating the mat of shreds into individual identities, which facilitates shred handling and distribution (Kindstedt, 1995).

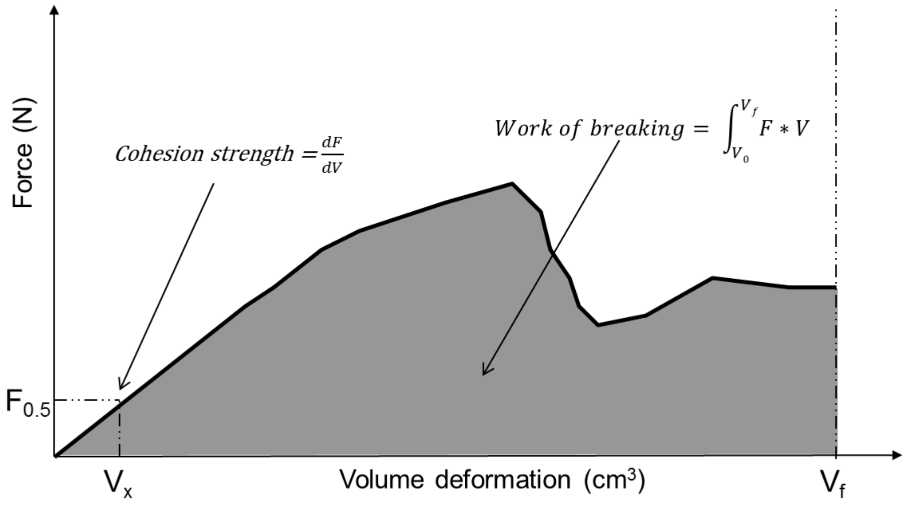

***Figure** 5.5* **Typical curve of force (F) versus volume deformation (V) of a cheese shred cake during the matting experiment. The cohesion strength is the initial slope of the curve measured at 0.5 N. The work of breaking is the force required to perform the test.**

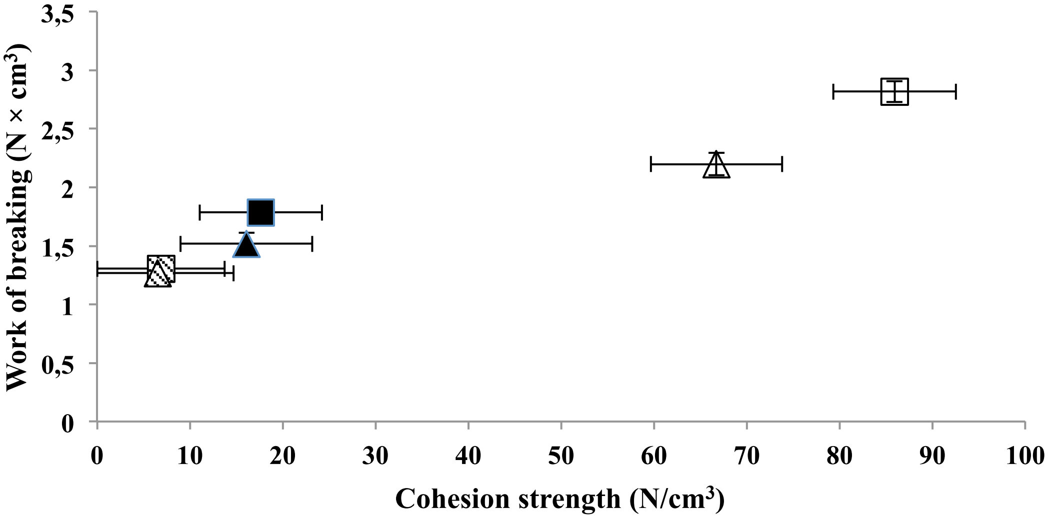

Figure 5.6 **Matting behaviour of the pizza Mozzarella cheese shred cakes represented by the cohesion strength and the work of breaking. Results are presented for cheese made from milk with (0.25% WP-D; ▲) or without (0% WP-D; ■) denatured whey protein (WP-D). The temperature during cake preparation was 4 (hatched symbols), 13 (filled symbols), or 22 °C (open symbols). All cakes were tested at 4 °C. Error bars represent standard error of mean.**

The result of the matting test was best represented as a texture map reporting the work of breaking plotted against the cohesion strength of the shred cakes (Figure 5.6). The *F*- and *P*-values associated with factors influencing the cohesion strength and the work of breaking are presented in Table 5.3. The cohesion strength varied with the temperature of the shreds at cake preparation ($P < 0.01$). As the temperature increased, the cohesion strength increased significantly (Figure 5.6). The softening of shreds at high temperatures promoted a higher compaction during cake formation. Moreover, the partial melting of fat at higher temperatures released free fat on the shred surfaces that solidified during cooling and increased the overall cohesion strength of the cakes. The work of breaking of the cakes also increased with the temperature but varied with the WP-D concentration in the cheeses ($P < 0.01$). The cakes prepared at room temperature (22 °C) fractured into big clumps but required a great amount of force to reach the fracture point. In comparison, the shred cakes prepared at 4 °C were puffy and light and fell apart easily. The addition of WP-D to the cheeses decreased the work of breaking by 15% and 22% for the cakes prepared at

13 and 22 °C, respectively. No effect of WP-D was detected at the lowest temperature (4 °C). The cause of this phenomenon is not clear, but a hypothetical explanation could be that the higher moisture content of the cheeses that contained WP-D had a lubricating effect on the shred surfaces and, thus, increased the breaking properties of the cakes.

5.5.3 Melting Experiment

The melting profiles were obtained from the rheological experiment by measuring the G* and the δ as a function of temperature. The melting profiles were pooled and presented as the means of the effect of the principal factors: WP-D concentration in milk, renneting pH, aging, and premelting treatment (Figure 5.7). As expected, increasing temperature systematically decreased G* and increased δ. The dynamic moduli are generally good indicators of the number and strength of the bonds holding the cheese matrix together (Lucey et al., 2003). The melting of cheese is a continuous process, leading to a more liquid-like (higher δ) and weaker (lower G*) material (Gunasekaran and Ak, 2002). It is generally accepted that 2 concomitant physical changes contribute to the melting of cheese: (1) the progressive liquefaction of the milk fat and (2) the reinforcement of hydrophobic bonds, which has a contracting effect on individual molecules and, thus, globally reduces the contact area between the proteins forming the cheese matrix as the temperature increases (Lucey et al., 2003; Johnson and Lucey, 2006; Guggisberg et al., 2007).

5.5.3.1 Effect of WP-D

As it would be expected, the G* measured at 5 °C was slightly lower for the cheeses with WP-D, but this difference was not significant. Indeed, no effect of the addition of WP-D to cheese on the melting profiles could be detected for temperatures under 60 °C (Figure 5.7b). Despite the higher moisture of cheeses with WP-D, their protein-to-DM ratio and fat content, 2 major compositional factors affecting meltability (Johnson and Lucey, 2006), were similar to cheeses without WP-D. However, a lower δ (at >60 °C) and a slightly higher G* (at 80 °C) were measured on the cheeses supplemented with WP-D. It has been shown that the δ values measured under melting temperatures were lower with increasing WP aggregate concentrations in cheese (Schenkel et al., 2011). As previously mentioned, the contraction of casein particles at high temperatures contributes to the melting of cheese. Whey protein particles are highly cross-linked through intermolecular disulfide bonds, limiting the capacity of those particles to reorganize and contract at high temperatures, which may explain the reduced melting performance of the cheeses containing WP-D. Lelièvre (1995) also suggested that whey protein aggregates complex to caseins and that the polymer chains formed could impair cheese meltability.

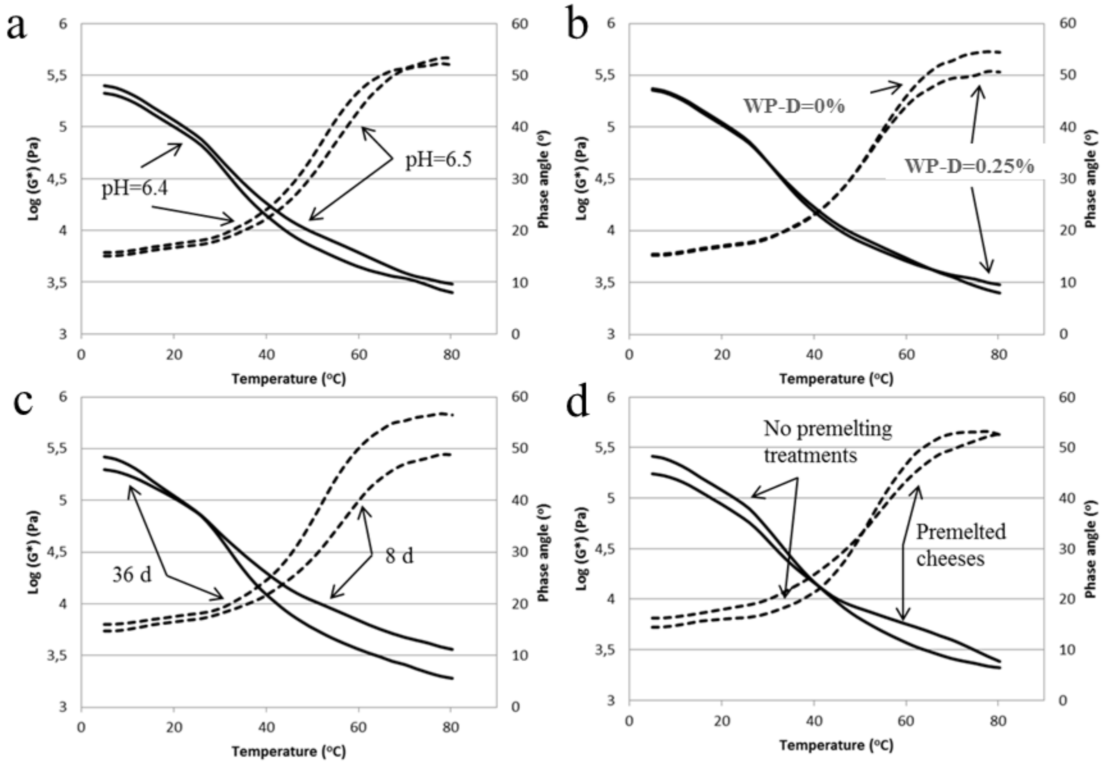

Figure 5.7 **Effect of denatured whey protein (WP-D) in milk (a), renneting pH (R; b), cheese aging (c), and premelting treatment (d) on the melting profiles of pizza Mozzarella cheeses assessed by the complex modulus (G*; solid lines) and the phase angle (δ; dashed lines).**

5.5.3.2 Effect of Renneting pH

Modifying the renneting pH of milk affected the melting profiles of the cheeses (Figure 5.7a). At low temperatures (5-30 °C), the G* of cheeses made with milk renneted at pH 6.4 was slightly lower than those made with milk renneted at 6.5. The same trend was observed for temperatures at which milk fat in cheese is expected to be in the liquid state (>40 °C; Vithanage et al., 2009). It is likely that the strength and number of cross-linkages between the caseins forming the cheese network influenced the physical properties of the cheeses, as reflected by the complex modulus (Gunasekaran and Ak, 2002). O'Mahony et al. (2006) demonstrated that the storage and loss moduli of melted cheeses decreased with decreasing concentration of CCP. Therefore, the results from Figure 5.7a were expected, considering the effect of the renneting pH on both the CCP content and proteolysis in the cheeses (Figure 5.1). For temperatures ranging from 40 to 60 °C, higher δ values were observed for the cheeses renneted at pH 6.4 compared with those renneted at 6.5. Higher δ values are associated with a more liquid-like behavior under mechanical deformation at high temperatures, which improves flow properties during melting.

5.5.3.3 Effect of Aging

The aged cheeses (36 d) showed a lower G* and a higher δ compared with the young cheeses (8 d) for all the temperatures tested (Figure 5.7c). This result is in keeping with the general agreement that cheeses develop better melting properties as they age. Numerous studies showed that the proteolysis of caseins and the loss of CCP, both of which occur during refrigerated aging, are primary factors that increase cheese meltability (Kindstedt, 1993; Joshi et al., 2004b; Choi et al., 2008).

5.5.3.4 Effect of Premelting Treatment

The melting profiles of the cheeses were affected by a premelting treatment before testing (Figure 5.7d). The premelted cheeses were softer (lower G*) and less elastic (higher δ) at lower temperatures (<40 °C) than the cheeses that had not undergone the premelting treatment. Interestingly, the opposite trend was observed for temperatures above 50 °C. Premelting the cheese modified its physical properties and rheological behavior depending on the temperature. Monitoring and understanding the changes induced by the baking of cheese could help to better control the desirable physical attributes of cooled or reheated cheese-topped dishes. Under classic baking conditions, moisture and fat losses explain most of the textural changes in cheese after solidification (Gunasekaran and Ak, 2002; Kim et al., 2011). In the present study, the premelting treatment was applied in a closed environment, and the composition of the cheeses was unchanged. The higher G* observed above 50 °C for the premelted cheese is probably associated with a reinforcement of the protein matrix, given that the contribution of milk fat to the G* above 40 °C is assumed to be minimal (Vithanage et al., 2009; Yang et al., 2011). Some studies previously suggested that protein interactions, perhaps through calcium bonding, play a role in the physical properties of melted and post-melted cheeses (Metzger et al., 2001; Pastorino et al., 2002; Kim et al., 2011). In agreement with our study, Kuo et al. (2001) also measured variations in the meltability of Mozzarella cheeses with different heating histories. Changes in fat distribution during premelting could possibly explain the lower G* and higher δ in those cheeses below 40 °C. Previous studies reported the formation of large pools during the baking of Mozzarella cheeses, driven by the reorganization of the protein matrix (Kindstedt, 1993; Auty et al., 1999). According to Laplace's pressure theory, increasing fat globule size for a constant volume fraction reduces the contribution of lipids to cheese firmness (Everett and Auty, 2008).

5.5.3.5 Softening Point

The softening point temperature of cheese represents the transition phase where the dissipated mechanical energy is equal to the energy stored in the cheese structure (Gunasekaran and Ak, 2002). An increase in the softening point temperature has been negatively correlated with cheese meltability as measured by empirical tests (Stenz et al., 2006; Ko and Gunasekaran, 2008). The renneting pH and WP-D conditions did not significantly affect the softening point temperature of the cheeses. However, the average softening point temperature decreased from 66 to 57 °C for the cheeses aged 8 and 36 d ($P < 0.01$). Increased proteolysis and decreased CCP concentration are among the factors known to be responsible for increased meltability (flow) improvement (Kindstedt et al., 1995). Both factors were observed during the aging of the Mozzarella cheeses (Figure 5.1). Premelting of the cheeses significantly increased the average softening point temperature by 3 °C ($P < 0.01$), thus confirming that cheese meltability was impaired by heat-induced changes.

5.6 Conclusions

The aging of pizza Mozzarella cheeses that were manufactured from milk with or without WP-D and renneted at 2 different pH values resulted in products that varied in terms of their moisture, CCP, and soluble-nitrogen contents. These manufacturing conditions also generated major effects on the physical properties of both the unmelted and melted cheeses. A lower milk renneting pH and cheese aging can effectively decrease fines production during shredding. Moreover, our results show that controlling these 2 manufacturing conditions is essential when WP-D is included in cheese milk formulations. In contrast, when the cheeses were aged, the proportion of long shreds was lower, thus affecting overall shreddability. To reduce the adhesion of the cheese to the blade and to maximize the shred size, pizza Mozzarella cheese should be shredded at low temperatures. In addition, shredded cheese should also be packaged and stored at low temperatures to reduce defects associated with the matting of shreds. The renneting pH of milk and the aging of cheese should also be controlled to produce pizza Mozzarella cheese with desirable melting properties. In addition, the physical properties of cheeses that have been premelted were investigated. A freshly baked pizza topped with Mozzarella cheese can be eaten right away or reheated the next day, and the cheese is expected to perform well in both conditions. This work demonstrates that the premelting of cheese leads to important changes in its physical properties (G^* and δ), probably through heat-induced microstructural rearrangements. Further studies will investigate the mechanism behind heat-induced textural, structural changes and its relationship with consumers' perceptions.

5.7 Acknowledgements

This work was financially supported by the Natural Sciences and Engineering Research Council of Canada (Ottawa, ON, Canada), the Fonds de recherche du Québec – Nature et technologies (Québec City, QC, Canada), and Agropur Dairy Cooperative (Longueuil, QC, Canada). The authors thank their colleagues at Agriculture and Agri-Food Canada (Saint-Hyacinthe, QC, Canada) and Agropur Dairy Cooperative for their scientific and technical expertise.

Chapter 6 Shreddability of pizza Mozzarella cheese predicted using physicochemical properties

6.1 Résumé

Une étude des propriétés physiques du fromage pizza Mozzarella a été entreprise en utilisant des tests de compression uniaxiale, de découpe par fil et de rhéologie dynamique sous faible cisaillement. Cette caractérisation a permis de construire des modèles prédictifs de l'aptitude au râpage des fromages en utilisant des données recueillies sur sa texture et sa composition. Pour ce faire, des fromages expérimentaux ont été produits avec du lait avec (0.25% [m/m]) ou sans ajout de protéines sériques dénaturées, et avec un pH d'emprésurage de pH 6.5 ou 6.4. En tout, 11 descripteurs de texture ont été mesurés sur des fromages à 4, 13, ou 22 °C, et entreposés pendant 8, 22, ou 36 jours. Les propriétés physiques étaient fortement influencées par la présence de protéines sériques dénaturées et par le pH d'emprésurage, mais ces propriétés étaient aussi dépendantes de la température du fromage. Une réduction des paramètres associés à la rigidité du fromage était aussi observée avec l'augmentation du temps d'entreposage. Aucun des descripteurs de texture ou de composition ne pouvait à lui seul prédire l'aptitude au râpage de façon satisfaisante. Par ailleurs, l'utilisation d'une méthode de sélection objective des descripteurs a permis de prédire la production de fines au râpage ($R^2 = 0.82$), le pourcentage de longues râpures ($R^2 = 0.67$), et dans une moindre mesure, l'adhésion du fromage ($R^2 = 0.45$) en utilisant 4 descripteurs ou moins. Une analyse en composante principale des résultats a permis d'établir un contraste clair entre certaines caractéristiques observées lors du râpage : soit l'adhésion du fromage d'une part, et d'autre part la longueur des râpures et la quantité de fines produites. Les résultats de cette étude permettent de mieux cibler les caractéristiques de texture et de composition nécessaires pour le développement de fromage ayant des propriétés mécaniques optimales lors de son râpage.

6.2 Abstract

This study used rheological techniques such as uniaxial compression, wire cutting, and dynamic oscillatory shear to probe the physical properties of pizza Mozzarella cheeses. Predictive models were built using compositional and textural descriptors to predict cheese shreddability. Experimental cheeses were made using milk with (0.25% [w/w]) or without denatured whey protein and renneted at pH 6.5 or 6.4. The cheeses were aged for 8, 22, or 36 d and then tested at 4, 13, or 22 °C for textural attributes using 11 descriptors. Adding denatured whey protein and reducing the milk renneting pH strongly affected cheese mechanical properties, but these effects were usually dependent on testing temperature. Cheeses were generally weaker (softer) as they aged. None of the compositional or rheological descriptors taken alone could predict the shredding behavior of the cheeses. Using the stepwise method, an objective selection of a few (<4) relevant descriptors made it possible to predict the production of fines ($R^2 = 0.82$), the percentage of long shreds ($R^2 = 0.67$), and to a lesser degree, the adhesion of cheese to the shredding blade ($R^2 = 0.45$). The principal component analysis markedly contrasted the adhesion of cheese to the shredding blade with other shredding properties such as the production of fines or long shreds. The predictive models and the principal component analysis can help manufacturers to select relevant descriptors for the development of cheese with optimal mechanical behavior under shredding conditions.

6.3 Introduction

The ability of cheese to be shredded, sliced, or diced is a critical concern for cheese manufacturers. Given the continuous growth of the cheese ingredient sector, a size reduction process that has not been optimized may reduce cheese quality and result in significant economic losses. When shredding problems do occur, manufacturers commonly describe them as excessive shattering of cheese into fine particles (fines), uneven distribution of shred sizes, or sticking of cheese to the processing equipment (Kindstedt, 1995; Childs et al., 2007). In all cases, shredding defects are related to inappropriate mechanical properties of the cheese. It is well known that cheese for pizza must meet narrow specifications for meltability, stretchability, and free oil release (Kindstedt et al., 2010). In addition, the physicochemical properties of cheese to the point of shredding must be controlled for optimal shredding.

Despite the obvious importance of cheese shreddability to the cheese industry, the factors influencing shreddability have received little attention, and thus their effects remain largely unclear. According to Kindstedt (1995) and Childs et al. (2007), the lack of a suitable method to characterize or predict shredding properties is responsible for the limited information in this field. Indeed, most reports on the subject are based on empirical observations (Kindstedt, 1995; Rankin et al., 2006; Lucey, 2008). Some authors have proposed that shreds be visually characterized for quality control purposes (Apostolopoulos and Marshall, 1994; Ni and Gunasekaran, 2004). Childs et al. (2007) directly measured the production of fines and the adhesion to the blade in Mozzarella cheeses with varying fat and dry matter contents using controlled, small-scale shredding equipment. In chapter 5, this method was adapted to evaluate the effects of different cheese-making conditions on the shreddability of pizza Mozzarella cheese. Despite those advances, there is a clear need for physicochemical descriptors that could predict the shredding properties of cheese before it is shredded.

From a materials science approach, shreddability is an overall indication of the complex mechanical behavior of cheese when it is submitted to high shear. In other words, the shredding behavior of a cheese is assumed to be strongly dependent on its rheological properties, such as plastic deformation, fracture, and friction (Gunasekaran and Ak, 2002;

Goh et al., 2005; Childs et al., 2007). Over the last few decades, the rheological properties of cheese have been characterized using an impressive number of descriptors, testing apparatuses, and protocols (International Dairy Federation, 1991; Gunasekaran and Ak, 2002), including the extensive use, for research and quality control purposes, of tests that induce large deformation. More recently, advanced rheological techniques such as dynamic rheometry performed under small deformation were successfully applied to study macromolecular interactions in cheese structure (Lucey et al., 2003; Choi et al., 2008; Tunick, 2011). Some authors proposed that the information obtained under low deformation could shed some light on the physical properties observed under larger deformation (Drake et al., 1999; Muliawan and Hatzikiriakos, 2007). It is well accepted that the rheological properties of cheese are governed by the structural elements of the material (e.g., fat, casein, and minerals) and by the way in which they are arranged in a substructure (i.e., the microstructure and the number, strength, and nature of interacting bonds). Therefore, the combination of different rheological techniques is, in the present authors' view, of primary importance for obtaining an overall global portrait of the effect of intrinsic and extrinsic factors on cheese texture and shredding behavior.

In a previous study, the present authors showed that the shredding behavior of pizza Mozzarella cheese was strongly affected by cheese-making conditions (Chapter 5). The first objective of the present study was to characterize the rheological properties of pizza Mozzarella cheese using descriptors from both small and large deformation techniques. The second objective was to use rheological and compositional descriptors to build predictive models for the shredding behavior of pizza Mozzarella cheese.

6.4 Material and methods

6.4.1 Pizza Mozzarella Cheeses

Pizza Mozzarella cheeses were produced in pilot plant as described in chapter 5. In that study, the effects of 36 combinations of cheese-making, aging, and testing conditions on the shreddability and meltability of pizza cheese were determined. For that purpose, a factorial split-split-plot experimental design was applied. Briefly, the cheese milks were standardized with 2 concentrations (0% or 0.25% [w/w] in milk) of denatured whey protein (WP-D) and were then renneted at a pH 6.5 (R6.5 cheese) or 6.4 (R6.4 cheese). The draining pH of the R6.4 cheeses was also decreased by 0.1 unit compared to that of the R6.5 cheeses. The WPC used originated from a single batch and was produced using a proprietary process from Agropur Cooperative (Granby, QC, Canada). After manufacture, the cheeses were allowed to age for 8, 22, or 36 d (subplot) at 4 °C, and then physical analyses were performed on the cheeses at 4, 13, or 22 °C (sub-subplot). Three independent cheese batches were made. The detailed composition and the shredding and melting properties of the cheeses were previously reported in chapter 5.

6.4.2 Rheological Analysis

The rheological properties of the cheeses were measured at 3 temperatures (4, 13, or 22 °C) using large and small deformation systems. Large deformation testing was performed with a uniaxial texture analyzer (TA-XT2; Stable Micro Systems, Scarsdale, NY). Three protocols were used: (1) the texture profile analysis (TPA) test; (2) the single lubricated compression (SLC) test; and (3) the wire-cutting test.

For the TPA and SLC tests, cylindrical cheese samples (20 mm in diameter, 25 mm in height) were compressed using a 38-mm Plexiglas cylinder fixture mounted on the moving head of the TA-XT2 texture analyzer. The TPA test consisted of a double compression (50% Hencky strain) that had a relaxation interval of 15 s and was performed at a constant crosshead speed of 1 mm s^{-1}. Hardness, adhesiveness, and cohesiveness were calculated from the TPA profiles, as proposed by Bourne (1978).

For the SLC test, the cheese sample surfaces were coated with paraffin oil (Cat. No. BP2629-1; Fisher Scientific, Whitby, On, Canada). In this case, the deformation reached 80% of the cheese height and was applied at a constant strain rate of 2.5% s^{-1}. The Young's modulus (Y_m) was determined as the initial slope of the stress-strain curve, calculated between 2.5% and 5% Hencky strain. As reported by Charalambides et al. (2001), at larger strains, the stress-strain curve of Mozzarella cheese is characterized by an inflection point followed by a fairly linear portion between 50% and 70% deformation. This last portion of the stress-strain curve represents the cheese flow under imposed deformation, and the slope corresponds to the flow modulus (F_m). No fracture was observed within this strain range for all conditions tested.

For the wire-cutting test, stainless-steel wires measuring 0.35 or 0.9 mm in diameter and mounted on a U-shaped tool were used to cut cheese cubes (25-mm edges) at 0.5 mm s^{-1}. Kamyab et al. (1998) demonstrated that the force measured at steady-state cutting (F_c) for a constant length of cheese (B) is proportional to the wire diameter (d), the yield stress of cheese (σ_y), and the friction coefficient at the cutting interface (μ) with an intercept that represents the fracture toughness (F_t).

Equation 6.1 Wire-cutting test parameters calculation

$$F_c/B = F_t + C_r \times d$$

$$\text{where } C_r = (1 + \mu)\, \sigma_y$$

When F_c/B is represented as a function of different wire diameters, the resulting slope is a linear function that represents the forces opposed to cutting: the yield stress of cheese and friction energy with the wire (Eq. 6.1). This complex rheological descriptor was called the cutting resistance (C_r). Moreover, the intercept (F_t) of the experimental data for F_c/B versus wire diameter (d) represents the fracture toughness of cheese. Kamyab et al. (1998) observed good agreement between the fracture toughness measurements obtained from the wire-cutting test and the tensile test of single-edge notched cheeses, the latter being a common fracture test for engineering materials.

Small amplitude oscillatory measurements were performed using a Physica MCR 301 rheometer (Anton Paar, Graz, Austria) equipped with 25-mm parallel plates (PP25, Anton Paar) with a Peltier temperature controller (C-PTD 200 Peltier system; Anton Paar). Sandpaper (silicon carbide, 180B grit; Mastercraft, Montreal, QC, Canada) was glued to the plates to prevent slipping. A cheese disc (25 mm in diameter, 3 mm in height) was loaded at 1 N normal force and equilibrated for 5 min. Preliminary results revealed that the linear viscoelastic region limit was greater than 0.2% strain for all conditions tested, and subsequent analyses were performed at 0.1% strain. The mechanical spectrum of each cheese was obtained by recording the complex modulus (G^*) and the phase angle (δ) during a frequency sweep test. Subramanian et al. (2006) showed that the frequency dependence of the moduli can be fitted to a power law model. The mechanical spectrum was characterized using equations 6.2 and 6.3, as follows:

Equation 6.2 Mechanical spectrum of the complex modulus

$$G^* = G^*_1 \, \omega^{G^*fd}$$

Equation 6.3 Mechanical spectrum of the phase angle

$$\delta = \delta_1 \, \omega^{\delta fd}$$

Where ω is the frequency (Hz) and G^*_1 and δ_1 represent the complex modulus and phase angle measured at 1 Hz, respectively. The G^*_{fd} and δ_{fd} descriptors are the power indexes, which represent the frequency dependence of the moduli between 1 and 50 Hz.

6.4.3 Predictive Modeling of Cheese Shreddability

Compositional and rheological descriptors were used as potential explicative variables to build predictive models for cheese shreddability. The modeling procedure consisted of 2 steps: (1) the validation of the linearity of the independent variables (explicative variables) against the dependent variables (shreddability variables); and (2) the selection of a limited number of meaningful explicative variables to build predictive models.

The linearization of the explicative variables was checked using the Box–Cox method (Sakia, 1992; Bingham and Fry, 2010). This procedure calculates the mathematical transformation that would optimize the linearity of a data set. If needed, the data from a descriptor were transformed as suggested by the test. The linearity and normality of the data were validated using residual and normality plots.

The variables to be included in the models were selected using the stepwise selection method (Bingham and Fry, 2010). For this procedure, the variables were added or removed (if necessary) from the model 1 step at a time. An ANOVA was performed at each step, and the algorithm was repeated until no further variable could be added or removed to minimize the model error. Residual plots were checked to ensure model validity.

6.4.4 Statistical Analyses

Statistical analyses were conducted using the SAS software package (v. 2.0.3, 2008; SAS Institute Inc., Cary, NC). The effects of WP-D addition, renneting pH, aging, and cheese testing temperature on the rheological properties of the cheeses were analyzed using PROC MIXED. Effects were declared significant when the probability level of F was $P \leq 0.01$. The Box–Cox test and the selection of variables for modeling were carried out using PROC REG. In addition, PROC FACTOR was used to perform a principal component analysis (PCA) based on rheological, compositional, and shreddability variables.

6.5 Results

6.5.1 Composition of Cheese

Detailed composition of cheese was previously reported in chapter 5. In that study, the compositional analysis revealed that making cheese with 0.25% WP-D increased the total moisture content of about 2.4%. It has been shown that the WP-D added was effectively retained in cheese as highlighted by a higher retention coefficient of protein compared to cheese made without WP-D. Lowering the renneting pH slightly decreased the protein content in cheese (−0.5%) and brought down the ash content by about 0.14%.Cheeses made with milk renneted at pH 6.4 contained less colloidal calcium and more water soluble nitrogen than R6.5 cheeses throughout the aging period.

Table 6.1 **Values of *F* and values of *P* (in parentheses) for the effects of added denatured whey protein, renneting pH, aging, and testing temperature on the rheological descriptors of cheeses.**

		Large deformation rheology							Small amplitude oscillatory shear			
		Texture profile analysis[b]			Single lubricated compression[c]		Wire-cutting[d]		Frequency sweep[e]			
Factor[a]	df	H	Adh	Coh	Y_m	F_m	C_r	F_t	G^*_1	G^*_{fd}	δ_1	δ_{fd}
Whole plot												
WP-D	1	25.7*(<0.01)	0.37 (0.56)	13.0* (0.01)	12.0* (0.01)	11.5 (0.02)	42.5*(<0.01)	8.5 (0.03)	54.4*(<0.01)	2.5 (0.16)	0.7 (0.44)	1.1 (0.33)
RpH	1	35.4*(<0.01)	0.39 (0.55)	12.3* (0.01)	11.7* (0.01)	57.0*(<0.01)	13.6* (0.01)	9.3 (0.02)	14.8* (0.01)	18.1*(<0.01)	50.1*(<0.01)	6.9 (0.04)
WP-D × RpH	1	1.2 (0.30)	1.65 (0.25)	1.9 (0.22)	0.2 (0.89)	2.8 (0.15)	0.5 (0.51)	0.0 (0.90)	2.7 (0.15)	5.2 (0.06)	9.5 (0.02)	2.7 (0.15)
Error	8											
Subplot												
A	2	54.2*(<0.01)	27.2*(<0.01)	76.6*(<0.01)	49.4*(<0.01)	10.5*(<0.01)	25.3*(<0.01)	4.9 (0.03)	2.1 (0.16)	3.5 (0.06)	9.0*(<0.01)	15.9*(<0.01)
A × WP-D	2	0.1 (0.92)	0.3 (0.75)	1.8 (0.21)	0.2 (0.81)	2.6 (0.11)	2.1 (0.17)	0.7 (0.51)	0.3 (0.75)	1.8 (0.22)	1.6 (0.24)	0.1 (0.92)
A × RpH	2	1.6 (0.24)	4.3* (0.04)	2.4 (0.14)	1.6 (0.24)	0.2 (0.80)	0.9 (0.45)	0.1 (0.88)	1.8 (0.20)	1.0 (0.40)	2.4 (0.12)	17.7*(<0.01)
A × WP-D × RpH	2	2.1 (0.16)	1.7 (0.22)	0.1 (0.87)	0.9 (0.44)	0.0 (0.97)	0.9 (0.45)	0.7 (0.52)	0.2 (0.84)	0.9 (0.43)	0.7 (0.49)	2.1 (0.16)
Error	16											
Sub-subplot												
T	2	543.7* (<0.01)	50.0* (<0.01)	68.7* (<0.01)	289.4* (<0.01)	280.7* (<0.01)	55.7* (<0.01)	36.6* (<0.01)	230.* (<0.01)	157.1* (<0.01)	169.3* (<0.01)	72.8* (<0.01)
T × WP-D	2	11.7*(<0.01)	1.8 (0.18)	0.4 (0.65)	13.4*(<0.01)	2.3 (0.12)	4.6 (0.02)	0.0 (0.97)	6.6*(<0.01)	0.1 (0.89)	0.4 (0.69)	2.1 (0.13)
T × RpH	2	5.0* (0.01)	0.1 (0.87)	2.8 (0.07)	3.9 (0.03)	2.9 (0.07)	0.2 (0.84)	1.5 (0.24)	4.6 (0.02)	3.5 (0.04)	3.3 (0.05)	3.3 (0.05)
T × WP-D × RpH	2	0.7 (0.52)	0.9 (0.43)	0.6 (0.57)	0.0 (0.97)	0.5 (0.63)	1.6 (0.22)	0.4 (0.65)	0.2 (0.83)	0.9 (0.41)	0.4 (0.71)	1.1 (0.36)
T × A	4	0.5 (0.70)	3.2 (0.02)	6.0*(<0.01)	5.6*(<0.01)	1.7 (0.17)	1.7 (0.18)	0.4 (0.82)	3.8 (0.03)	3.4 (0.02)	6.7*(<0.01)	1.5 (0.22)
T × A × WP-D	4	0.8 (0.52)	0.7 (0.61)	0.6 (0.68)	0.2 (0.96)	0.9 (0.50)	1.4 (0.26)	1.3 (0.28)	0.4 (0.78)	1.6 (0.20)	0.5 (0.70)	1.3 (0.27)
T × A × RpH	4	0.3 (0.89)	0.1 (0.99)	2.2 (0.09)	1.4 (0.24)	1.4 (0.25)	0.4 (0.82)	0.2 (0.93)	0.5 (0.75)	1.2 (0.34)	2.6 (0.05)	2.8 (0.04)
T × A × WP-D × RpH	4	0.9 (0.46)	1.1 (0.37)	0.8 (0.51)	0.2 (0.94)	0.4 (0.81)	0.2 (0.91)	0.5 (0.77)	0.3 (0.85)	1.7 (0.17)	2.1 (0.09)	0.6 (0.66)
Error	48											

[av] Split-split-plot design with added denatured whey protein (WP-D) and renneting pH (RpH) as the main plot in the factorial 2 × 2 design. The subplot accounted for the effect of aging of the cheeses (A), and the sub-subplot accounted for the effect of the cheese temperature at testing (T).

[b] Rheological descriptors from the texture profile analysis test: hardness (H), adhesiveness (Adh), and cohesiveness (Coh).

[c] Rheological descriptors from the single lubricated compression test: Young's modulus (Y_m) and flow modulus (F_m).

[d] Rheological descriptors from the wire-cutting test: cutting resistance (C_r) and fracture toughness (F_t).

[e] Rheological descriptors from the frequency sweep test: complex modulus measured at 1 Hz (G^*_1), frequency dependence of the complex modulus (G^*_{fd}), phase angle measured at 1 Hz (δ_1), and frequency dependence of the phase angle (δ_{fd}).

$^*P \leq 0.01$

6.5.2 Rheological Properties of Cheeses

The effects of cheese-making factors on the rheological properties of the cheeses are presented in Table 6.1. Highly significant ($P \leq 0.01$) effects on texture are shown in Figures 6.1 to 6.4 and are described in detail for each testing procedure below.

6.5.2.1 Texture Profile Analysis Test

Hardness measured by TPA represents the force necessary to reach the first compression peak of deformation. The hardness of cheeses at different testing temperatures varied with WP-D concentration and renneting pH (Figure 6.1a). As a general trend, adding denatured WP-D to the cheese milk or reducing the renneting pH decreased cheese hardness. When cheese temperature increased, hardness decreased more for the 0% WP-D cheeses and the R6.5 cheeses (-1.5 N $°C^{-1}$) than for the 0.25% WP-D cheeses and the R6.4 cheeses (-1.2 N $°C^{-1}$). Hardness also decreased when the cheeses were aged at 22 d (30 N) rather than 8 d (37 N) but did not change afterward (28 N at 36 d) (Figure 6.1a).

Adhesiveness measured by TPA represents the energy necessary to overcome the attractive forces between the cheese surface and the testing fixture (International Dairy Federation, 1991). As illustrated in Figure 6.1b, the older cheeses (22 and 36 d) were generally more adhesive (approximately +30%) than were the young cheeses (8 d). It was also observed that adhesiveness dropped by 33% when the cheeses were tempered at 22 °C in comparison with lower temperatures (Figure 6.1b). Adding WP-D or modifying the renneting pH had no impact on cheese adhesiveness (Table 6.1).

The TPA test also allows the measurement of cheese cohesiveness, an index of the strength of the internal bonds making up the body of cheese (Gunasekaran and Ak, 2002). Cohesiveness varied depending on the age and temperature of the cheeses (Figure 6.1c). The young cheeses (8 d) were more cohesive than the older cheeses were at all temperatures. Moreover, the cohesiveness of the cheeses aged for 8 or 22 d increased when the temperature rose to 22 °C in comparison with lower temperatures. In contrast, cohesiveness in the older cheeses was not significantly impacted by temperature. The R6.5 and 0% WP-D cheeses showed slightly but significantly higher cohesiveness (+4.4%) in comparison with that of the R6.4 and 0.25% WP-D cheeses (Figure 6.1c).

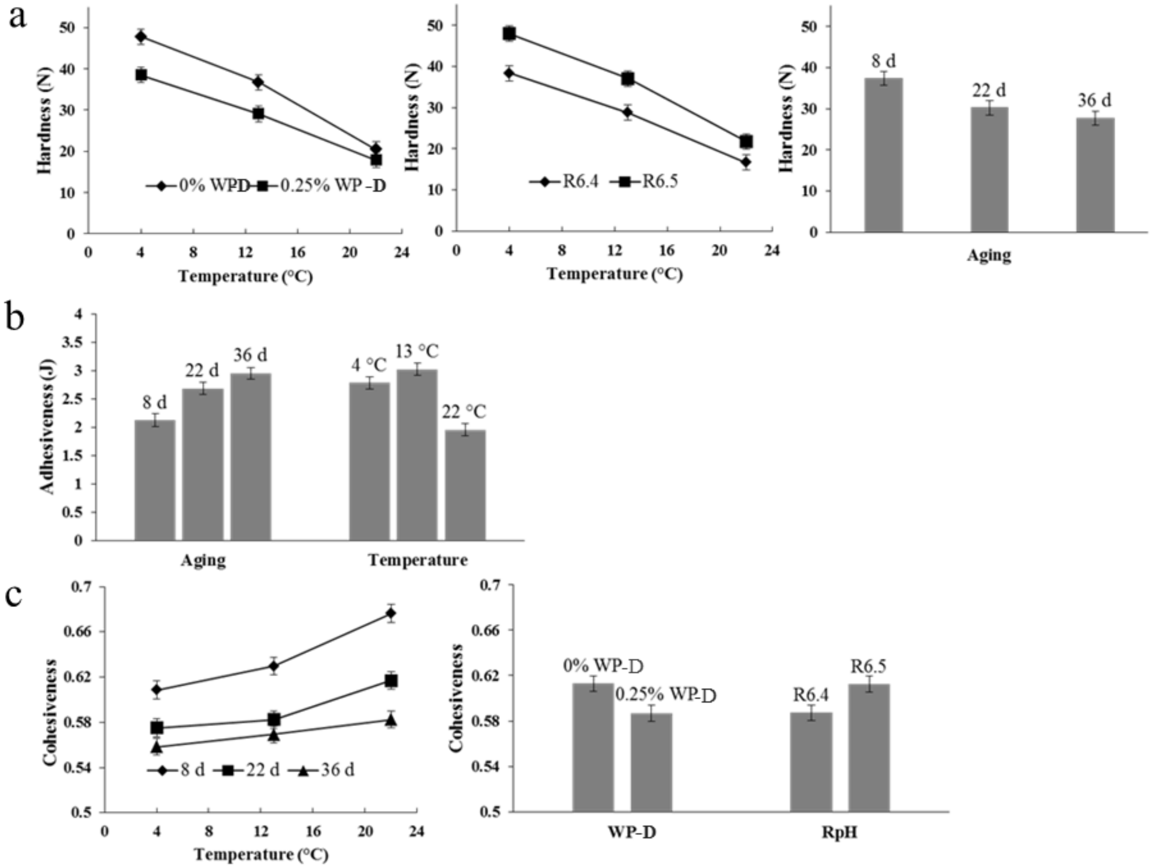

Figure 6.1 **Effects of added denatured whey protein (WP-D), renneting pH (RpH; R6.4 = renneting at pH 6.4; R6.5 = renneting at pH 6.5), aging, and testing temperature on 3 cheese texture descriptors, (a) hardness, (b) adhesiveness, and (c) cohesiveness, as determined using texture profile analysis. Error bars represent the standard errors of the means.**

6.5.2.2 Single Lubricated Compression Test

Unlike the TPA test, the SLC test allows the friction effect at the cheese/plate interface to be ignored, and a homogeneous deformation throughout the sample is therefore expected (Ak and Gunasekaran, 1993; Charalambides et al., 2001). A true stress-strain curve was built, and the initial slope (Young's modulus, or Y_m), is an index of the stiffness of the material (Gunasekaran and Ak, 2002). As shown in Figure 6.2a, the Y_m of the 0% WP-D cheeses was higher at 4 °C (+41%) and decreased more with increasing temperature

(−9.9 kPa °C^{-1}) in comparison with the Y_m of the 0.25% WP-D cheeses (−6.5 kPa °C^{-1}). The Y_m of the young cheeses (8 d) was higher than that of the older cheeses at 4 and 13 °C, but no significant difference was observed at 22 °C (Figure 6.2a). The mean Y_m of the R6.5 cheeses was consistently higher (+25%) than that of the R6.4 cheeses (Figure 6.2a).

The SLC test also allows the calculation of the flow modulus, or F_m. The F_m represents the plastic behavior of cheese under large and continuous deformation. The larger the F_m value, the higher the stress input is required to induce continuous deformation. The F_m of the R6.5 cheeses was always higher (+31%) than that of the R6.4 cheeses (16.4 kPa) (Figure 6.2b). During aging, the mean F_m decreased, from 21.6 kPa at 8 d to 18.2 and 17.6 kPa at 22 and 36 d, respectively. The F_m of pizza Mozzarella cheeses decreased markedly with increased temperature, from 28.9 kPa at 4 °C to 7.9 kPa at 22 °C (Figure 6.2b).

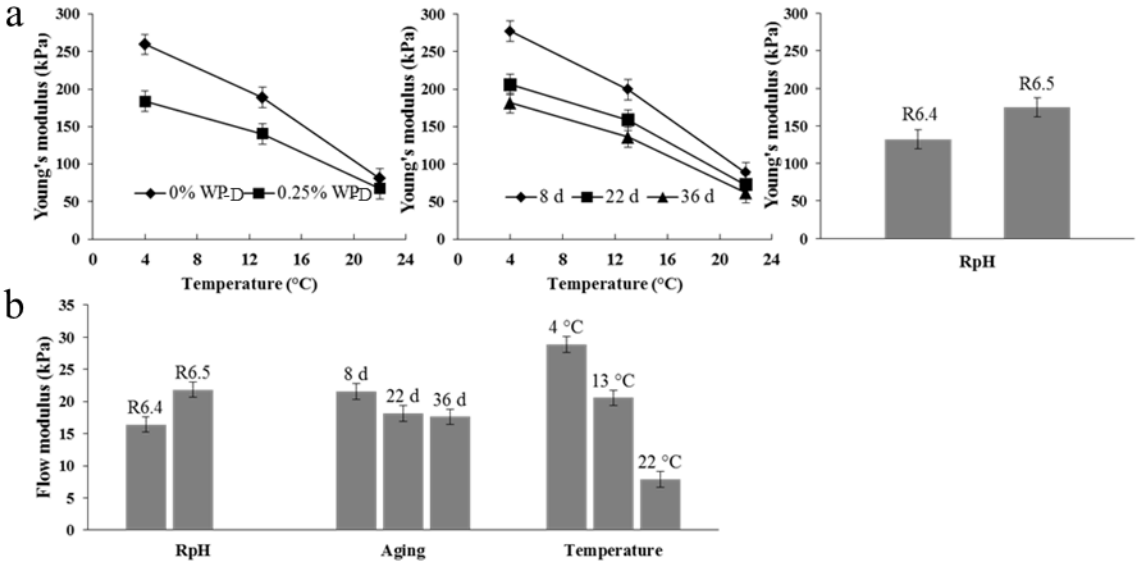

Figure 6.2 **Effects of added denatured whey protein (WP-D), renneting pH (RpH; R6.4 = renneting at pH 6.4; R6.5 = renneting at pH 6.5), aging, and testing temperature on 2 cheese texture descriptors, (a) Young's modulus and (b) flow modulus, as determined using the single lubricated compression test. Error bars represent the standard errors of the means.**

6.5.2.3 Wire-Cutting Test

The energy necessary to cut cheese depends on the friction, flow (plastic/viscous), and fracture behavior of the cheese matrix (Kamyab et al., 1998; Goh and Kindstedt, 2005). The present study uses the broad term cutting resistance, or C_r, to represent the energy necessary to overcome the friction and plasticity behavior of cheese during wire-cutting. The C_r was highly affected by all principal factors, namely WP-D, renneting pH, aging, and temperature. Adding WP-D to the cheeses or lowering the renneting pH decreased the mean C_r by about 31% and 17%, respectively (Figure 6.3a). The mean C_r was higher for the cheeses tested at 8 d of age (124 kPa) than for the cheeses aged longer, for 22 d (92 kPa) or 36 d (79 kPa). The increase in temperature caused a major decrease in the C_r, especially between 13 and 22 °C (Figure 6.3a).

The fracture toughness, or F_t, of a material represents the amount of energy per unit area necessary to create new crack surfaces (Charalambides et al., 1995; Gunasekaran and Ak, 2002). The cheese F_t decreased from 5.5 to 3.8 and 2.7 J m^{-2} when the testing temperature increased from 4 to 13 and 22 °C, respectively (Figure 6.3b).

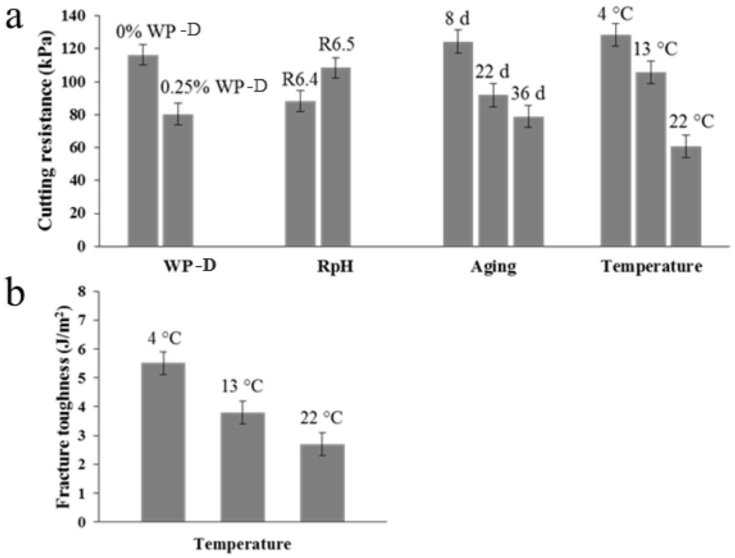

Figure 6.3 **Effects of added denatured whey protein (WP-D), renneting pH (RpH; R6.4 = renneting at pH 6.4; R6.5 = renneting at pH 6.5), aging, and testing temperature on 2 cheese texture descriptors, (a) cutting resistance and (b) fracture toughness, as determined using the wire-cutting test. Error bars represent the standard errors of the means.**

6.5.2.4 Frequency Sweep Test

The complex modulus measured at 1 Hz, or G^*_1, is an indicator of the number and strength of the bonds forming the cheese matrix (Lucey et al., 2003). The effects of temperature and WP-D concentration are presented in Figure 6.4a. The G^*_1 of the 0.25% WP-D cheeses was lower and decreased less with increased temperature (−6.1 kPa $°C^{-1}$) in comparison with the G^*_1 of the 0% WP-D cheeses (−8.2 kPa $°C^{-1}$). The mean G^*_1 of the R6.4 cheeses was approximately 15% lower than that of the R6.5 cheeses (Figure 6.4a).

The frequency dependence of the complex modulus, or G^*_{fd}, is a measure of how fast the bonds interacting in the cheese matrix can reorganize or recover upon deformation (Tunick, 2011). A true gel will have a low G^*_{fd}, since recovery is fast. In contrast, slowly recovering bonds would result in higher frequency dependence. As shown in Figure 6.4b, 2 main factors influenced the cheese G^*_{fd}: the renneting pH and the temperature. Lowering the renneting pH to 6.4 slightly increased the frequency dependence of the pizza Mozzarella cheeses. However, the G^*_{fd} was considerably increased when the cheese temperature was increased.

The phase angle, or δ, represents the relative importance of the viscous dissipation against the elastic restitution of the total energy transferred to the cheese matrix during oscillatory testing. The phase angle measured at 1 Hz, or δ_1, was impacted mainly by renneting pH, aging, and cheese temperature (Table 6.1). Figure 6.4c presents the interacting effects of aging and temperature on δ_1. The δ_1 of the young cheeses (8 d) was lower at 4 °C and was more temperature-dependent than the δ_1 of the longer-aged cheeses. Generally, the increase in δ_1 seemed to be greater when the temperature increased from 13 to 22 °C than when it increased from 4 to 13 °C. Lowering the renneting pH induced a significant increase in the δ_1, indicating a more liquid-like behavior (Figure 6.4c). However, it must be stressed that the δ of the cheeses ranged from about 14° to about 17° under all the cheese-making conditions tested. By definition, a material that presents equal viscous and elastic components has a δ of 45°. Clearly, the pizza Mozzarella cheeses under study presented a dominant elastic behavior.

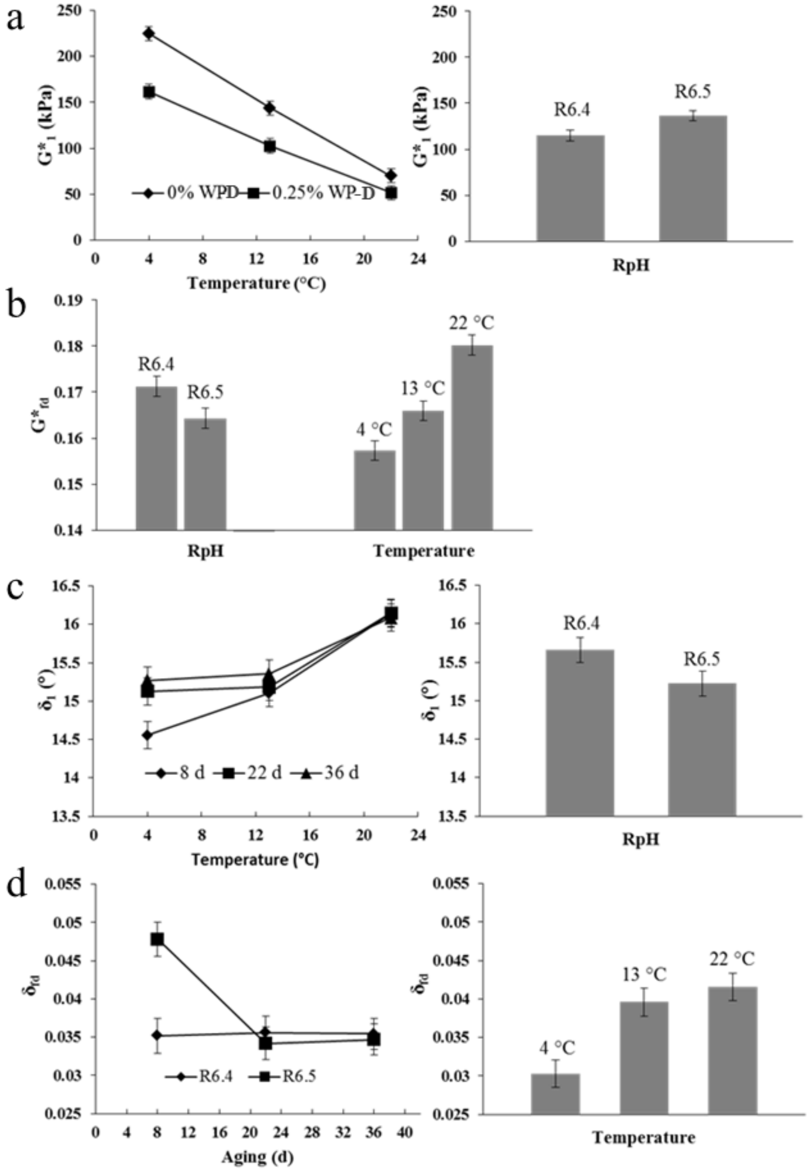

Figure 6.4 **Effect of added denatured whey protein (WP-D), renneting pH (RpH; R6.4 = renneting at pH 6.4; R6.5 = renneting at pH 6.5), aging, and testing temperature on 4 cheese texture descriptors, (a) complex modulus measured at 1 Hz (G^*_1), (b) frequency dependence of the complex modulus (G^*_{fd}), (c) phase angle measured at 1 Hz (δ_1), and (d) frequency dependence of the phase angle (δ_{fd}), as determined using the frequency sweep test. Error bars represent the standard errors of the means.**

The frequency dependence of the phase angle, or δ_{fd}, was also influenced by renneting pH, aging, and cheese temperature (Table 6.1). The effects of those factors are presented in Figure 6.4d. The δ_{fd} values were similar during aging for all cheeses except the R6.5 cheese at 8 d, which had a higher δ_{fd}. The δ_{fd} values of the cheeses generally increased when the temperature increased from 4 to 13 °C but did not vary on further increases in temperature. Despite significant differences between the δ_{fd} values of the various cheeses, the recorded values were very low (ranging from 0.028 to 0.055). These lower values mean that both the elastic and viscous components varied in more or less the same way with increased frequencies. Some authors reported that the phase angles of cheeses were constant over a wide range of frequencies (Gunasekaran and Ak, 2002; Subramanian et al., 2006).

6.5.3 Predictive Models for Shreddability of Pizza Mozzarella Cheese

6.5.3.1 Input of Variables

The second objective of this study was to use rheological and compositional descriptors to build predictive models for the shredding behavior of pizza Mozzarella cheese. Table 6.2 reports the basic statistics of the dependent variables (shredding descriptors) and the independent variables (compositional and rheological descriptors). The compositional and shredding properties of all the experimental units characterized in the present study were previously reported in chapter 5 and were used to build the models. Along with the 11 rheological descriptors, 3 compositional descriptors—moisture (M), colloidal calcium phosphate (CCP), and water-soluble nitrogen on total nitrogen (WSN)—were included as potential predictive variables. Protein and fat content were not considered, since they were controlled factors in the cheese-making experiments. In addition, 3 shredding descriptors—fines production (F%), long shreds production (LS%), and adhesion of cheese to the shredding blade (AdhB)—were used as dependent variables.

6.5.3.2 Modeling Cheese Shreddability

According to the Box–Cox power transformation analysis, the H, Y_m, F_m, C_r, F_t, and G^*_1 data were transformed using a logarithmic function. Cohesiveness data were elevated to the square in order to correct the dissymmetry (Table 6.2). Linearity and normality of the data were confirmed by probability and residual plots.

Table 6.2 **Basic statistics of shredding, compositional, and rheological descriptors of cheeses.**

Input variable	Symbol	*n*	Statistics				
			Min value	Max value	Mean	Standard deviation	Asymmetry coefficient
Dependent variable: shredding descriptors							
Fines production (% [w/w])[a]	F%	108	1.08	11.6	6.9	0.2	−0.24
Long shreds production (% [w/w])[a]	LS%	108	29.0	86.0	50.6	10.2	0.80
Adhesion to the blade (mg cm^{-2})[a]	AdhB	108	0.25	4.2	2.1	0.65	0.75
Predictive variable: compositional descriptors							
Moisture (%)[a]	M	36	43.8	48.6	46.0	1.4	0.12
Colloidal calcium phosphate (mg g^{-1} casein)[a]	CCP	36	6.9	37.1	18.6	7.4	0.19
Water-soluble nitrogen (%)[a]	WSN	36	4.3	19.1	11.8	3.8	0.10
Predictive variable: rheological descriptors							
Hardness (N)	H	108	9.5	60.3	31.5	12.7	0.26
Cohesiveness (–)	Coh	108	0.18	0.68	0.55	0.06	−2.04
Adhesiveness (J)	Adh	108	1.1	4.2	2.6	0.8	0.13
Young's modulus (kPa)	Y_m	108	35.4	431.6	151.9	81.6	0.74
Flow modulus (kPa)	F_m	108	27.8	282.8	98.2	47.4	1.18
Cutting resistance (kPa)	C_r	108	1.7	31.2	10.7	5.4	1.14
Fracture toughness (J m^{-2})	F_t	108	0.2	10.6	4.0	2.2	0.97
Complex modulus measured at 1 Hz (kPa)	G^*_1	108	38.2	306.2	123.7	64.3	0.73
Frequency dependence of the complex modulus (–)	G^*_{fd}	108	0.14	0.20	0.17	0.01	0.36
Phase angle measured at 1 Hz (°)	δ_1	108	14.1	17.4	15.4	0.7	0.58
Frequency dependence of the phase angle (–)	δ_{fd}	108	0.02	0.07	0.04	0.01	0.80

[a] Data from chapter 5.

The predictive models for shreddability are presented in Table 6.3. The *F*-values for the model parameters resulting from the type II sum of squares of the variance analysis are also presented in this table. The *F*-values represent the relative contribution of each parameter to the total variance explained by the model. The R^2, or coefficient of determination, indicates the ability of the model to explain the variability of the shredding properties measured on the cheeses. All predictive descriptors selected by the stepwise method added significant contributions ($P < 0.05$) to the models (data not shown).

$Table$ *6.3* Predictive models for the production of fines, production of long shreds, and adhesion to the blade during shredding.

				Predictive variables in model[a]				F-values for model parameters				
Predictive model		R^2	RMSE[b]	***A***	***B***	***C***	***D***	***A***	***B***	***C***	***D***	***Intercept***
Fines production	$= -0.38\boldsymbol{A} - 96.01\boldsymbol{B} + 0.07\boldsymbol{C} - 3.95\boldsymbol{D} + 46.10$	0.82	0.89	WSN	G^*_{fd}	CCP	G^*_1	184.9	33.1	24.8	21.7	31.1
Long shreds production	$= 2.09\boldsymbol{A} + 32.08\boldsymbol{B} - 0.17\boldsymbol{C} + 236.9\boldsymbol{D} - 57.67$	0.67	4.87	WSN	H	CCP	G^*_{fd}	87.6	26.1	9.8	8.0	6.8
Adhesion to the blade	$= -1.57\boldsymbol{A} - 1.7 \times 10^{-4}\boldsymbol{B} - 15.24\boldsymbol{C} + 5.42$	0.45	0.37	H	Adh	δ_{fd}	-	49.2	5.9	5.6	-	134.2

[a] WSN = water-soluble nitrogen as a percentage of total nitrogen; G^*_{fd} = frequency dependence of the complex modulus; CCP = colloidal calcium phosphate; G^*_1 = Log (complex modulus measured at 1 Hz); H = Log (hardness); Adh = adhesiveness; δ_{fd} = frequency dependence of the phase angle. Units for the descriptors can be found in Table 6.2.

[b] Root mean square error of the model.

6.6 Discussion

6.6.1 Factors Influencing Rheological Descriptors of Cheeses

6.6.1.1 Effect of WP-D Addition and Milk Renneting pH

Modifying the WP-D concentration in milk affected the hardness, cohesiveness, Y_m, C_r, and G^*_1 of the pizza Mozzarella cheeses (Table 6.1). Overall, cheese containing WP-D tends to have a softer/weaker body texture. The cheeses manufactured with 0.25% WP-D contained approximately 2.4% more moisture than did the cheeses made without added WP-D (see section 5.5.1), a difference that could explain the weaker structure. Denatured WP is well known to retain water effectively in the *para*casein matrix, and this property has been used to soften the texture of reduced-fat cheeses (Schenkel et al., 2013b; Skeie et al., 2013). Because the strength of the cheese structure is promoted mainly by its protein and solid fat phases, the additional free water found in WP-D cheeses would be expected to reduce the relative importance of structural components in terms of overall cheese texture (Lelièvre, 1995). Another possible cause for the softer body of the 0.25% WP-D cheeses is the fact that denatured WP-D does not contribute to, and indeed probably impairs, the *para*casein network. A recent study by Schenkel et al. (2013b) tends to support the idea that WP-D can physically disrupt the *para*casein network and act as an inert filler in the cheese matrix. Lee et al. (2013) presented transmission electron micrographs that clearly showed that denatured WP-D was included as large aggregates that reduced the homogeneity of the cheese mass. Interestingly, the present results show that the G^*_{fd} was not influenced by WP-D concentration (Table 6.1), in contrast with a strong decrease in G^*_1 with 0.25% WP-D (Figure 6.4a). These results suggest that the addition of WP-D to the cheeses did not affect the relaxation properties of the matrix, and thus the incorporated particles did not have a detrimental effect on the integrity of the *para*casein network under low deformation. Under large deformation, however, the addition of WP-D reduced the cohesiveness of the cheeses.

Modifying the renneting pH during cheese-making affected the mean values of 8 rheological descriptors (Table 6.1). The general trend suggests that the cheeses made with milk renneted at pH 6.5 had a stronger network, and therefore a firmer body, than did the

R6.4 cheeses. Decreasing the renneting pH was previously shown to considerably reduce the insoluble colloidal calcium phosphate that is responsible for cross-linking between *para*casein strands in cheese (Johnson and Lucey, 2006). On a molecular level, the *para*casein network of the R6.4 cheese was prone to a greater and permanent structural rearrangement upon deformation, because the matrix contained fewer high-energy intermolecular bonds (i.e., calcium phosphate cross-links) in comparison with the R6.5 cheeses. The relaxation property of the R6.4 cheese protein matrix in reaction to a small deformation cycle was thus less pronounced than that of the R6.5 cheese. As a result, the G* of the R6.4 cheese increased more rapidly with increases in frequency (i.e., higher G^*_{fd}), since relaxation could not fully occur between the increasingly shorter periods of strain deformation cycles. The effect of lowering the renneting pH was also clearly perceptible under large deformation, as shown by decreases in hardness, cohesiveness, F_m, and C_r.

Incorporating denatured WP-D into milk or varying the renneting pH during cheese-making are efficient texture modifiers. It must also be stressed that in the present study, those factors affected the cheese texture independently from one another (Table 6.1). This information is important for cheese makers so that they can better anticipate the impact of cheese-making adjustments on the physical properties of the final product.

6.6.1.2 Effect of Cheese Aging

Aging the cheeses over a period of 36 d had a significant impact on many rheological descriptors (Table 6.1). As expected, cheese body texture was observed to be softer and weaker with aging. Previous studies demonstrated that the variations in the texture of Mozzarella cheese during aging were attributable mainly to the gradual absorption of water by protein fibres (Kindstedt and Guo, 1997; Guinee et al., 2002; Monteiro et al., 2011), the solubilization of colloidal calcium phosphate (O'Mahony et al., 2005; Johnson and Lucey, 2006), and the proteolysis of caseins caused by residual rennet activity (Sheehan and Guinee, 2004; Kindstedt et al., 2010). The last 2 parameters were monitored on the cheeses under study and showed significant changes upon aging as previously reported in chapter 5.

A series of interconnected events may have led to the textural changes observed in the present study. The solubilization of colloidal calcium phosphates and the release of β-casein in the aqueous phase of cheese during the first days of cold storage have been observed in concomitance with the gradual hydration of the *para*casein fibers (Guo and Kindstedt, 1995; Guinee et al., 2002; Joshi et al., 2004b; Monteiro et al., 2011). The more hydrated *para*casein network improves enzyme accessibility and increases the proteolytic activity (Feeney et al., 2002). The resulting casein fragments are less cross-linked and more mobile and therefore contribute less to the elastic behavior of the cheese matrix (Lucey et al., 2003).

6.6.1.3 Effect of Cheese Testing Temperature

As expected, the temperature greatly affected the rheological properties of the cheeses measured under small and large deformation (Table 6.1). Applying thermal energy to the cheeses affected mainly the number, strength, and nature of the bonds forming the matrix. The total number and/or strength of the bonds were drastically reduced with increasing temperature, as indicated by a strong decrease in G* as the temperature rose (Figure 6.4a). The 2 major constituents contributing to the structure of cheese are the milk fat fraction and the *para*casein matrix. Milk fat in its crystalline form is closely packed and behaves in a solid-like manner. However, the gradual transition of partly solid fat toward its complete melting, at around 40 °C, contributes to intermolecular mobility because of the decreasing numbers of effective bonds. In addition, the *para*casein matrix can be seen as a complex balance of both associative interactions (hydrophobic interactions, ionic and electrostatic bridging, and hydrogen bonding) and repulsive interactions (electrostatic repulsion) that determines its structural properties (Lucey et al., 2003). During heating, given that temperature has various impacts on these interactions, the overall balance is affected and generally leans toward a decrease in the number and strength of total bonds in the *para*casein matrix (Lucey et al., 2003; Choi et al., 2008).

As reported previously, adhesiveness was generally higher for older cheeses at 4 and 13 °C but decreased independently of cheese age when measured at 22 °C (Figure 6.1b). At a higher temperature, the increasing presence of liquid fat on the cheese surface acted as a lubricant and reduced cheese adhesiveness. The partial melting of fat with increased temperature may also allow the protein matrix to reorganize with fewer permanent disruptions or microfractures during large deformation in comparison with conditions where fat is mostly in a solid state. As a result, cohesiveness was improved for the young cheeses when temperature increased (Figure 6.1c). However, when the protein matrix already showed some resilience properties (i.e., in an older cheese), the impact of melted fat on cheese cohesiveness became insignificant.

As a general trend, the texture differences between the cheeses manufactured with different WP-D concentrations (Figures 6.1a, 6.2a, and 6.4a), renneting pH values (Figure 6.1a), and aging periods (Figures 6.2a and 6.4c) were smaller when measured at 22 °C than when measured at lower temperatures. Other studies reported smaller differences between rheological measurements with increased temperature (Guinee et al., 2002; Joshi et al., 2004c). Recently, Rogers et al. (2010) estimated the contribution of the fat phase and the protein matrix to the storage modulus (G′) of Cheddar cheese. Those authors found that changes in both phases, that is, the melting of fat and the reduction of the total bonds in the *para*casein matrix, contributed to the decrease in G′ during heating from 10 to 25 °C. However, the contribution of fat to the elastic modulus decreased faster than did the contribution of the protein matrix upon heating. Those observations are in agreement with the present results and support the critical role played by fat in cheese structure and rheological properties.

6.6.2 Prediction of Shreddability using Compositional and Rheological Descriptors

Some rheological and compositional descriptors satisfactorily described the production of fines ($R^2 = 0.82$) and long shreds ($R^2 = 0.67$) during shredding (Table 6.3). According to the models, the cheeses with higher WSN concentrations and lower CCP contents showed reduced fines production and an increased long shreds ratio. A relatively low G^*_1 with a high frequency dependence further decreased fines production. In contrast, a high G^*_{fd} and a high hardness value were indicators of a high proportion of long shreds. According to the *F*-values, controlling and monitoring the proteolysis of pizza Mozzarella cheese by means of the WSN percentage is one of the key factors in reducing fines and increasing the proportion of long shreds. Long and uniform shreds are visually appealing and preferred by consumers (Apostolopoulos and Marshall, 1994; Rankin et al., 2006). Long shreds are also easier to handle and show consistent melting properties. In contrast, fine particles of cheese that are produced during shredding are likely to burn faster when baked because of their large surface-to-volume ratio. Fines are also prone to being blown away in industrial forced-air oven, resulting in yield losses and necessitating frequent maintenance to clean the cooking equipment. In the conditions used in the present study, cheese fines accounted for up to 11% (w/w) of the total cheese mass (Table 6.2). Reducing fines production and increasing shred size and uniformity can provide large financial benefits for cheese manufacturers and users.

The adhesion of cheese to the shredding blade could not be satisfactory predicted by compositional and rheological descriptors ($R^2 = 0.45$). However, the results suggest that a cheese with a higher hardness value is less likely to stick to the shredding blade. That finding is in agreement with the observation that old cheeses are generally soft and pasty and are, therefore, likely to cause sticking problems during shredding (Kindstedt, 1995).

The models presented in Table 6.3 are empirical and therefore represent the best linear regressions that could be produced using a defined modeling procedure and the experimental data from the study. The models provide insights into the variables that are important for each of the shredding descriptors of interest. This information is especially valuable, since the models were built using a wide range of physicochemical characteristics (Table 6.2).

6.6.3 Relationship between Cheese Shreddability and Compositional and Rheological Descriptors

The loading scores for the rheological, compositional, and shreddability variables from the PC analysis are presented in Figure 6.5. The PC analysis allowed the number of variables to be reduced by finding linear combinations (PCs) of those variables in order to explain most of the variability. The cross-validation technique established that the components captured 66% of the total variance in the pooled data. Two significant components explained most of this variation as the rest of the variability could not be grouped into significant components (eigenvalues < 1). Of the variability explained by PCs, 61.8% was represented by PC1 and 36.2% by PC2. The variable loading score represents the shared variability of this variable with the communal variability of the component. Variables with a loading score above |0.50| are generally considered to have significant loading on a component (Jolliffe, 2002).

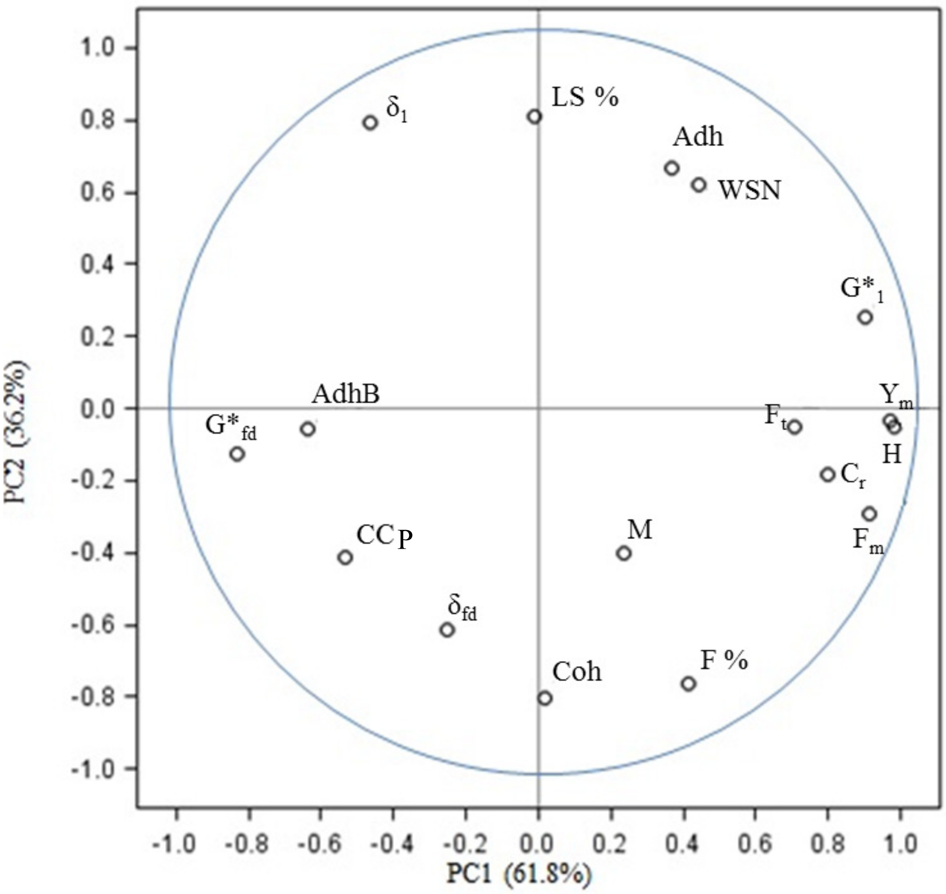

Figure 6.5 **Principal component (PC) biplot of the rheological, compositional, and shredding descriptors of cheeses: LS% = percentage of long shreds; Adh = adhesiveness; WSN = water-soluble nitrogen; G^*_1 = complex modulus measured at 1 Hz; Y_m = Young's modulus; F_t = fracture toughness; H = hardness; C_r = cutting resistance; F_m = flow modulus; M = moisture; F% = percentage of fines; Coh = cohesiveness; δ_{fd} = frequency dependence of the phase angle; CCP = colloidal calcium phophate; G^*_{fd} = frequency dependence of the complex modulus; AdhB = adhesion to the blade; δ_1 = phase angle measured at 1 Hz.**

Based on that cut-off, 6 variables—hardness, Y_m, F_m, G^*_1, C_r, and F_t—were found to be loaded positively on PC1, and 3 variables—G^*_{fd}, adhesion to the blade, and CCP—were found to be loaded negatively. The variables loaded positively on PC1 similarly differentiated the cheeses, as seen by the cluster formed by those variables (Figure 6.5). In particular, hardness and Y_m were strongly correlated, suggesting that the first PC is somehow related the strength of the cheese matrix. The adhesion of cheese to the shredding blade was related to PC1 and poorly related to PC2. These findings are in agreement with the empirical model that suggested that hardness was an important descriptor for predicting adhesion to the blade (Table 6.3). Childs et al. (2007) suggested that cheese can be a pressure-sensitive adhesive material if its viscoelastic response is below the Dahlquist criterion and depending on the surface energy of the cheese and the blade. Apparently, materials with low elastic properties (i.e., $G' < 10^5$ Pa) have more tack and are therefore likely to stick to the shredding blade. Soft cheeses are also likely to endure higher deformation and spread, and thus a larger surface is exposed to contact with the blade. Interestingly, G^*_1 was well correlated with PC1 and other large deformation rheological measurements. This finding reinforces the idea that it is possible to relate the physical properties of a cheese undergoing large deformation processing (e.g., shredding, slicing, chewing) by probing the structure of the cheese under very small strain using techniques such as dynamic rheometry.

The production of long shreds and the production of fines were loaded on PC2 with values of −0.81 and 0.75, respectively, but were poorly related to PC1. Cohesiveness and δ_1 were also strongly loaded on PC2, as were, to a lesser degree, WSN and adhesiveness measured by TPA. The second PC seems to group variables in which the rearrangement properties of the cheese matrix upon deformation are important. As reported in Table 6.3, the WSN and CCP contents in the cheeses were the most relevant descriptors for modeling the production of fines and long shreds. Lower cross-linking potential between caseins, as shown by high WSN and low CCP contents, would improve cheese resilience during deformation. Consequently, cheese cohesiveness would be high enough to sustain the shredding process and thus reduce the breaking of shreds into smaller particles.

The PC analysis confirmed that the production of fines and the production of long shreds in cheeses are poorly related to a single variable in PC1 such as hardness, Y_m, and descriptors measured by the wire-cutting test. Moreover, the PC analysis showed that adhesion to the blade is unrelated to the cheese characteristics responsible for the production of fines and long shreds. These findings stress the importance of considering multiple physicochemical properties in order to predict the rather complex behavior of pizza Mozzarella cheese when it is subjected to the shredding process.

6.7 Conclusions

It was possible to achieve a wide range of cheese rheological properties using combinations of different cheese-making conditions. Adding WP-D and changing the milk renneting pH were shown to influence the mechanical properties of cheese. At that point, one can choose to vary the post-manufacture storage time to further modify the cheese texture. These manufacturing leverages work well together, since the effects of aging do not statistically interact with the effects of changing the renneting pH or adding WP-D. Finally, it was shown that cheese temperature had a very strong impact on rheological properties. Unlike the other cheese-making variables, which impact the *para*casein properties, the temperature influences the texture by altering the solid fat content of cheese.

All these conditions can be combined together in order to make cheese that will show optimal shredding behavior. This study proposed to build an empirical model for the prediction of cheese shreddability, by selecting a few important physicochemical variables. Both compositional and rheological descriptors were important for explaining the production of fines and long shreds during shredding. For instance, monitoring the proteolysis and the amount of colloidal calcium phosphate in cheese are great tools for controlling shreddability when they are combined with simple rheological measurements (i.e., hardness, G^*_1). The predictive models and PC analysis can help manufacturers to select appropriate descriptors to facilitate the development of cheese with optimal mechanical behavior under shredding conditions.

6.8 Acknowledgements

This work was financially supported by the Natural Sciences and Engineering Research Council of Canada (Ottawa, ON, Canada), the Fonds de recherche du Québec – Nature et technologies (Québec City, QC, Canada), and Agropur Dairy Cooperative (St-Hubert, QC, Canada).

Chapter 7 Relationship between baked-cheese sensory properties and melted-cheese physical characteristics

7.1 Résumé

Les propriétés physiques du fromage en cours de refroidissement suite à sa cuisson ont été très peu étudiées comparativement au processus de fonte. Conséquemment, la relation entre la texture perçue lors de la dégustation du fromage cuit et les caractéristiques rhéologiques de ce fromage demeure incomprise. Cette étude évalue les propriétés rhéologiques et sensorielles de fromages Pizza Mozzarella fabriqués selon une technologie fromagère traditionnelle ou stabilisée. Les fromages étaient testés après 10, 25, 40 ou 55 jours de maturation. Une évaluation sensorielle descriptive a été développée afin de caractériser la perception de texture des fromages cuits sur pizzas. La fermeté, la résistance au bris, le caractère caoutchouteux et la libération de liquide perçue lors de la dégustation variaient selon le type et l'âge des fromages cuits. Les différents traitements influençaient aussi significativement les propriétés rhéologiques sous petites déformations du fromage fondu, puis refroidi à différentes températures. Un test rhéologique appliquant plusieurs cycles de fluage et de recouvrance, sous petites et grandes contraintes, a aussi été adapté pour le fromage en cours de refroidissement. Les résultats de ce test ont démontré la plus forte corrélation avec les descripteurs sensoriels du fromage cuit sur pizzas. Ce travail démontre aussi qu'une déshydratation partielle des fromages permet de mieux relier la perception sensorielle des fromages aux résultats d'analyses rhéologiques.

7.2 Abstract

Little is known about the effects of manufacturing conditions on sensory properties of baked Mozzarella cheese. Moreover, instrumental analysis of cheese at room temperature does not fully correlate with the sensory properties of cheese after baking on a cooking dish. In this study, regular and stabilized pizza cheeses were aged 10, 25, 40 or 55 d before testing. A descriptive sensory analysis was performed on cheese baked on a pizza model and was related to the rheological properties of cheese melted under controlled conditions. The results showed that the sensory firmness, breakdown resistance, rubberiness and liquid release varied among cheese types and ages. Differences among cheese types and ages were also observed in small-strain oscillatory shear moduli measured on melted cheeses cooled at different temperatures. The Multiple Stress Creep Recovery test, using low and high stress loadings, was adapted to characterize the properties of the melted cheeses and proved to provide the best correlation with baked-cheese sensory terms. Partial dehydration of cheese before melting, applied to mimic the water loss during baking process, was shown to increase correlations.

7.3 Introduction

Around 38% of the cheese produced in North America is destined for the food services sector (Euromonitor, 2014). Cheese is increasingly used as an ingredient in various menu applications, and shredded Mozzarella cheese topping for pizza remains a leading market segment. Restaurant chains use commercial forced-air ovens to bake the cheese topping at temperatures over 250 °C for a short time (4–6 min) (Wadhwani et al., 2011). Baked pizzas are generally served to consumers at temperatures between 50 °C and 70 °C depending on the cooling period and environment (e.g., in a cardboard box, under infrared light, at room temperature) (Chen et al., 2009). Baking conditions inevitably induce changes in the microstructure, composition, appearance and flowing properties of cheese (Rudan and Barbano, 1998; Metzger et al., 2000; Wang and Sun, 2002a).

Most cheese producers and commercial end-users (e.g., pizza chains) have developed their own empirical tests to evaluate baking performance (Chen et al., 2009). Stretching, browning, oiling-off and melt appearance are generally measured and compared to pre-set performance standards that meet each end-user preference. However, consumer acceptance depends not only on the melting quality and appearance of baked cheese but also on the textural sensations experienced during mastication (Metzger and Barbano, 1999; Moskowitz, 2001). Among other things, poorly melted cheese may feel tough, whereas excessive melting may result in a soupy texture (Wang and Sun, 2002a). The proteolysis occurring during the aging is known to increase the melting behavior of Mozzarella cheese (Kindstedt, 1995; Lucey et al., 2003; Sheehan et al., 2004; Olivares et al., 2009). Descriptive sensory evaluation has been successfully used to characterize the texture of unbaked cheese (Drake et al., 1999; Brown et al., 2003). However, this approach is time-consuming and requires the development of relevant sensory terms, standard references and a rigorous sample-preparation protocol. To our knowledge, a formal descriptive sensory evaluation of baked cheese has never been attempted. Consequently, the impacts of cheese-making conditions, including the effect of aging, on the sensory texture of baked-cheese remain to be determined.

A fundamental understanding of the relationship between the mechanical and sensory properties of cheese is of great interest, because such an understanding would allow sensory perceptions to be predicted by rapid and accurate instrumental measurements. The mechanical properties of cheese are recognized as critical factors that impact the perception of texture (Foegeding and Drake, 2007; Chen, 2014), but physiological and psychological factors must also be considered (Koç et al., 2013; Morell et al., 2014). Foegeding and Drake (2007) stressed that instrumental measurements performed under experimental conditions similar to those in place during the sensory evaluation is key to improving the relationship between mechanical and sensory properties. Along with this view, texture profile analysis has been extensively used for food texture research and quality control, because two-cycle compression mimics the mastication process. Fracture and torsion tests have also been used to understand bolus breakdown during the high-stress compressions of oral processing (Çakir et al., 2012b). Unfortunately, these techniques are not suitable for measuring baked cheese because of challenges related to sample preparation and representativeness (Truong et al., 2002) as well as poor temperature control. Small-deformation rheometry, such as oscillatory shear and creep tests, are widely used to characterize the viscoelastic nature of cheese (Lucey et al., 2003; Olivares et al., 2009). However, the relevance of these descriptors for predicting mouth sensory terms can be questioned, given that the deformation applied during oral processing greatly exceeds the linear viscoelastic limits of cheese (Foegeding and Drake, 2007). Recently, Melito et al. (2013) probed the non-linear behaviour of unmelted cheeses using the large-amplitude oscillatory shear technique and found good correlations with sensory measurements. Hence, there is a need to develop a rheological technique that would relate to the sensory texture of baked cheese. However, baked cheese is highly heterogeneous and is not well suited for rheological analysis. Testing of melted cheese, rather than baked cheese, is expected to provide more consistent results with a greater control of the heating conditions and sample preparation.

The first objective of this study was to measure the compositional and sensory properties of commercial cheeses baked at different stages of aging. The second objective was to evaluate the relationship between small- and large-deformation rheological parameters measured on melted cheese and the sensory properties of baked cheese.

7.4 Material and methods

7.4.1 Cheese Sampling and Aging

Two types of culture-acidified pizza Mozzarella cheese were supplied by a local plant (Agropur Cooperative, Granby, QC, Canada). Cheese-making followed the general steps described by Chen et al. (2009), but regular cheeses (RCs) and stabilized cheeses (SCs) were made using different proprietary cheese-making processes. Whereas SCs are expected to retain fairly constant physical properties over a long refrigeration period (up to 60 d), RCs retain desirable properties over a relatively short time period (~10–20 d) but can be frozen and used on demand. Hence, different baking performances were expected from the RCs and SCs as they aged. Five vats of milk were used to produce each cheese type and were assigned randomly as vat *no.* 1-5 (replicates). Two cheese blocks (~2.27 kg) were randomly selected from each vat. Seven days after production, the blocks were trimmed by removing the outer surface (~10 mm) and cut into five representative samples. One sample from each block was kept for compositional analysis and the others were vacuum-sealed (VAK*3.0 film; Winpak Ltd., Winnipeg, MB, Canada) and aged for 10, 25, 40 or 55 d at 4 °C. The aged samples were frozen (–40 °C) and then thawed at 4 °C for 72 h for testing. Aged samples issued from a given vat were randomly assigned for either the baking or the melting experiments.

7.4.2 Cheese Composition Analyses

Moisture, nitrogen and fat contents in the cheeses were determined using the forced-air drying, macro-Kjeldahl, and Mojonnier methods, respectively (Wehr and Frank, 2004). Protein content was estimated using a nitrogen conversion factor of 6.38. Sodium chloride content was determined using a Chloride Analyzer 926 (Sherwood Scientific, Cambridge, UK), and pH was measured with an AR15 Accumet pH meter (Fisher Scientific, Whitby, ON, Canada). These analyses were done on the two combined grated samples issued from a given vat at d 7 (2 cheese types × 5 vats; $n = 10$). Water-soluble nitrogen was extracted from aged cheeses as described by Watkinson et al. (2001) and was quantified by the Kjeldahl method. The percentage of water-soluble nitrogen in total nitrogen (WSN) was calculated as a proteolysis index.

7.4.3 Cheese-Baking Method

Baked cheeses were prepared on a pizza model. Homemade pizza dough (0.40 g cm^{-2}) and pizza sauce (0.20 ml cm^{-2}) (Gattuso original pizza sauce; Heinz Canada, North York, ON, Canada) were laid on the bottom of a circular, 8-cm-diameter pan (4456; Fox Run, Vaughan, ON, Canada). Then, a dividing layer (parchment paper; Selection, Montreal, QC, Canada) was added to prevent contact between the sauce and cheese. This layer was perforated consistently with pin-sized holes (9 holes cm^{-2}) to allow sauce and dough moisture to evaporate during baking. The dividing layer was covered uniformly with freshly shredded cheese (0.32 g cm^{-2}) (Grater 02, 4-mm blade holes; SANTOS, Lyon, France). The prepared pizzas were equilibrated to 8 °C and then baked at 230 °C in a commercial forced-air oven for 210 s (MT1820F/M; G.S. Blodgett Corp., Burlington, VT, USA).

7.4.4 Baked-Cheese Properties

7.4.4.1 Weight Loss

The cheese weight loss after baking, which is attributed to water evaporation, was determined. First, the baked pizza models were cooled in an environmental chamber set to 40 °C for 30 min. Then, the cheese was removed from the dividing layer and weighed on an analytical balance. Because of its hydrophobic nature, the dividing layer did not have any pizza sauce material on it. However, the layer was coated with some free fat that had been released by the cheese during baking. To quantify this free fat, the dividing layer was immersed in 20 ml of hexane (H292SK4; Fisher Scientific) and agitated on an orbital plate shaker at 100 rpm for 10 min. The dividing layer was removed, and the solvent was allowed to evaporate under low heat on a hot plate. The amount of free fat retrieved was used to calculate the weight loss that could be attributed to moisture evaporation during baking, as follows:

Equation 7.1 Weight loss calculation of cheese during baking

$$\%\ \text{weight loss} = \left(1 - \frac{\text{weight of baked cheese} + \text{weight of free fat}}{\text{weight of cheese before baking}}\right) \times 100$$

7.4.4.2 Expressible Serum and Oil

The capacity of the baked-cheese matrix to retain serum and oil (i.e., liquid milk fat) was determined. First, the baked pizzas were equilibrated to 40 °C as previously described. Then, the cheese was removed from the dividing layer, weighed in a polycarbonate tube, and centrifuged at 12,000 rpm (21,300*g*) for 30 min (Avanti centrifuge J-26 XPI, JA-18 rotor; Beckman Coulter, Brea, CA, USA). The supernatant consisted of a cloudy aqueous phase (i.e., serum phase) and an oil phase. The liquid phases were stirred with 10 ml of hexane so that the oil–hexane phase could be differentiated, and then carefully transferred to a glass Petri dish. The weight of the oil phase was determined after the hexane had evaporated. Then, the aqueous phase was discarded and the pellet was weighed. The serum phase weight was determined by the difference between the weight of the baked cheese and the weight of the pellet and the oil phase. The amounts of the serum and oil phases were reported as percentages (w/w) of baked cheese.

7.4.4.3 Sensory Properties

The descriptive sensory evaluation of the baked cheeses was carried out by an experienced panel of 10 assessors based at the Food Research and Development Centre (Agriculture and Agri-Food Canada, St-Hyacinthe, QC, Canada). The members of the sensory panel, consisting of one man and nine women, were recruited based on personal interest and experience (>5 years) in the sensory evaluation of cheese. In preliminary sessions, the panelists were invited to choose a limited number of relevant sensory terms based on the evaluation of a wide range of baked cheeses. The sensory terms used for the descriptive evaluation of the baked cheeses are summarized in Table 7.1. Firmness was evaluated during the first bite, whereas breakdown resistance, rubberiness and liquid release were evaluated as chewdown terms.

Then, various foods were evaluated as potential references for a specific sensory term. The panelists were invited to agree on an intensity score ranging from 1 to 15 for each reference food associated with a sensory term (Table 7.1). Repeated training sessions were held to minimize inter-panelist variability in the scores and to achieve agreement between the panelists' intensity perception and set reference values.

Table 7.1 **Sensory terms used for the texture evaluation of baked cheeses and scores attributed to reference food.**

Sensory term	Definition	Evaluation stage	Reference food and score [a]	
Firmness	The amount of force required to bite through the cheese, assessed using the molars.[b]	Assessed during the first compression.	Velveeta brand processed cheese	2
			Low-fat Jarlsberg	8
			Parmesan	14
Breakdown resistance	The property of cheese to deform rather than break into particles under normal mastication. Low scores represent samples that fractured easily under stress. High scores represent samples that deformed.	Assessed during the first 3 chews.	Feta cheese	1
			Parmesan	3
			Low-fat Jarlsberg	7
			Old Cheddar	11
			Velveeta brand processed cheese	15
Rubberiness	The extent to which the cheese returns to its initial form after biting.[b]	Assessed during the first 3 chews.	Low-fat Jarlsberg	2
			Mild Cheddar	6
			Parmesan	8
			Brined whelk	15
Liquid release	The degree to which liquids (watery or fat) are released during mastication.[c]	Assessed during the first 5 chews.	Cracker	1
			Raw potato	4
			Black olives (brined)	7
			Asian pear	10
			Grapefruit	15

[a] Scores varied from 1 to 15 for the lowest to the highest intensity, respectively. The reference foods for the firmness and breakdown resistance terms were evaluated at 8 °C. The cheeses used as references for the rubberiness term were warmed at 60 °C before evaluation. The whelk was evaluated at 4 °C, and the other references were evaluated at room temperature.
[b] Definitions from Foegeding and Drake (2007).
[c] Definition from Chen et al. (2009).

Baked cheeses were prepared for five independent sensory sessions (replicated cheese vats). During each session, 8 cheeses (2 cheese types × 4 ages) were evaluated successively in random order. Immediately after the pizza models were baked, the dividing layer were folded in half to hide the appearance of the cheese surface, and the pizzas were kept at 60 °C for 15 min in a covered glass Petri dish. When they received the samples, the panelists cut the folded cheese in half and evaluated the first piece for firmness-rubberiness and the other for breakdown resistance-liquid release within a 1-min period. The panelists' scores were collected using the FIZZ Réseau system (v. 2.40A; Biosystèmes, Dijon, France).

7.4.5 Cheese-Melting Method

Melted cheeses were prepared for rheological testing. The melting procedure was developed to approximate the heating treatment imposed during baking while providing constant sample preparation conditions which were critical for rheological evaluations. Disks of cheese (35-mm diameter) were cut from a cheese slice (~2.2-mm thick). The disks were vacuum-packed (−101.6 kPa) in sealed plastic bags and heated for 15 min in a water bath at 90 °C before being immediately tested for rheological properties. This melting method almost eliminated phase separation and prevented the sample from overflowing. However, unlike in baking, no water evaporation occurs during the melting process. To take into account the effect of water loss on cheese properties, a partial dehydration treatment was applied before the cheese was melted. Dehydration was performed at a low temperature (8 °C) and under a controlled atmosphere. Cheese slices (2.2-mm thick) were laid on a fine grid over a saturated sodium chloride solution and sealed in an airtight desiccator. The weight loss of the samples was regularly monitored until the target moisture loss was achieved (~4 d). This target was based on the mean moisture loss of baked-cheeses measured during preliminary tests. Then, the slices were wrapped in plastic wrap and kept at 4 °C for 24 h to ensure homogeneous moisture content throughout the sample.

7.4.6 Melted-Cheese Properties

The properties of the melted cheeses were measured using a HAAKE MARS II rheometer (Thermo Fisher Scientific, Newington, NH, USA). Immediately after being melted at 90 °C, the cheese samples were loaded between serrated plates (PP35 Ti LS; Thermo Fisher Scientific; 35-mm diameter) at a gap height of 1.2 mm, and any cheese that exceeded the plate surfaces was trimmed and discarded. The lower plate temperature was maintained at 90 °C using a Peltier effect temperature control device (UTC DC30; Thermo Fisher Scientific). The sample edges were covered with paraffin oil (BDH3338; Fisher Scientific), and a solvent trap (222-1515; Thermo Fisher Scientific) was used to prevent moisture loss. A 5-min resting period before measurement allowed the temperature to equilibrate and the structure of the sample to relax.

7.4.6.1 Small-Strain Oscillatory Shear Rheology

The physical properties of the melted cheeses were assessed by a temperature sweep experiment. Initially at 90 °C, the cheese temperature decreased to 20 °C at a rate of 3 °C min^{-1} while a strain amplitude of 0.01 and a frequency of 1 Hz were being applied. The complex modulus (G*) and the phase angle (δ) at 90 °C, 60 °C, 40 °C and 20 °C were selected for analysis. The G* is a good indicator of the number and strength of bonds structuring the cheese matrix (Lucey et al., 2003). The δ represents the viscoelastic behaviour of the cheese. Definite liquid-like behaviour can be detected by a δ that is much greater than 45°, whereas a δ that is much less than 45° designates solid-like behaviour. A δ of 45° is generally recognized as the liquid–solid transition point. No signs of slippage or of non-linear behaviour were detected during the experiments, as monitored by the raw torque signal and angular displacement.

7.4.6.2 Multiple Stress Creep Recovery

The Multiple Stress Creep Recovery (MSCR) test is an ASTM International standard method intended to characterize the mechanical properties of asphalt binder (ASTM, 2010). In the present study, the protocol was modified slightly to account for the properties of melted cheese. Briefly, the melted cheese sample was cooled at a rate of 3 °C min^{-1} from 90 °C to 60 °C and kept at that temperature for 120 s. Then, the sample was instantaneously loaded at a constant stress for 1 s (creep portion). The stress was instantaneously removed, and the sample was allowed to recover for 10 s (recovery portion). Ten successive creep/recovery cycles were performed at 50 Pa followed immediately by ten cycles at 2000 Pa. The lower and higher stress levels were chosen because they are, respectively, under and over the linear viscoelastic region limits of the cheeses at 60 °C.

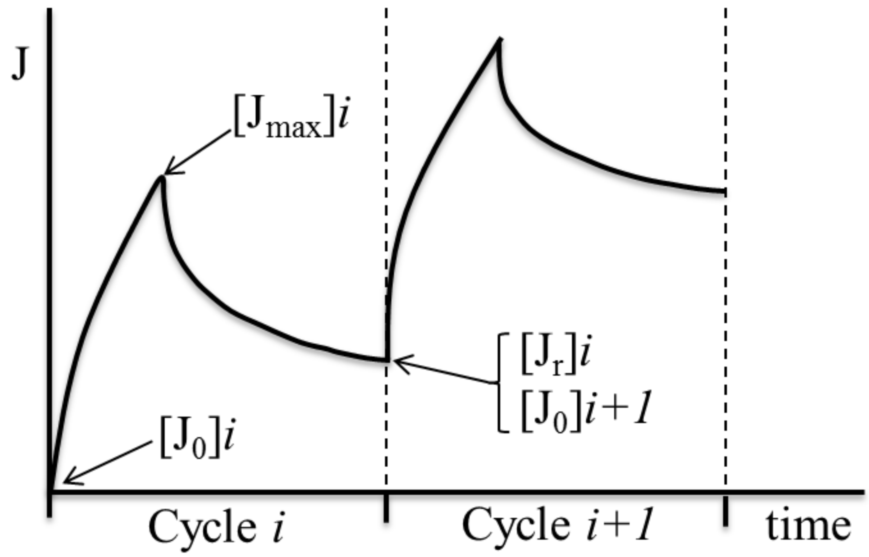

Figure 7.1 **Schematic representation of two creep/recovery cycles in the Multiple Creep Stress Recovery test (not to scale). The cheese compliance (J) was plotted as a function of time. A total of 20 successive cycles were performed: cycle numbers (*i*) from 1 to 10 were loaded at a constant stress of 50 Pa, whereas cycle numbers from 11 to 20 were loaded at 2000 Pa. The initial compliance (J_0), maximum compliance (J_{max}) and recovered compliance (J_r) were analyzed for each cycle.**

During stress (σ) loading, the sample undergoes deformation (γ) to a certain extent as a function of time. The compliance (J) is used in creep analysis to report the deformation of a material per stress unit (i.e., $J = \gamma/\sigma$; unit: Pa^{-1}). For truly elastic material measured within the linear viscoelastic region, the removal of the load, which corresponds to the imposition of an equal but negative load, would eventually result in the complete recovery of the sample. However, rather than store all the energy (elastic character), cheese also dissipates part of it through viscous flow (viscous character). Microstructural reorganization or structural damage can also occur during the loading portion and can contribute to permanent deformation. Hence, only partial recovery compliance is expected for viscoelastic material such as cheese (Brown et al., 2003; Olivares et al., 2009). The compliance measured for the i^{th} cycle of an MSCR test is therefore dependent on the compliance measured at the end of the recovery portion of the previous cycle ($i - 1$).

A schematic representation of two creep/recovery cycles (cycle number *i* from 1 to 20) obtained with the MSCR test is presented in Figure 7.1. The initial compliance (J_0), maximum compliance (J_{max}) and recovered compliance (J_r) of each creep/recovery cycle were used to calculate the following values:

The mean total creep compliance (J_{tot}) at 50 or 2000 Pa:

Equation 7.2 Mean total creep compliance (J_{tot}) calculation at 50 and 2000 Pa

$$J_{tot}\ [50\ \mathrm{Pa}] = \frac{\sum_{i=1}^{i=10} (J_{max}-J_0)\, i}{10}$$

$$J_{tot}\ [2000\ \mathrm{Pa}] = \frac{\sum_{i=11}^{i=20} (J_{max}-J_0)\, i}{10}$$

Equation 7.3 Fatigue calculation at 50 and 2000 Pa

$$\mathrm{Fatigue}\ [50\ \mathrm{Pa}] = \frac{\sum_{i=1}^{i=9}\left((J_{max}-J_0)i{+}1\ \ -\ \ (J_{max}-J_0)i\right)}{9}$$

$$\mathrm{Fatigue}\ [2000\ \mathrm{Pa}] = \frac{\sum_{i=11}^{i=19}\left((J_{max}-J_0)i{+}1\ \ -\ \ (J_{max}-J_0)i\right)}{9}$$

Equation 7.4 Mean recovery percentage (R%) calculation at 50 or 2000 Pa

$$\mathrm{R\%}\ [50\ \mathrm{Pa}] = \frac{\sum_{i=1}^{i=10}\left(\frac{J_{max}-J_r}{J_{max}-J_0}\times 100\right) i}{10}$$

$$\mathrm{R\%}\ [2000\ \mathrm{Pa}] = \frac{\sum_{i=11}^{i=20}\left(\frac{J_{max}-J_r}{J_{max}-J_0}\times 100\right) i}{10}$$

Equation 7.5 Calculation of the recovery percentage difference between the two loads applied (R% diff.)

$$\mathrm{R\%\ diff.} = \mathrm{R\%}\ [2000\ \mathrm{Pa}] - \mathrm{R\%}\ [50\ \mathrm{Pa}]$$

7.4.7 Statistical Analysis

Data analysis of the water-soluble nitrogen content, and baked- and melted-cheese experiments were performed based on a split-plot design with cheese type and aging as main plot and subplot, respectively. The water-soluble nitrogen content and baked-cheese properties were evaluated on five replicated cheese vats (2 cheese type × 4 ages × vats 1 to 5; $n = 40$) whereas the melted-cheese properties were measured on three of those vats (2 cheese type × 4 ages × vats 1 to 3; $n = 24$). The effect of panelist differentiation was included as a blocked factor for the sensory evaluation design. The analysis of variance was performed with PROC MIXED of the SAS software program (v. 9.3; SAS Institute Inc., Cary, NC, USA). A *t*-test was performed to evaluate composition difference between the 2 cheese types and multiple mean comparisons were analysed using the Tukey-Kramer test. Effects were declared significant when the probability level of F was $P < 0.05$. All data from sensory and rheological evaluations of cheeses (2 cheese types × 4 ages) issued from vats 1 to 3 were used for the correlation analysis ($n = 24$). The Pearson correlation coefficients of intra-sensory parameters, intra-rheological parameters and also between sensory and rheological parameters were calculated using PROC CORR of SAS software program.

7.5 Results

7.5.1 Cheese Composition and Aging

Table 7.2 presents the mean compositions of the two types of cheese. The RCs had a slightly higher moisture content and lower salt-to-moisture ratio than the SCs ($P = 0.03$ and $P = 0.04$, respectively). The RCs also contained more fat (~2%) and less protein (~3.5%) than the SCs did ($P < 0.01$). Hence, the fat-to-protein ratio (~0.84 for RCs and ~0.66 for SCs) was the most important compositional difference measured between the cheeses. The pH of the two types of cheese was similar ($P > 0.05$). Figure 7.2 presents the interaction effect between cheese types and aging time on water-soluble nitrogen content in the cheeses ($P < 0.01$). During the 45-d aging period, the WSN content in the RCs increased from 4.1% to 11.2% at an average rate of 0.16% d^{-1}. The water-soluble nitrogen content in the SCs was slightly lower (3.1%) after 10 d and increased by only 0.06% d^{-1} thereafter.

Table 7.2 **Mean compositions and standard deviations of regular and stabilized cheeses.**

	Cheese type	
Composition	Regular	Stabilized
Moisture (% [w/w])*	50.8 ± 0.3	50.2 ± 0.4
Protein (%[w/w])*	23.2 ± 0.9	26.7 ± 1.4
Fat (%[w/w])*	19.4 ± 0.4	17.5 ± 0.4
Salt-to-moisture (%)*	2.3 ± 0.2	2.7 ± 0.3
pH	5.3 ± 0.1	5.4 ± 0.1

Asterisks indicate significant different between mean ($P < 0.05$).

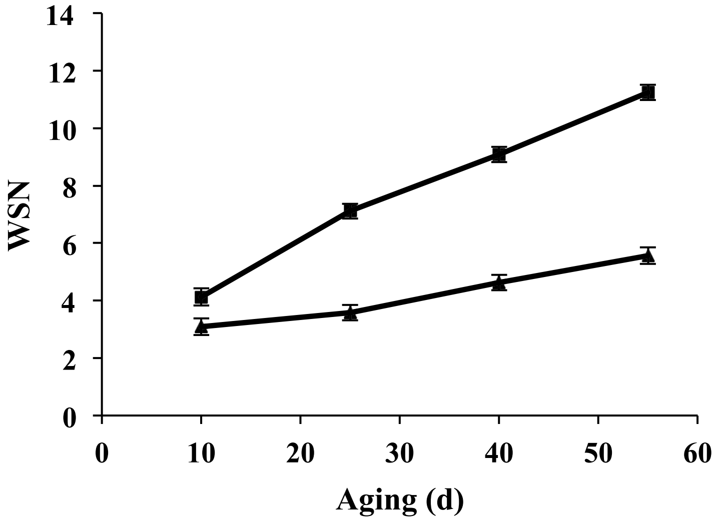

Figure 7.2 **Effect of aging time on the percentage of water-soluble nitrogen in total nitrogen content (WSN) for regular (■) and stabilized (▲) cheeses. Error bars represent the standard error of the means.**

7.5.2 Baked-Cheese Properties

7.5.2.1 Moisture loss

Moisture loss during baking was influenced by aging time ($P = 0.01$). Dehydration decreased as the cheeses aged: the younger cheeses (10 and 25 d) lost about 15.7% of their weight in moisture evaporation versus 13.5% for the older cheeses (40 and 55 d). The effect of cheese type and the interaction between cheese type × aging on moisture loss was not significant ($P = 0.30$ and $P = 0.10$, respectively).

7.5.2.2 Expressible Serum and Oil phases

Changes in the amount of serum and oil phases expelled from the baked cheeses were observed during the aging but these changes depended also on cheese type ($P \leq 0.01$) (Figure 7.3). A large amount of serum phase was released by the young RC (10 d), but free serum decreased by about 33% when the cheeses were further aged. In comparison, the serum phase expelled by the SCs was constant during aging (~17%) and lower than the amount for the RCs even after 55 d of aging. The expressible oil phase of the RCs gradually decreased after 25 d of aging, whereas no change was observed for the SCs. Both the aqueous and oil phases had a significant impact on the total expressible liquids.

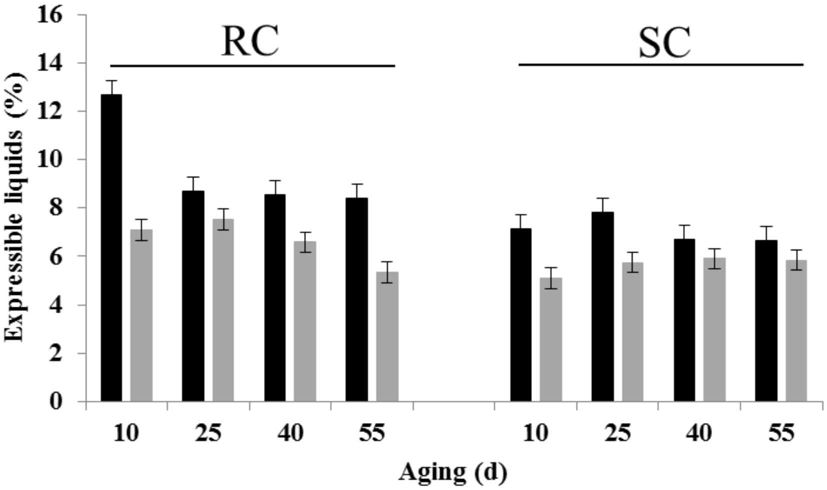

Figure 7.3 **Effects of cheese type and aging on the percentage of serum (dark bars) and oil (light bars) phases expressed from baked cheeses after centrifugation. Cheese types: regular cheese (RC) and stabilized cheese (SC). Aging time: 10, 25, 40 and 55 d. Error bars represent the standard error of the means.**

7.5.2.3 Sensory Properties

The sensory scores given were influenced largely by baked-cheese type and age (Table 7.3). The SCs were generally firmer, more breakable, more rubbery and drier than the RCs were. The intensity of the sensory attributes of the SCs was fairly constant during aging. The sensory texture of the RCs, however, was definitely influenced by aging time. Indeed, the RCs were softer, more deformable, less rubbery and drier as aging time increased.

***Table 7.3* Sensory scores and statistical analysis of baked regular and stabilized cheeses at different aging times**

	Sensory scores[1]								Effect of factors		
	Regular cheese				Stabilized cheese				*F*-value and *P*-value (in parentheses)		
Sensory term	10 d	25 d	40 d	55 d	10 d	25 d	40 d	55 d	Cheese type	Aging	Cheese type × Aging
Firmness	5.8^{c}	4.8^{b}	4.0^{ab}	3.5^{a}	7.6^{d}	7.2^{d}	6.9^{d}	7.3^{d}	308.8* (<0.01)	21.3* (<0.01)	10.9* (<0.01)
Breakdown resistance	8.6^{abc}	9.7^{cd}	10.1^{de}	11.2^{e}	7.5^{a}	7.9^{ab}	8.7^{bcd}	8.2^{ab}	46.9* (<0.01)	16.7* (<0.01)	5.5* (<0.01)
Rubberiness	5.9^{bc}	5.3^{ab}	5.1^{ab}	4.4^{a}	7.1^{d}	6.6^{cd}	6.7^{cd}	7.2^{d}	79.8* (<0.01)	5.1* (<0.01)	6.6* (<0.01)
Liquid release	5.5^{b}	5.3^{ab}	5.3^{ab}	4.7^{a}	4.6^{a}	4.6^{a}	4.5^{a}	4.9^{ab}	10.8* (<0.01)	0.9 (0.40)	3.7* (0.01)

[1] Values represent the mean scores assigned by trained panelists (*n* = 10). The sensory terms were scored on a point scale from 1 to 15. Different letters within a row indicate a significant difference between means ($P < 0.05$) determined using the Tukey-Kramer test for mean comparison.
Asterisks indicate significant effects at $P < 0.05$.

7.5.3 Melted-Cheese Properties

7.5.3.1 Small-Strain Oscillatory Shear Rheology

The analysis of variance results for the effects of cheese type and aging on the physical properties of the melted cheeses at different temperatures during cooling are presented in Table 7.4. No interacting effect between cheese type and aging was detected in the magnitude of the G* or δ ($P \geq 0.05$). The cooling profiles of the melted cheeses were pooled and presented as the means of the effect of cheese type (Figure 7.4a and 7.4b) or aging (Figure 7.4c and 7.4d). During cooling, the number and strength of interactions increased (i.e., the G* increased); those increases indicate an overall solidification of the cheeses as the temperature decreased (Figure 7.4a). Indeed, the magnitude of the G* increased by about 100 times between 90 °C and 20 °C. Both types of cheese exhibited similar cooling behaviour, but the G* was about two times higher for the SCs than for the

RCs at all temperatures. The effect of cheese type on the δ (Figure 7.4b) was not significant at 90 °C, 60 °C and 40 °C but was significant at 20 °C (Table 7.4). This finding is surprising, considering that the difference observed between the two types of cheese was greater at higher temperatures (Figure 7.4b). This result is explained by data variability, which was greater at higher temperatures. As a general trend, the δ seemed to be slightly higher for the RCs, meaning that these cheeses exhibited a more liquid-like behaviour.

Table 7.4 **Values of *F* and *P* (in parentheses) for the effects of cheese type and aging on the complex modulus (G*) and the phase angle (δ) measured at different temperatures during the cooling of melted cheeses.**

		Effect of factors		
Moduli	°C	Cheese type	Aging	Cheese type × Aging
G*	90	35.5* (0.03)	23.1* (<0.01)	4.6 (0.05)
	60	57.7* (0.02)	20.2* (<0.01)	4.08 (0.07)
	40	61.5* (0.02)	26.1* (<0.01)	2.9 (0.12)
	20	66.7* (0.02)	21.1* (<0.01)	1.8 (0.25)
δ	90	6.8 (0.12)	6.8* (0.02)	0.4 (0.7)
	60	9.4 (0.09)	24.8* (<0.01)	2.7 (0.14)
	40	15.4 (0.06)	22.4* (<0.01)	3.2 (0.12)
	20	41.0* (0.02)	33.2* (<0.01)	0.7 (0.57)

Asterisks indicate significant effects at $P < 0.05$.

The effect of cheese aging is presented in Figure 7.4c and 7.4d. Similar trends in the magnitude of the G* were found for all cooling temperatures (Figure 7.4c). The G* of the cheeses aged for 10 and 25 d did not differ, but further aging to 40 or 55 d caused a decrease in the G* of 26% and 42%, respectively. The older cheeses were less elastic than the younger cheeses were, as shown by the general increase in the δ with aging time (Figure 7.4d). More constant δ values were found in the 90 °C to 70 °C range. At those temperatures, only the cheeses aged for 40 or 55 d were above the liquid–solid transition value (δ = 45°). The change in cheese properties toward a much more elastic behaviour began when the cooling temperature approached 60 °C. The rate of change in the δ peaked around 50 °C and gradually slowed when the cooling temperature reached approximately 40 °C.

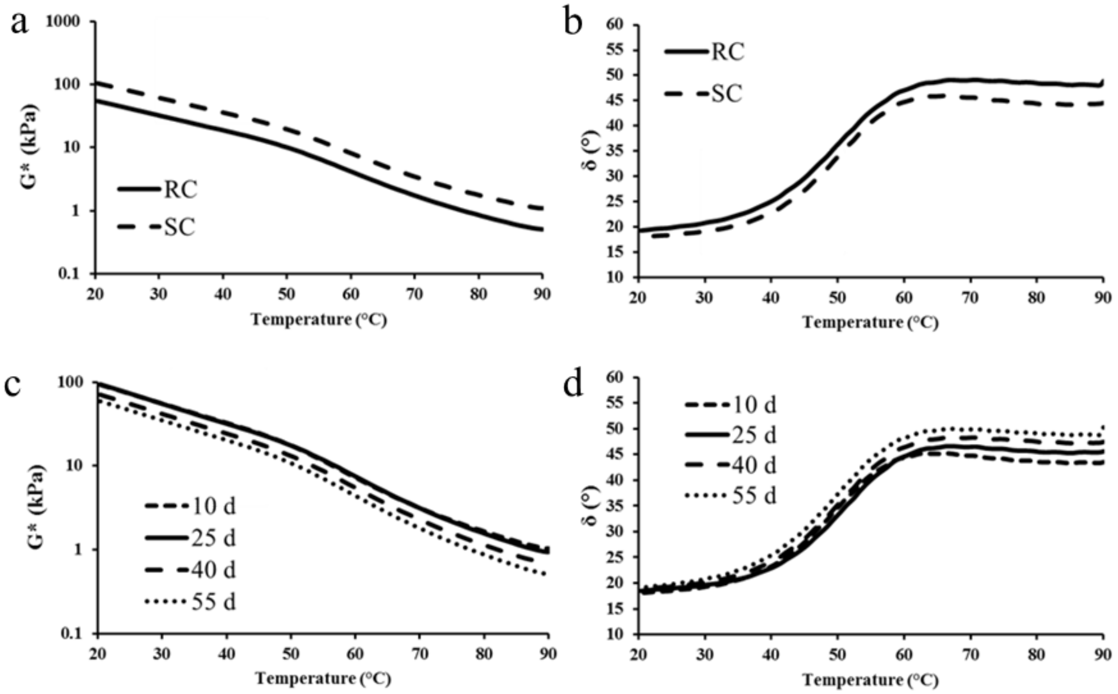

Figure 7.4 **Effects of cheese type (a and b) and aging (c and d) on the complex modulus (G*; a and c) and the phase angle (δ; b and d) of melted cheeses during cooling from 90 °C to 20 °C. Cheese types: regular cheese (RC) and stabilized cheese (SC). Aging time: 10, 25, 40 and 55 d.**

The effect of moisture loss on the physical properties of the melted cheeses was also investigated (data not shown). In this experiment, RCs and SCs aged for 10, 25, 40 or 55 d were partially dehydrated to an average weight loss of 16.7% before melting. Hence, the weight loss of the partially dehydrated cheeses that was attributed to moisture evaporation was slightly higher than the moisture evaporation measured in the baked cheeses (~15%).

The effect of cheese type on the G* of the partially dehydrated cheeses was similar to the effect observed on the non-dehydrated melted cheeses. The G* of the partially dehydrated SCs was exactly twice the G* of the partially dehydrated RCs for all cooling temperatures analyzed ($P \leq 0.01$). When the magnitude of the G* for the partially dehydrated and non-dehydrated cheeses was compared at 90 °C, 60 °C, 40 °C and 20 °C, the G* of the partially dehydrated cheeses was higher, with increase factors of 1.6, 1.8, 1.6 and 1.5, respectively. A slightly higher δ was observed for the partially dehydrated RCs than for the partially dehydrated SCs during cooling ($P < 0.05$). These values were similar to those measured in the non-dehydrated RCs and the non-dehydrated SCs. No effect of aging was detected on the G* or δ of the melted partially dehydrated cheeses during cooling ($P \geq 0.05$). In other words, partial dehydration did not affect the viscoelastic behaviour (i.e., the δ) of the cheese matrix but increased its strength (i.e., the G*) by about 60%, no matter the age of the cheeses.

7.5.3.2 Multiple Stress Creep Recovery

The analysis of variance results for the effects of cheese type and aging on the parameters measured by MSCR at 60 °C are presented in Table 7.5. The effects of cheese type on the J_{tot} (Eq. 7.2) and fatigue (Eq 7.3) are shown in Figure 7.5a and 7.5c, respectively. Figure 7.5a shows that the melted SCs were deformed to a lesser extent than the melted RCs were at a given stress load. The J_{tot} values for the SCs were 53% and 40% lower than the J_{tot} values for the RCs at stress loads of 50 and 2000 Pa, respectively. The fatigue, representing the impact of repeated creep/recovery cycles on the J_{tot}, was similar for both types of cheese at 50 Pa (Figure 7.5c). At 2000 Pa, however, the fatigue was approximately 32 times higher for the RCs than for the SCs. This result shows that the cheese matrix was permanently damaged by the higher stress load but not by the lower stress load. The negative fatigue values (50 Pa) indicate a hardening behaviour when multiple creeps were imposed. This finding suggests that the relaxation of the bonds forming the cheese matrix was slower than the duration of the creep/recovery cycle. When the higher stress was applied, however, the cheeses were deformed to a greater extent as repeated creep cycles were imposed. The viscous flow and the weakening of the cheese matrix induced by permanent deformation are believed to have caused fatigue.

Table 7.5 **Values of *F* and *P* (in parentheses) for the effects of cheese type and aging on the total creep compliance (J_{tot}), the fatigue, and the recovery percentage (R%) of melted cheeses at 60 °C. The Multiple Stress Creep Recovery test was done at two stresses: 50 Pa and 2000 Pa.**

	Effect of factors		
MSCR parameters	Cheese type	Aging	Cheese type × Aging
J_{tot} [50 Pa]	29.9* (0.03)	13.1* (<0.01)	0.22 (0.88)
J_{tot} [2000 Pa]	54.6* (0.02)	36.9* (<0.01)	4.62 (0.05)
Fatigue [50 Pa]	1.0 (0.42)	0.21 (0.89)	4.1 (0.07)
Fatigue [2000 Pa]	101.8* (0.01)	32.8* (<0.01)	4.7 (0.05)
R% [50 Pa]	11.1 (0.08)	1.3 (0.35)	20.9* (<0.01)
R% [2000 Pa]	20.5 (0.05)	38.3* (<0.01)	8.8* (0.01)

Asterisks indicate significant effects at $P < 0.05$.

The effects of aging on the J_{tot} and fatigue are shown in Figure 7.5b and 7.5d, respectively. The cheeses aged for 10 or 25 d showed similar J_{tot} values (Figure 7.5b), but further aging increased the J_{tot}. The older cheeses also appeared to be more sensitive to deformation at the higher stress load. When the higher stress was applied, the effect of aging on fatigue was clearly seen: the J_{tot} increased more between cycles with repeated loadings when the cheeses were older (Figure 7.5d). The effect of aging on the R% (Eq. 7.4) was dependent on cheese type (Figure 7.5e). High R% values, ranging from 91% to 95%, were measured under the lower stress load. Surprisingly, the R% values increased slightly for the RCs but decreased slightly for the SCs with aging time. Generally, the R% [2000 Pa] values were much lower than the values recorded at 50 Pa. About 77% recovery was observed for the RCs aged for 10 and 25 d, but the R% values decreased markedly, to 65% and 51%, after 40 and 55 d of aging, respectively. The impact of aging on the R% values of the SCs was smaller, indicating a higher degree of structure and elasticity. The values for R% diff. (Eq. 7.5) between the two loads were 17%, 19%, 31%, and 47% for the RCs aged for 10, 25, 40, and 55 d, respectively, but only 6%, 6%, 11%, and 13% for the melted SCs (data not shown).

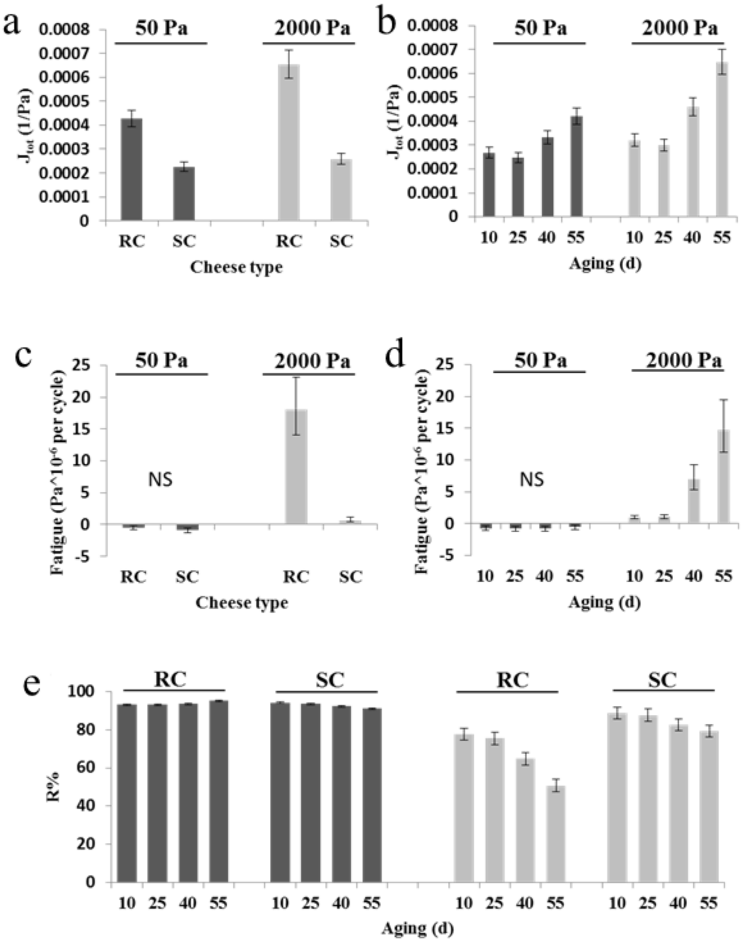

Figure 7.5 **Effects of cheese type (a, c and e) and aging (b, d and e) on the total creep compliance (J_{tot}; a and b), the fatigue (c and d) and the recovery percentage (R%; e) of melted cheeses at 60 °C. Multiple creep/recovery cycles were done at two stresses: 50 Pa (dark bars) and 2000 Pa (light bars). Cheese types: regular cheese (RC) and stabilized cheese (SC). Aging time: 10, 25, 40 and 55 d. Error bars represent the standard error of the means.**

The partially dehydrated cheeses were also tested by MSCR (data not shown). They were less deformed overall by the stress loads. The partially dehydrated RCs presented a higher J_{tot} (0.00027 Pa^{-1}) than did the partially dehydrated SCs (0.00012 Pa^{-1}) ($P < 0.01$). The J_{tot} values measured at the different stresses were similar. This finding suggests that the linear viscoelastic region limit of the partially dehydrated cheeses was close to or higher than the highest stress studied. Consequently, no impact of repeated loading (i.e., fatigue) was measured on the partially dehydrated cheeses. However, the stress level seemed to affect the recovery properties of the partially dehydrated cheeses. The R% [50 Pa] was not affected by cheese type or aging and averaged 93%. At 2000 Pa, however, the R% values for the partially dehydrated cheeses averaged 80% and 88% for the RCs and SCs, respectively ($P = 0.03$). The R% [2000 Pa] decreased slightly during aging, and this trend was similar for both cheese types ($P = 0.04$). The R% diff. values never exceeded 19% and 6% for the partially dehydrated RCs and the partially dehydrated SCs, respectively (data not shown).

7.6 Discussion

7.6.1 Impact of Baking on Cheese

In the present study, the cheese was baked on a pizza model with a perforated dividing layer so that (1) the pizza ingredients could be homogeneously portioned and distributed for consistent experiments, (2) the pizzas could be baked in conditions that simulated common practices in the foodservice industry, and (3) the sensory evaluation of the baked cheese could be done without the interfering taste and texture of the pizza sauce (Wang et al., 1998). Baked-cheese characteristics are known to be affected by many factors such as the cooking conditions, the other pizza ingredients and the intrinsic physicochemical properties of the cheese (Chen et al., 2009). Few studies have investigated the impact of baking on cheese composition. Rudan and Barbano (1998) reported up to 50% total moisture loss after baking Mozzarella cheese alone in an uncovered aluminium pan. However, those authors did not consider the presence of other ingredients, even though they are important contributors to the heating dynamics during pizza baking (Dumas and Mittal, 2002). Forced-air ovens generate convective heating, which is known to greatly dehydrate the cheese surface (Rudan and Barbano, 1998; Wadhwani et al., 2011). The present study found that the weight loss of approximately 15% was due to moisture evaporation. Consequently, the baked cheeses had a relative dry matter content that was ~1.2 times higher than that of the initial, unbaked cheese. Heat also causes the contraction of the *para*casein matrix and the expansion of the liquefied fat, which may be released from the cheese matrix (Kuo et al., 2001; Pastorino et al., 2002; Kim et al., 2011). Under the studied baking conditions, the mechanically expressible liquid phases represented 14% to 20% of the baked-cheese weight. Depending on the physical properties and microstructure of the cheese matrix, mastication is expected to expel some of these liquids, which contribute to the perception of juiciness and to the lubrication of the bolus (Foegeding et al., 2011).

As shown in Table 7.3, cheese type and aging influenced the baked-cheese properties. The RCs had a higher fat-to-protein ratio than the SCs did at similar moisture contents. Hence, the RCs contained fewer structuring components (i.e., protein) and higher flowing components (i.e., liquefied fat), resulting in a weaker and less elastic cheese matrix overall. For RCs, firmness and rubberiness decreased and breakdown resistance increased markedly during aging whereas the sensory texture of SCs remained constant over aging. Changes in the sensory properties of RCs are likely to be attributed to the more intense protein breakdown activity during aging compared to the SCs (Figure 7.2). Water-soluble nitrogenous factions cleaved from the protein matrix no longer contribute to the structure of cheese (Lucey et al., 2003). Our results on sensory properties are in agreement with other studies that reported increased meltablilty of baked cheese with aging (Lucey et al., 2003; Sheehan et al., 2004). Increased meltability precludes a softer and more deformable (increase flow) cheese which is likely to be perceived during consumption. The impact of increasing concentration of cleaved peptides fractions in the aqueous phase on the release of liquid during chewing is not clear, but the panelists assessed lower liquid release during aging, and this only for the RCs which had the higher proteolysis activity (Table 7.3). Other age-related factors such as changes in calcium equilibrium or casein solubilization may have influenced the sensory properties of baked cheeses but was not measured in this study (Lucey et al., 2003; Guggisberg et al., 2007).

7.6.2 Relationships Between Baked-Cheese Properties

The sensory terms used for the evaluation of baked-cheese texture were all correlated, and Pearson correlation coefficients are presented in Table 7.6. The firmness and breakdown resistance terms were negatively correlated. As reported by Brown et al. (2003), firmer cheese is likely to fracture into more pieces during mastication, whereas softer cheese will be more deformable, preventing it from structure breakdown. A strong positive correlation between the firmness and rubberiness terms was also found (Table 7.6). A high rubberiness character implies that a definite elastic behaviour is present. The elastic character increases with the strength and number of elastic interactions within the matrix, which also contribute to the overall firmness of the cheese. Indeed, Çakir et al. (2012a) showed that strongly cross-linked inter-protein interactions in reduced-fat cheese resulted in a firmer and more elastic cheese in comparison with a full-fat cheese. Liquid release during chewing was correlated to a lesser extent with the other sensory terms ($r < 0.65$). However, the trend indicates that firm and rubbery baked cheeses with a high tendency to break also released more liquid. Surprisingly, the sensory terms were poorly correlated with the mechanically expressible serum or oil phase (Table 7.6). A significant positive correlation was found between the extractible serum phase and sensory liquid release. The amount of serum phase drove the amount of total expressible liquid ($r = 0.93$) in comparison with the oil phase ($r = 0.63$) (data not shown), indicating that serum release during mastication may have contributed more to the sensation of juiciness.

Table 7.6 **Pearson correlation coefficients (*r*) between sensory terms and expressible liquids measured on baked cheeses (*n* = *24*).**

	Sensory terms				Expressible liquids	
	Firmness	Breakdown resistance	Rubberiness	Liquid release	Serum	Oil
Firmness	1.00	**−0.86*****	**0.94*****	**−0.54***	−0.31	−0.19
Breakdown resistance		1.00	**−0.83*****	**0.49***	0.28	0.16
Rubberiness			1.00	**−0.49***	−0.37	−0.07
Liquid release				1.00	**0.48***	0.36
Expressible serum phase					1.00	0.29
Expressible oil phase						1.00

*Significant correlation at $P < 0.05$.
**Significant correlation at $P < 0.005$.
***Significant correlation at $P < 0.0001$.

It must be stressed that the correlations observed between terms do not necessarily imply redundancy. The relationships between sensory properties may vary when the product's characteristics or external conditions are changed. Given that many combinations of sensory properties can be found in food systems, it was of interest to investigate the relationships between sensory terms measured on baked cheese.

7.6.3 Relationship between Melted-Cheese Rheological Parameters

The correlation analysis among some rheological parameters measured on cheese that was melted and then cooled to 60 °C is presented in Table 7.7. The R% diff. was used to account for both the R% [50 Pa] and the R% [2000 Pa]. The fatigue [50 Pa] was considered to have little relevancy (Fig. 7.5C and 7.5D) and was therefore excluded from the correlation analysis. Strong correlations were found between all rheological parameters presented in Table 7.7. The parameters obtained by a given test, whether oscillatory shear or MSCR, were best correlated among themselves. The G* and δ were negatively correlated (Eqs. 2.5 and 2.6); they indicate two different properties: the strength of the cheese network and its viscoelastic nature, respectively. Negative correlations between the G* and compliance-related parameters were expected, given that they represent the inverse ratio of stress and strain (Brown et al., 2003). The variations between the correlation coefficients in Table 7.7 clearly indicate that different properties were measured under low or high stress. The high-stress parameters, the J_{tot} [2000 Pa], R% diff. and fatigue [2000 Pa], were strongly correlated and accounted for the behaviour of the melted cheeses in the non-linear viscoelastic region. The correlations between those parameters and the G*, δ or J_{tot} [50 Pa], which are measured within the linear viscoelastic region, were generally weaker. The partial dehydration of cheese increased (~6%) the correlations involving the J_{tot} but decreased the others.

Table 7.7 **Pearson correlation coefficients (*r*) between the rheological parameters of melted cheeses: the complex modulus (G*), the phase angle (δ), the total creep compliance (J_{tot}), the fatigue and the recovery percentage difference (R% diff.). Correlation coefficients obtained with partially dehydrated cheeses are in parentheses (*n* = 24).**

	G* [60 °C]	δ [60 °C]	J_{tot} [50 Pa]	J_{tot} [2000 Pa]	Fatigue [2000 Pa]	R% diff.
G* [60 °C]	1.00	−0.89*** (−0.89***)	−0.85*** (−0.92***)	−0.83*** (0.89***)	−0.79*** (−0.73***)	−0.87*** (−0.86***)
δ [60 °C]		1.00	0.81*** (0.89***)	0.82*** (0.87***)	0.80*** (0.77***)	0.84*** (0.88***)
J_{tot} [50 Pa]			1.00	0.97*** (0.99***)	0.85*** (0.92***)	0.96*** (0.98***)
J_{tot} [2000 Pa]				1.00	0.94*** (0.96***)	0.99*** (0.98***)
Fatigue [2000 Pa]					1.00	0.95*** (0.95***)
R% diff.						1.00

*Significant correlation at $P < 0.05$.
**Significant correlation at $P < 0.005$.
***Significant correlation at $P < 0.0001$.

7.6.4 Relationship between Melted-Cheese Rheological Parameters and Baked-Cheese Sensory Terms

Table 7.8 presents the correlation analysis between the baked-cheese sensory terms and the parameters measured by oscillatory shear or MSCR on the melted cheeses. The G* measured at 60 °C was strongly correlated with the sensory firmness ($r = 0.88$). Both descriptors evaluate the force required to deform the sample, and therefore, a good correlation was expected. It is recognized that sensory firmness can be satisfactorily described by instrumental measurements. However, the relationships with more complex chewdown terms are generally hard to capture by common rheological methods such as oscillatory shear (Foegeding and Drake, 2007). Indeed, the G* was less correlated with breakdown resistance and rubberiness and poorly correlated with liquid release. Mastication is a complex process that involves the compression of food under forces that are well over the range measured by oscillatory shear techniques.

***Table 7.8* Pearson correlation coefficients (*r*) between the sensory terms of baked cheeses and the rheological parameters of melted cheeses: the complex modulus (G*), the phase angle (δ), the total creep compliance (J_{tot}), the fatigue and the recovery percentage difference (R% diff.). Correlations coefficients obtained with partially dehydrated cheeses are in parentheses (*n* = 24).**

	Firmness	Breakdown resistance	Rubberiness	Liquid release
G* [60 °C]	**−0.88***** **(0.85***)**	**−0.72***** **(−0.66**)**	**0.79***** **(0.87**)**	**−0.49*** **(−0.61**)**
δ [60 °C]	**−0.70***** **(−0.74***)**	**0.66**** **(0.60**)**	**−0.64**** **(−0.73***)**	0.18 **(0.51*)**
J_{tot} [50 Pa]	**−0.82***** **(−0.87***)**	**0.70**** **(0.74***)**	**−0.81**** **(−0.91***)**	0.31 **(0.48*)**
J_{tot} [2000 Pa]	**−0.85***** **(−0.86***)**	**0.76***** **(0.74***)**	**−0.85***** **(−0.90**)**	0.30 **(0.44*)**
Fatigue [2000 Pa]	**−0.82***** **(−0.76***)**	**0.76***** **(0.72***)**	**−0.82***** **(−0.81***)**	0.32 (0.28)
R% diff.	**−0.88***** **(−0.86***)**	**0.79***** **(0.75***)**	**−0.87***** **(−0.88***)**	0.36 **(0.45*)**

*Significant correlation at $P < 0.05$.
**Significant correlation at $P < 0.005$.
***Significant correlation at $P < 0.0001$.

Correlations with sensory terms improved when MSCR parameters were used instead of oscillatory shear parameters (Table 7.8). On average, the Pearson correlation coefficients were 5.6% higher. The R% diff. was strongly correlated with the sensory rubberiness ($r = 0.87$). When deformed, the bonds within the cheese matrix can stretch, rearrange, or break permanently (Masi and Addeo, 1986). Rubberiness can be related to the ability of the cheese matrix to store energy within its structure and to restore the energy when the stress is removed. However, the bonds within the matrix must be undamaged by the imposed stress in order to restore energy efficiently. In MSCR, the stress was applied repeatedly and on a time scale (1 s) that can be related to the action of chewing. Some permanent deformation occurred during creep cycles under the higher load and resulted in a lower recovery potential. It seems that the short, repeated stress/recovery cycles of MSCR satisfactorily simulated the action of mastication on the melted cheeses. The R% diff. was also well correlated with the sensory firmness ($r = 0.88$). For firm cheese, lower deformation is expected at a given imposed stress, and therefore, the strain to recover from in a given time period is lower for a firm cheese than for softer cheese. The R% diff., J_{tot} [2000 Pa] and fatigue [2000 Pa] were satisfactorily correlated with the breakdown resistance of the baked cheeses ($r \geq 0.76$). This last sensory term relates to the propensity of food to fracture during mastication, which is normally difficult to measure instrumentally on melted cheese because of sample preparation limitations (Truong et al., 2002). The correlations between the J_{tot} [50 Pa] and firmness, breakdown resistance and rubberiness were weaker than the correlations obtained when high-stress parameters were used (*r* decreased by ~4.5%). Hence, the non-linear behaviour probed by the higher stress level in MSCR was important for better predicting texture perception. However, the sensory perception of liquid release was weakly correlated with the MSCR parameters.

The partial dehydration of cheeses before melting and rheological evaluation improved the correlations with sensory terms overall, with an average Pearson correlation coefficient increase of 3.9% (Table 7.8). The application of rheological measurements to melted cheeses that have similar moisture contents to baked cheeses proved to increase the predictability of sensory properties. Our results agree with the view of Foegeding and Drake (2007) that rheological properties should be characterized under conditions that cover those applied for sensory evaluation. However, only slight impact of dehydration was observed in correlation for the high-stress MSCR descriptors (+0.6%) whereas dehydration of the samples globally increased the *r* of the other rheological descriptors of 12.2%. The dehydrated cheeses were firmer and more structured, and thus their linear viscoelastic region limit was increased. As discussed before, the measuring stresses had a negligible impact on the MSCR descriptors for the partially dehydrated cheeses. Hence, the testing at 2000 Pa does not seem to probe the behaviour of melted partially dehydrated cheese in the non-linear viscoelastic region, and therefore, the increased potential of high-stress MSCR descriptors to correlate with sensory terms is reduced. This result suggests that increasing the measuring stresses during the MSCR test on partially dehydrated cheeses could further improve the prediction of sensory terms.

7.7 Conclusions

Aged Mozzarella cheeses were baked on a pizza model under standardized conditions that simulated the preparation procedures used by commercial food chains. The cheeses lost approximately 15% of their weight through moisture evaporation during baking, but the younger cheeses were more prone to dehydration than older ones. As a result, baking cheese increased solids content and modified its physical and sensory properties. During heating, the cheese matrix underwent changes that resulted in the release of aqueous serum and fat, which contributed to the unique sensory properties of the baked cheeses. The descriptive sensory evaluation satisfactorily discriminated the effects of cheese type and age. Extensive proteolysis during aging and higher fat-to-protein ratio in the RCs led to a softer, more deformable and less rubbery texture than that of the SCs after baking. Similar observations were made on the melted cheeses using rheological analyses. Hence, high correlations were found between the rheological descriptors of the melted cheeses and the sensory descriptors of the baked cheeses. The application of the Multiple Stress Creep Recovery test to melted cheeses was best suited for predicting the baked-cheese sensory texture terms. This test may be used in future studies to probe the physical properties of melted cheeses under short, repeated and both low and high stress loads. Taking into account the moisture loss that occurs during baking by the partial dehydration of melted cheeses had shown to further improve the correlations with sensory properties. These results stress the importance of considering the final product characteristics and the conditions during oral processing when developing appropriate predictive rheological techniques.

7.8 Acknowledgements

This work was financially supported by the Natural Sciences and Engineering Research Council of Canada (Ottawa, ON, Canada), the Fonds de recherche du Québec – Nature et technologies (Québec City, QC, Canada) and Agropur Dairy Cooperative (St-Hubert, QC, Canada). The authors are grateful to Jacinthe Fortin and the sensory panelists at Agriculture and Agri-Food Canada’s Food Research and Development Centre (St-Hyacinthe, QC, Canada) for their professionalism and hard work.

Chapter 8 General Conclusions

The goal of the work presented in this thesis was to gain a better understanding on the compositional and technological factors that can be controlled by the cheese-maker in order to produce Mozzarella cheese with desired physical properties. The general hypothesis of this doctoral thesis was that *the key attributes of Mozzarella cheese required for its uses as ingredient food are primarily governed by its composition, microstructure and physical properties, and that these properties can be modulated using simple and common cheese-making strategies*.

The work carried out allowed us to validate our hypothesis using innovative methodological approaches that were focused on the characterization of cheese properties in conditions that simulated the ones used by end-users. We were able to demonstrate that microstructure and physical properties of Mozzarella can be controlled by carefully optimize the milk standardization with denatured whey proteins, pH during cheese-making steps and the thermo-mechanical treatments applied on curd. Furthermore, the aging of cheese and external conditions such as temperature and shear are factors that have shown to have profound impacts on cheeses properties. In turn, changes in microstructure and physical properties definitely impacted some key attributes of ingredient cheese such as its machinability and its texture during and after baking conditions.

8.1 Achievements and Original Contributions

The work presented in this thesis covers many events typically encountered during the shelf life of Mozzarella cheese used as an ingredient. Figure 8.1 presents an overview of the fields of interests of the different core chapters presented in this thesis. Considering all these main events is important since each one of them has an impact on the following ones. This thesis presents for the first time a global approach to study the different factors that impact the cheese physical properties during these main events.

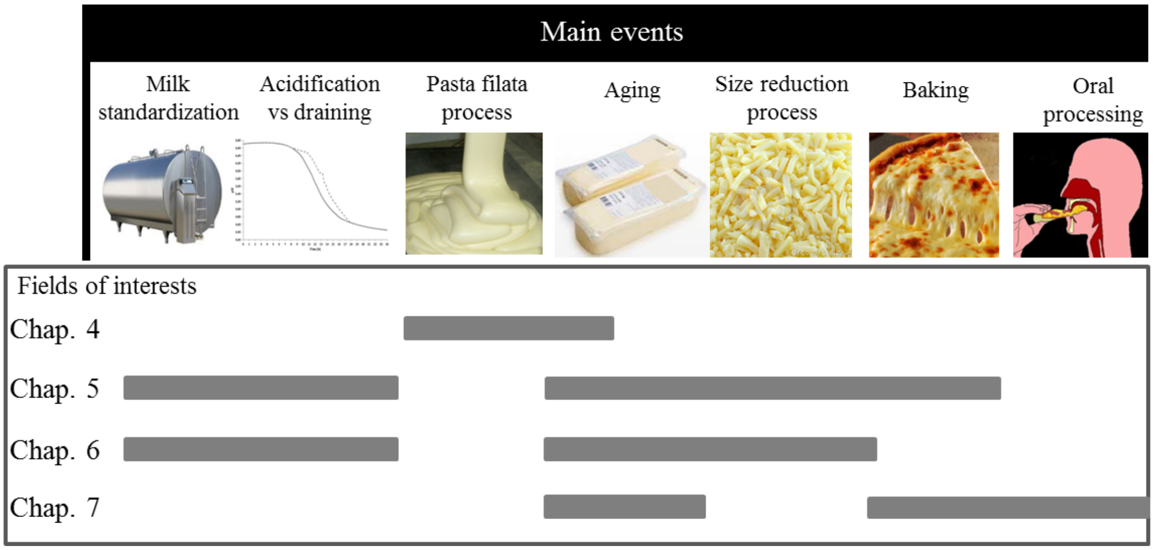

Figure 8.1 **Fields of interests of the core chapters of the thesis related to the main events occurring during the life span of Mozzarella cheese ingredient.**

The first objective of this work was to evaluate the impact of the thermo-mechanical treatments applied to the curd during the pasta filata process on the composition, microstructure, and rheological properties of cheese (Chapter 4). To our knowledge, we studied for the first time the impact of independently controlled conditions of the pasta filata process (i.e., temperature, time of residence, auger speed). This was done using a mixing model system in which important scale-up processing variables were measured (Specific mechanical energy, heat load and curd's final temperature). Knowing the limits of this model to fully mimic the industrial process, we were yet able to expose several important trends that can help manufacturers to better evaluate the impact of their process. We demonstrated that the amount of mechanical energy imparted is strongly linked to the loss of fat into the stretching brine, while the thermal component of the treatment had insignificant effect within common temperature operation range of the process. The major impact of the processing conditions on the composition and microstructure of the final product was also exposed. This part also highlighted the difficulty to isolate effects of multiple factors on the physical properties of cheese as a result of the complex changes that occurs during the thermo-mechanical treatment. The work presented in Chapter 4 brings enough evidences to support the hypothesis that the pasta filata process conditions can be optimized in order to produce Mozzarella cheese with desired characteristics.

The second objective of this work aimed at measuring the impact of some common cheese-making strategies on the machinability of cheese (Chapter 5). The effect of 3 strategies was investigated as part of different events of the cheese-making process: the addition of denatured whey protein to cheese milk, the control of curd mineralization by the modifying of pH at renneting and draining steps, and the aging of cheese. The experimental design used for this work allowed us to evaluate the interaction effect of these strategies, and therefore bringing further insights on the global approach that could be used by cheese manufacturers to optimize the shreddability of their product. Practically, this work showed that the synergy effect of milk supplementation, mineralization control and aging may considerably improve shredding performance. This work was also an opportunity to improve or develop original methodologies to characterize the shredding properties of cheeses. To our view, these new methods brought significant contribution to this area of research since most of the few previous researches reported empirical observations about factors impacting the cheese shreddability.

The third objective of this thesis was to develop predictive models of cheese machinability based on its composition and its texture (Chapter 6). This work was based on the hypothesis that the cheese-making factors studied in Chapter 5 induced significant impact on cheese physical properties. This hypothesis was verified by measuring 10 textural descriptors from 4 different testing methodologies, which could then be related to the shredding performance of the cheese. The use of an objective selection method for the predictive parameters allowed us to target only few compositional and textural parameters that were relevant to cheese shreddability. Moreover, the use of the principal component analysis approach revealed that the sticking of cheese to the shredding blade and the quality of the shreds (presence of fines or long shreds) are independent phenomena, and therefore that their optimisation requires different strategies. This work has practical importance for the cheese manufacturers as it helps them to choose appropriate quality control descriptors in order to predict shredding performance prior to the process.

Finally, the fourth objective of the work aimed to better understand the impact of baking Mozzarella cheese on its physical properties and sensory perception (Chapter 7). The hypothesis that cheeses made with different cheese-making strategies and ages would have different physical properties, and that it would also have an impact on baked cheese, was imposed by our previous observations (Chapters 4 to 6) and the literature. This hypothesis was verified by (1) a descriptive sensory analysis of cheese baked in realistic conditions, and (2) by measuring melted cheese rheological properties using original protocols under both small and large ranges of shear-deformation. This work also contributed to a better understanding of the relationship between physical properties and sensory perception of texture. Moreover, we verified the hypothesis that variations in cheeses composition, mainly attributed to moisture loss during baking impact the physical properties of melted cheese. Results of this work showed the potential of using compositional or technological factors to change melted cheese textural characteristics and proposed relevant methods to the food research community in order to better quantify these changes.

Overall, the work presented in this thesis supports the idea that an adequate control of the physical properties of cheese using different cheese-making strategies may improve significantly the performance of cheese under shredding and baking conditions.

8.2 Significance of the Results

This thesis work is expected to have impacts on many aspects of the cheese technology field. As mentioned previously, many events of importance occur during the shelf life of cheese ingredient from the farm to the fork (Figure 8.1). The work presented in this thesis led to several advancements in many of these events. This broad area of research allowed us to study the impact of many different cheese-making strategies to produce cheese with specifics key attributes. Practically, the results obtained in this thesis showed that different factors can contribute to attain a desired texture, but also that a combination of many factors may be required. This knowledge reveals a particular importance for cheese producers whom may be constrained by infrastructure and equipment limitations or instances regulation, and therefore who must envisage different strategies in order to develop an ingredient cheese with designated attributes.

In line with this view, this thesis work led to a better understanding of the impacts of milk standardization and transformation, and of external conditions such as shear and temperature on the quality attributes of Mozzarella cheese. Table 8.1 summarizes the key impacts of different factors studied in our research that can be used as single or combined strategies to control various aspects of cheese properties.

Table 8.1 **Summary of the main factors studied and their key impacts on cheese properties.**

Factors	Key impact observed
Addition of WP-D in cheese milk	• Increase cheese moisture • Impact on cheese structure • May negatively impact shredding properties
Control of cheese mineralization through the renneting and draining pH	• Impact on proteolysis during aging • Impact on cheese structure • May alter shredding and melt properties
Control of the pasta filata process parameters	• Impact on cheese composition and solid loss • Impact on the distribution of water and fat within cheese microstructure
Aging of cheese	• Impact on cheese structure of the *para*casein matrix • May improve shredding and melt properties to a certain level • Negatively impact the handling of shredded cheese
Shear or deformation on physical behavior	• Impact on the loss of solid during the pasta filata process • Impact the physical behavior of cheese depending of the extent of the shear (e.g., fracture or flow)
Temperature	• Impact the ability of cheese to be sheared (e.g., during pasta filata process, shredding, or mastication) • Impact the microstructure of the protein and fat and the partition of the aqueous phase within the cheese • Impact the physicochemical reactions during aging of cheese

A better understanding of the impact of WP-D on key attributes of machinability or meltabilily was needed since related research was scarce and impacts reported were sometimes contradictory. This lack of knowledge may be explained by a low practical interest of some countries in this research field since the use of this ingredient is strictly controlled, and by the fact that these particles probably induce many confounding impacts on cheese final characteristics. The knowledge gained in this work is expected to benefit the dairy transformation sector, and especially in Canada where the use of denatured whey protein in Mozzarella cheese is generalized.

The major impact of mineralization on Mozzarella cheese's melting attributes is now widely accepted amongst cheese scientists. The results of this thesis confirmed its importance as a key factor for the control of cheese properties. In particular, this work investigated for the first time its impact on the large deformation and fracture behavior in a context of size reduction process. Moreover, the significant differences observed between cheeses made with milk renneted at pH's that only differed by 0.1 unit confirmed the effectiveness of this strategy, and therefore validated previous observations made by Dr Britten's team and by Johnson and Lucey (2006).

The pasta filata process distinguishes Mozzarella cheese from other cheese-making technologies. This is a rather complex process since it involves simultaneous thermal and mechanical actions. Despites that it can be expected to have dramatic impact on the cheese properties, these impacts were not clearly identified, and moreover the causes of these impacts (i.e., the thermal or the mechanical component of the process) were not defined in the few studies available in literature. The work presented in this thesis allowed to better understand the composition and microstructure changes as a result of the pasta filata process. This study is expected to give precious orientations on how this process can be optimized according to the product's attributes needed, or simply for reduction of solids loss during stretching.

Aging of Mozzarella cheese is well recognized as a critical factor for the improvement of key attributes required for its use as food ingredient. However, in practice this factor can be subjected to economical or technical limitations. This project not only confirmed the major impact of this factor on cheese physical properties, but also explored different cheese-making strategies to modify the evolution of these properties during the aging process (e.g., amount of bulk water after pasta filata process, colloidal calcium content, and global approach to limit coagulant residual activity). We expect that the results presented in this thesis will support cheese-making strategic decisions in order to make the most efficient use of this aging period.

This thesis work is also expected to provide a better understanding of the impact of large shear or deformation on cheese properties. Particularly, this work investigated the relationship between cheese properties subjected to common large deformation processes such as pasta filata process, size reduction and mastication. This understanding is, to our view, critical for the development of enhanced cheese characteristics and sensory perception.

As expected, we validated that temperature is one of the most important factors that affect cheese physical properties. This work's investigation covered different events where we can have a control on cheese temperature: during the pasta filata process, during shredding and storage of the shreds, and of course during melting and cooling of baked cheese. The profound impact of temperature on cheese composition, microstructure, and in definitive on its texture was clearly demonstrated by this thesis work. These results should promote the control of cheese temperature as an opportunity to develop new properties.

Along the research path of the last few years, several original approaches and methodology were developed in order to resolve questions not yet answered by the dairy industry and the academic sectors. These new advances in the way we characterize food texture is expected to stimulate further exciting research.

Finally, the current popularity of Mozzarella cheese as a food ingredient is expected to bring this thesis work significant interest from both the academic and the industrial dairy sector. To this day, recently published papers (Chapters 5 and 6 in this thesis) were downloaded more than 800 times in 34 countries (My Research Dashboard, 2015). This interest is expected to grow during the years to come.

8.3 Remaining Questions and Perspectives

Some important remaining questions and perspectives arise from the results presented in this thesis. In chapter 4, we observed a major impact of the thermo-mechanical treatments on cheese composition and microstructure. According to the current state of knowledge found in literature, this was expected to also induce variation in cheese physical properties, but dramatic changes were not observed. We then made the assumption that confounding effect where present which resulted in a globally low impact on physical properties. This hypothesis was not verified in this work. Further work would be required to understand the relative impact of these combined factors.

In this thesis work, we have made the choice to study certain processes using model systems (e.g., mixing unit, shredding device, and pizza baking model). Using these approaches, we better controlled external factors at the expense of a certain loss of representativeness if compared with the real processing conditions. This inevitably leads to some limitations about generalizing this thesis results to the numberless variety of processing conditions found in the cheese sector. Indeed, the impact of using stretching brine to stretch the curd (as in Chapter 4) may not lead to similar conclusions than if hot steam was used instead as it is now common when using the dry cooker technology. Many other examples could be given regarding the size reduction process and practices used for ingredient cheese preparation and consumption. That being said, it is clear that further work should be done on specific applications, and it is reasonable to expect that future research hypothesis will be based on the observations presented in this thesis.

This work measured physical properties of cheese in many conditions of large shear or deformation. It must be acknowledge that many questions remain in this area of research since the physical behavior of viscoelastic material, such as cheese, is not clearly related to the shear extend beyond the so-called linear viscoelastic region. Clearly, this limits the analysis of the true material properties and our understanding of the cheese texture under common large shear-deformation processes. In chapter 7, we proposed to use the MSCR test to characterize large stress properties of melted cheese. This method has a great potential to bring further insight into our comprehension of cheese properties, but a deeper analysis of potential parameters was beyond this thesis scope. Fortunately, new rheological characterization approaches using large shear-deformation have been recently been proposed (Ewoldt et al., 2008; Melito, 2012) and should be given increased attention for future research.

A better knowledge of the physical behavior of cheese beyond its LVR should benefit our understanding of the relationship between mechanical properties and the sensation experimented during the consumption of food. A relatively new area of research called “Oral processing science” is a promising avenue to get these answers (Koç et al., 2013).

This example shows that multiple fields of research must work conjointly in order to better understand the extremely complex relationships between food texture, the mechanical action of eating and the sensory oral perception. Knowing that, it seems obvious that characterizing the sensory perception of baked cheese, which texture is strongly impacted during cooling, is a challenge that needs future work in order to be fully understood.

The results presented in this thesis were analysed using different statistical tools (e.g., variance analysis using mixed model, correlation, predictive modeling, and PCA). It is critical that the research data are treated adequately in order to efficiently communicate the results obtained. This is especially true when multiples interactions between factors are studied. It is expected that the use of multifactorial analysis such as PCA will be increasingly used in future studies since (1) the explaining potential of these methods is progressively democratized, and that (2) there is an increasing number of studies that wish to understand complex interactions between factors (Ma et al., 2013a; Schenkel et al., 2014). These thesis works have also shown that multiples factors can impact cheese properties, and therefore it seems that these statistical techniques can be appropriate in the context of cheese technology research.

References

Abhyankar, A. R., D. M. Mulvihill, and M. A. E. Auty. 2014. Combined confocal microscopy and large deformation analysis of emulsion filled gels and stirred acid milk gels. Food Struc. 1:127–136.

Ak, M. M., and S. Gunasekaran. 1993. Stress-stress curve analysis of Cheddar cheese under uniaxial compression. J. Food Sci. 57:1078–1081.

Akbulut, C., S. Govindasamy-Lucey, J. A. Lucey, J. J. Jaeggi, and M. E. Johnson. 2011. Impact of grating size on the texture and melting properties of reformed non-fat cheese. Milchwissenschaft 66:169–172.

Altan A., M. Turhan, and S. Gunasekaran. 2005. Short Communication: Comparison of covered and uncovered Schreiber test for cheese meltability evaluation. J. Dairy Sci. 88:857-861.

Apostolopoulos, C., and R. J. Marshall. 1994. A quantitative method for the determination of shreddability of cheese. J. Food Qual. 17:115–128.

ASTM. 2010. Standard test method for Multiple Stress Creep and Recovery (MSCR) of asphalt binder using shear rheometer. Designation D7405-10a, ASTM International, West Conshohocken, PA.

Auger, F., M.-H. Morel, J. Lefebvre, M. Dewilde, and A. Readl. 2008. A parametric and microstructural study of the formation of gluten network in mixed flour–water batter. J. Cereal Sci. 48:349–358.

Auty, M. A. E., M. A. Fenelon, T. P. Guinee, C. Mullins, and D. M. Mulvihill. 1999. Dynamic confocal scanning laser microscopy methods for studying milk protein gelation and cheese melting. Scanning 21:299–304.

Auty, M. A. E., M. Twomey, T. P. Guinee, and D. M. Mulvihill. 2001. Development and application of confocal scanning laser microscopy methods for studying the distribution of fat and protein in selected dairy products. J. Dairy Res. 68:417–427.

Ayyash, M. M., and N. P. Shah. 2011. The effect of substitution of NaCl with KCl on chemical and functional properties of low-moisture Mozzarella cheese. J. Dairy Sci. 94:3761–3768.

Bähler, B., and J. Hinrichs. 2013. Characterisation of Mozzarella cheese curd by means of capillary rheometry. Int. J. Dairy Technol. 66:231–235.

Bansal, N., P. F. Fox, and P. L. H. McSweeney. 2009. Comparison of the level of residual coagulant activity in different cheese varieties. J. Dairy Res. 76:290–293.

Barden, L. M., J. A. Osborne, D. J. McMahon, and E. A. Foegeding. 2015. Investigating the filled gel model in cheddar cheese through use of Sephadex beads. J. Dairy Sci. 98:1502–1516.

Barrangou, L. M., M. A. Drake, C. R. Daubert, and E. A. Foegeding. 2006. Sensory texture related to large-strain rheological properties of agar/glycerol gels as a model food. J. Texture Stud. 37:241–262.

Bast, R., P. Sharma, H. K. B. Easton, T. T. Dessev, M. Lad, and P. A. Munro. 2015. Tensile testing to quantitate the anisotropy and strain hardening of Mozzarella cheese. Int. Dairy J. 44:6–14.

Bertola, N. C., A. E. Bevilacqua, and N. E. Zaritzky. 1996. Textural changes and proteolysis of low-moisture Mozzarella cheese frozen under various conditions. LWT-Food Sci. Technol. 29:470–474.

Bingham, N. H. and J. M. Fry. 2010. Regression: Linear Models in Statistics. Springer, London, UK.

Boisard, L., C. Tournier, E. Sémon, E. Noirot, E. Guichard, and C. Salles. 2014. Salt and fat contents influence the microstructure of model cheeses, chewing/swallowing and in vivo aroma releases. Flavour Fragr. J. 29:95–106.

Bourne, M. C. 1978. Texture profile analysis. Food Technol. 32:62–66.

Brenner, T., and K. Nishinari. 2014. A note on instrumental measure of adhesiveness and their correlation with sensory perception. J. Texture Stud. 45:74–79

Bringe, N. A., and J. E. Kinsella. 1986. Influence of calcium-chloride on the chymosin-initiated coagulation of casein micelles. J. Dairy Res. 53:371–379.

Brown, J. A., E. A. Foegeding, C. R. Daubert, M. A. Drake, and M. Gumpertz. 2003. Relationships among rheological and sensorial properties of young cheeses. J. Dairy Sci. 86:3054–3067.

Bryant, C. M., and D. J. McClements. 1998. Molecular basis of protein functionality with special consideration of cold-set gels derived from het-denatured whey. Trends Food Sci. Technol. 9:143–151.

Cais-Sokolinska, D., and J. Picul. 2009. Cheese meltability as assessed by the tube test and Schreiber test depending on fat contents and storage time, based on curd-ripened fried cheese. Czech J. Food Sci. 27:301–308.

Çakir, E., H. Koç, C. J. Vinyard, G. Essick, C. R. Daubert, M. A. Drake, and E. A. Foegeding. 2012a. Evaluation of texture changes due to compositional differences using oral processing. J. Texture Stud. 43:257–267.

Çakir, E., H. Koç, C. J. Vinyard, G. Essick, C. R. Daubert, M. A. Drake, and E. A. Foegeding. 2012b. Interrelations among physical characteristics, sensory perception and oral processing of protein-based soft solid structures. Food Hydrocolloid 29:234–245.

Charalambides, M. N., J. G. Williams, and S. Chakrabarti. 1995. A study of the influence of ageing on the mechanical properties of Cheddar cheese. J. Mater. Sci. 30:3959–3967.

Charalambides, M. N., S. M. Goh, S. L. Lim, and J. G. Williams. 2001. The analysis of the frictional effect on stress-strain data from uniaxial compression of cheese. J. Mater. Sci. 36:2313–2321.

Castillo, M. 2010. Cheese making: cutting time prediction. Published online in Encyclopedia of Agricultural, Food, and Biological Engineering. D. R. Heldman, C. I. Moraru. 2nd ed. Taylor and Francis, New York, NY.

Chen, C. 2003. Development and application of a cheese shred/texture map delineated by cheese rheological, sensory and chemical analysis. Annual Report, Wisconsin Center for Dairy Research, Madison, WI.

Chen, C., D. Wolle, and D. Sommer. 2009. Mozzarella, Pages 459–487 in The sensory evaluation of dairy products, C. Clark, M. A. Drake, M. Costella, F. Bodyfelt. ed. Springer. London.

Chen, J. 2014. Food oral processing: Some important underpinning principles of eating and sensory perception. Food Struct. 1:91–105.

Chen, J., and J. R. Stokes. 2012. Rheology and tribology: two distinctive regimes of food texture sensation. Trends Food Sci. Tech. 25:4–12.

Childs, J. L., C. R. Daubert, L. Stefanski, and E. A. Foegeding. 2007. Factors regulating cheese shreddability. J. Dairy Sci. 90:2163–2174.

Choi, J., D. S. Horne, M. E. Johnson, and J. A. Lucey. 2008. Effects of the concentration of insoluble calcium phosphate associated with casein micelles on the functionality of directly acidified cheese. J. Dairy Sci. 91:513–522.

Colin-Cruz, M. L. A., O. Dublan-Garcia, A. Espinoza-Ortega, and A. Dominguez-Lopez. 2011. The effect of varying fat content on the microstructure of Oaxaca cheese, a typical pasta filata cheese. Int. J. Dairy Technol. 65:71–80.

Corredig, M., G. Ion Titapiccolo, Z. Gaygadzhiev, and M. Alexander. 2011. Rennet-induced aggregation of milk containing homogenized fat globules: effect of interacting and non-interacting fat globules observed using diffusing wave spectroscopy. Int. Dairy J. 21:679–684.

Cortez, M. A. S., M. M. Furtado, M. L. Gigante, and P. S. Kindstedt. 2008. Effect of pH on characteristics of low-moisture Mozzarella cheese during refrigerated storage. J. Food Sci. 73:S443–S448.

Costabel, L., M. S. Pauletti, and E. Hynes. 2007. Proteolysis in Mozzarella cheeses manufactured by different industrial processes. J. Dairy Sci. 90:2103–2112.

Creamer, L. K. 1976. Casein proteolysis in Mozzarella-type cheese. New Zeal. J. Dairy Sci. 11:130–135.

Dalgleish, D. G., and M. Corredig. 2012. Micelle of milk and its changes during processing. Ann. Rev. Food Sci. Technol. 3:449–467.

Dave, R. I., D. J. McMahon, C. J. Oberg, and J. R. Broadbent. 2003. Influence of coagulant level on proteolysis and functionality of Mozzarella cheeses made using direct acidification. J. Dairy Sci. 86:114–126.

Deeth, H. C., and M. J. Lewis. 2015. Practical consequences of calcium addition to and removal from milk and milk products. Int. J. Dairy Technol. 68:1–10.

Dejmek, P., and P. Walstra. 2004. The syneresis of rennet-coagulated curd. Pages 71–103 in Cheese: Chemistry, Physics and Microbiology. Vol 1. P.F. Fox, P. L. H. McSweeney, T. M. Cogan, T. P. Guinee, 3rd ed. Chapman and Hall, London, UK.

Delgadillo, R., U. Bahia, and R. Lakes. 2012. A nonlinear constitutive relationship for asphalt binders. Mater. Struct. 45:457–473.

De Kruif, C. G. 1999. Casein micelle interactions. Int. Dairy J. 9:183–188.

De Kuif, C. G., T. Huppertz, V. S. Urban, and A. V. Petukhov. 2012. Casein micelles and their internal structure. Adv. Colloid Interfac. 171–172:36–52.

De Souza Mendez, P. R., and R. L. Thompson. 2013. A unified approach to model elasto-viscoplastic thixotropic yield-stress material and apparent yield-stress fluids. Rheol. Acta 52:673–694.

Dimitreli, G., and A. S. Thomareis. 2008. Effect of chemical composition on the linear viscoelastic properties of spreadable-type processed cheese. J. Food Eng. 84:368–374.

Drake, M. A., P. D. Gerard, V. D. Truong, and C. R. Daubert. 1999. Relationship between instrumental and sensory measurement of cheese texture. J. Texture Stud. 30:451–476.

Drake M. A. 2009. Modern sensory practices, Pages 505–530 in The sensory evaluation of dairy products, C. Clark, M. A. Drake, M. Costella, and F. Bodyfelt. Ed., Springer. London.

Dumas, C., and G. S. Mittal. 2002. Heat and mass transfer properties of pizza during baking. Int. J. Food Prop. 5:161–177.

El-Bakry, M., E. Duggan, E. D. O'Riordan, and M. O'Sullivan. 2010. Small scale imitation cheese manufacture using a Farinograph. LWT-Food Sci. Technol. 43:1079–1087.

El-Bakry, M., and J. Sheehan. 2014. Analysing cheese microstructure: a review of recent developments. J. Food Eng. 125:84–96.

Euromonitor. 2014. Cheese: Key Markets, Categories and Value adding trends, Passport Market Report. Euromonitor International, London, U.K.

Everard, C. D., C. P. O'Donnell, C. C. Fagan, E. M. Sheehan, C. M. Delahunty, and D. J. O'Callaghan. 2005. Correlation between process cheese meltability determined by sensory analysis, computer vision method and Olson and Price test. Int. J. Food Prop. 8:267–275.

Everett, D. W., M. K. Rowney, M. W. Hichey, and P. Roupas. 2004. Salt-induced structural changes in Mozzarella cheese and the impact upon free oil formation in ripening cheese. Lait 84:539–549.

Everett, D. W. 2007. Microstructure of natural cheeses. Pages 170–209 in Structure of dairy products. A. Y. Tamine. Ed., Blackwell publishing Ltd, Oxford, UK.

Everett, D. W., and M. A. E. Auty. 2008. Cheese structure and current methods of analysis. Int. Dairy J. 18:759–773.

Ewoldt, R. H., A. E. Hosoi, and G. H. McKinley. 2008. New measures for characterizing nonlinear viscoelasticity in large amplitude oscillatory shear. J. Rheol. 52:1427–1458.

Farrell, H. M., R. Jimenez-Flores, G. T. Bleck, E. M. Brown, J. E. Butler, L. K. Creamer, C. L. Hicks, C. M. Hollar, K. F. Ng-Kwai-Hang, and H. E. Swaisgood. Nomenclature of the proteins of cows' milk–Sixth Revision. J. Dairy Sci. 87:1641–1674.

Feeney, E. P., T. P. Guinee, and P. F. Fox. 2002. Effect of pH and calcium concentration on proteolysis in Mozzarella cheese. J. Dairy Sci. 85:1646–1654.

Fenelon, M. A., and T. P. Guinee. 1997. The compositional, textural and maturation characteristic of reduced-fat Cheddar made from milk containing added Dairy-Lo™. Milchwissenschaft 52:385–389.

Fife, R. L., D. J. McMahon, and C. J. Oberg. 2002. Test for measuring the stretchability of melted cheese. J. Dairy Sci. 85:3539–3545.

Fife, R. L. 2003. The influence of fat and water on the melted cheese characteristics of Mozzarella cheese. Thesis. Utah State University, Logan, UT.

Floury, J., B. Camier, F. Rousseau, C. Lopez, J.-P. Tissier, and M.-H. Famelart. 2009. Reducing salt level in food: Part 1. Factors affecting the manufacture of model cheese systems and their structure-texture relationships. LWT- Food Sci. Technol. 42:854–862.

Floury, J., S. Jeanson, S. Aly, and S. Lortal. 2010. Determination of the diffusion coefficients of small solutes in cheese: a review. Dairy Sci. Technol. 90:477–508.

Foegeding, E. A., and M. A. Drake. 2007. Invited review: Sensory and mechanical properties of cheese texture. J. Dairy Sci. 90:1611–1624.

Foegeding, E. A., C. R. Daubert, M. A. Drake, G. Essick, M. Trulsson, C. J. Vinyard, and F. Van De Velde. 2011. A comprehensive approach to understanding textural properties of semi- and soft-solid foods. J. Texture Stud. 42:103–129.

Food and Drug Regulations. 2015. C.R.C., c. 870. Minister of Justice. Government of Canada. Published online: http://laws-lois.justice.gc.ca.

Fox, P. F. 1970. Influence of aggregation on the susceptibility of casein to proteolysis. J. Dairy Res. 37:173–180.

Ge, Q., M. Almena-Aliste, and P. S. Kindstedt. 2002. Reversibility of pH-induced changes in the calcium distribution and melting characteristics of Mozzarella cheese. Aust. J. Dairy Technol. 57:3–9.

Gernigon, G., M. Piot, E. Beaucher, R. Jeantet, and P. Schuck. 2009. Physicochemical characterization of Mozzarella cheese wheys and stretchwaters in comparison with several other sweet wheys. J. Dairy Sci. 92:5371–5377.

Giroux, H. J., C. Bouchard, and M. Britten. 2014. Combined effect of renneting pH, cooking temperature, and dry salting on the contraction kinetics of rennet-induced milk gels. Int. Dairy J. 35:70–74.

Goh, S. M., M. N. Charalambides, and J. G. Williams. 2003. Large strain time dependent behavior of cheese. J. Rheol. 47:701–716.

Goh, S. M., M. N. Charalambides, and J. G. Williams. 2005. On the mechanics of wire cutting of cheese. Eng. Fract. Mech. 72:931–946.

Gresti, J., M. Bugaut, C. Maniongui, and J. Bezard. 1993. Composition of molecular species of triacylglycerols in bovine milk fat. J. Dairy Sci. 76:1850–1869.

Guggisberg, D., U. Bütikofer, and B. Albrecht. 2007. Melting and solidification characteristics of Swiss Raclette cheese measured by small amplitude oscillatory shear measurements. J. Texture Stud. 38:297–323.

Guinee, T. P. 2002. The functionality of cheese as an ingredient: a review. Aust. J. Dairy Technol. 57:79–91.

Guinee, T. P. 2004. Salting and the role of salt in cheese. Int. J. Dairy Technol. 57:99–109.

Guinee, T. P., and D. J. O'Callaghan. 1997. The use of a simple empirical method for objective quantification of the stretchability of cheese on cooked pizza pies. J. Food Eng. 31:147–161.

Guinee, T. P., and P. F. Fox. 2002. Salt in cheese: physical, chemical and biological aspects. Pages 207–259 in Cheese: Chemistry, physics and microbiology. Vol. 1. Eds: Fox, P. F., McSweeney, P. L. H., Cogan, T. M., and T. P Guinee. 3rd ed., Elsevier academic press, London, UK.

Guinee, T. P., M. A. E. Auty, and C. Mullins. 1999. Observation of the microstructure and heat-induced changes in the viscoelasticity of commercial cheeses. Aust. J. Dairy Technol. 54:84–89.

Guinee, T. P., M. A. E. Auty, and M. A. Fenelon. 2000a. The effect of fat content on the rheology, microstructure and heat-induced functional characteristics of Cheddar cheese. Int. Dairy J. 10:277–288.

Guinee, T. P., E. O. Mulholland, C. Mullins, and M. O. Corcoran. 2000b.Effect of salting method on the composition, yield and functionality of low moisture Mozzarella cheese. Milchwissenschaft 55:135–138.

Guinee, T. P., D. Harrington, M. O. Corcoran, E. O. Mulholland, and C. Mullins. 2000c. The compositional and functional properties of commercial Mozzarella, Cheddar and analogue pizza cheese. Int. J. Dairy Technol. 53:51–56.

Guinee, T. P., E. P. Feeney, M. A. E. Auty, and P. F. Fox. 2002. Effect of pH and calcium concentration on some textural and functional properties of Mozzarella cheese. J. Dairy Sci. 85:1655–1669.

Guinee, T. P., and K. N. Kilcawley. 2004. Cheese as an ingredient. Pages 395-423 in Cheese: Chemistry, physics and microbiology. Vol. 1. Eds: Fox, P. F., McSweeney, P. L. H., Cogan, T. M., and T. P Guinee. 3rd ed., Elsevier academic press, London, UK.

Gunasekaran, S., and M. M. Ak. 2002. Cheese Rheology and Texture. CRC Press, Boca Raton, FL.

Gunasekaran S., M.-I. Kuo, and Y.-C. Wang. 1998. Evaluating melt characteristics of Mozzarella cheese by a linear viscoelastic test. Aust. J. Dairy Technol. 53:111–122.

Guo, M. R., and P. S. Kindstedt. 1995. Age-related changes in the water phase of Mozzarella cheese. J. Dairy Sci. 78:2099–2107.

Guo, M. R., J. K. A. Gilmore, and P. S. Kindstedt. 1997. Effect of sodium chloride on the serum phase of Mozzarella cheese. J. Dairy Sci. 80:3092–3098.

Gustavsson, F., M. Glantz, A. J. Buitenhuis, H. Lindmark-Mansson, H. Stalhammar, A. Andrén, and M. Paulson. 2014. Factors influencing chymosin-induced gelation of milk from individual dairy cows: Major effects of casein micelle size and calcium. Int. Dairy J. 39:201–208.

Hassan, A., M. E. Johnson, and J. A. Lucey. 2004. Changes in the proportions of soluble and insoluble calcium during the ripening of Cheddar cheese. J. Dairy Sci. 87:854–862.

Heymann, H., B. Machada, L. Torri, and A. L. Robinson. 2012. How many judges should one use for sensory descriptive analysis? J. Sens. Stud. 27:111–122.

Hickey, C. D., M. A. E. Auty, M. G. Wilkinson, and J. J. Sheehan. 2015. The influence of cheese manufacture parameters on cheese microstructure, microbial localization and their interactions during ripening: A review. Trends Food Sci. Technol. 41:135–148.

Hicsasmaz, Z., L. Shippelt, and S. S. H. Rizvi. 2004. Evaluation of Mozzarella cheese stretchability by the ring-and-ball method. J. Dairy Sci. 87:1993–1998.

Hinrichs, J. 2001. Incorporation of whey proteins in cheese. Int. Dairy J. 11:495–503.

Horne, D. S. 1998. Casein interaction: casting light on the black boxes, the structure in dairy products. Int. Dairy J. 8:171–177.

Horne, D. S. and J. M. Banks. 2004. Rennet-induced coagulation of milk. Pages 47–70 in Cheese: Chemistry, Physics and Microbiology. Vol 1. P.F. Fox, P. L. H. McSweeney, T. M. Cogan, T. P. Guinee, 3rd ed., Chapman and Hall, London, UK.

Horne, D. S., and J. A. Lucey. 2014. Revisiting the temperature dependence of the coagulation of renneted bovine casein micelles. Food Hydrocolloid. 42:75–80.

Huppertz, T. 2013. Chemistry of the caseins. Pages 135–160 in Advanced Dairy Chemistry: A Proteins: Basic Aspects. Vol. 1. P. L. H., McSweeney, P. F. Fox. 4th ed., Springer Science+Business Media, New York, NY.

IDF (International Dairy Federation). 1991. Rheological and fracture properties of cheese. Bull. IDF No. 268. IDF, Brussels, Belgium.

Inayat, S., M. Ayaz, T. N. Pasha, M. Abdullah, and A. Waheed. 2014. Evaluation of commercially available Mozzarella cheese for its melt and stretching behaviour through Farinograph-E. J. Anim. Plant Sci. 24:770–773.

Ismail, M., E.-T. Ammar, and R. El-Metwally. 2011. Improvement of low fat Mozzarella cheese properties using denatured whey protein. Int. J. Dairy Technol. 64:207–217.

Johnson, M. E., and J. A. Lucey. 2006. Calcium: a key factor in controlling cheese functionality. Aust. J. Dairy Technol. 61:147–153.

Jolliffe, I. T. 2002. Principal Component Analysis. 2nd ed. Springer-Verlag, New York, NY.

Joshi, N. S., K. Muthukumarappan, and R. I. Dave. 2002. Role of soluble and colloidal calcium contents on functionality of salted and unsalted part-skim Mozzarella cheese. Aust. J. Dairy Technol. 57:203–210.

Joshi, N. S., K. Muthukumarappan, and R. I. Dave. 2003. Understanding the role of calcium in functionality of part skim Mozzarella cheese. J. Dairy Sci. 86:1918–1926.

Joshi, N. S., K. Muthukumarappan, and R. I. Dave. 2004a. Modeling rheological characteristics and calcium content of Mozzarella cheese. J. Food Sci. 69:97–101.

Joshi, N. S., K. Muthukumarappan, and R. I. Dave. 2004b. Effect of calcium on microstructure and meltability of part skim Mozzarella cheese. J. Dairy Sci. 87:1975–1985.

Joshi, N. S., K. Muthukumarappan, and R. I. Dave. 2004c. Viscoelastic properties of part skim Mozzarella cheese: Effect of calcium, storage, and test temperature. Int. J. Food Prop. 7:239–252.

Kamyab, I., S. Chakrabarti, and J. G. Williams. 1998. Cutting cheese with wire. J. Mater. Sci. 33:2763–2770.

Keller, B., N. F. Olson, and T. Richardson. 1974. Mineral retention and rheological properties of Mozzarella cheese made by direct acidification. J. Dairy Sci. 57:174.

Kim, S.-Y., S. Lim, and S. Gunasekaran. 2011. Protein interactions in reduced-fat and full-fat Cheddar cheeses during melting. LWT Food Sci. Technol. 44:582–587.

Kindstedt, P. S. 1990. Rapid quantitative test for free oil (oiling off) in melted Mozzarella cheese. J. Dairy Sci. 73:867–873.

Kindstedt, P. S. 1993. Effect of manufacturing factors, composition, and proteolysis on the functional characteristics of Mozzarella cheese. Crit. Rev. Food Sci. Nutr. 33:167–187.

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

Kindstedt, P. S. 2004. Mozzarella cheese: 40 years of scientific advancement. Int. J. Dairy Technol. 57:85–90.

Kindstedt, P. S., and P. F. Fox. 1993. Effect of manufacturing factors, composition, and proteolysis on the functional characteristics of Mozzarella cheese. Crit. Rev. Food Sci. 33:167–187.

Kindstedt, P. S., and M. R. Guo. 1997. Recent developments in the science and technology of pizza cheese. Aust. J. Dairy Technol. 52:41–43.

Kindstedt, P. S., and M. R. Guo. 1998. A physic-chemical approach to the structure and function of Mozzarella cheese. Aust. J. Dairy Technol. 53:70–73.

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

Kindstedt, P. S., J. J. Yun, D. M. Barbano, and K. L. Larose. 1995. Mozzarella cheese: impact of coagulant concentration on chemical composition, proteolysis, and functional properties. J. Dairy Sci. 78:2591–2597.

Kindstedt, P. S., A. J. Hillier, and J. J. Mayes. 2010. Technology, biochemistry and functionality of pasta filata/pizza cheese. Pages 330–359 in Technology of Cheesemaking. 2nd ed. B. A. Law and A. Y. Tamine, ed. Wiley-Blackwell, Oxford, UK.

Ko, S., and S. Gunasekaran. 2007. Error correction of confocal microscopy images for in situ food microstructure evaluation. J. Food Eng.79:935–944.

Ko, S., and S. Gunasekaran. 2008. Analysis of cheese melt profile using inverse-Hill function. J. Food Eng. 87:266–273.

Ko, S., and S. Gunasekaran. 2014. Evaluation of cheese meltability using convection and conduction melt profilers. Int. J. Dairy Technol. 67:194–201.

Koç, H., C. J. Vinyard, G. K. Essick, and E. A. Foegeding. 2013. Food oral processing: conversion of food structure to textural perception. Annu. Rev. Food Sci. Technol. 4:237–366.

Kuo, M. -I., and S. Gunasekaran. 2009. Effect of freezing and frozen storage on microstructure of Mozzarella and pizza cheeses. LWT Food Sci. Technol. 42:9–16.

Kuo, M. -I., Y.-C. Wang, and S. Gunasekaran. 2000. A viscoelasticity index for cheese meltability evaluation. J. Dairy Sci. 83:412–417.

Kuo, M.-I., Y.-C. Wang, S. Gunasekaran, and N. F. Olson. 2001. Effect of heat treatments on the meltability of cheeses. J. Dairy Sci. 84:1937–1943.

Kuo, M. -I., S. Gunasekaran, M. Johnson, and C. Chen. 2001. Nuclear magnetic resonance study of water mobility in pasta filata and non-pasta filata Mozzarella. J. Dairy Sci. 84:1950–1958.

Läuger, J., and H. Stettin. 2010. Differences between stress and strain control in the non-linear behavior of complex fluids. Rheol. Acta 49:909–930.

Lawless, H. T., and H. H. Heymann. 1998. Sensory evaluation of food: principle and practices, Chapman and Hall, New York, NY.

Lee, M.-R., M. E. Johnson, and J. A. Lucey. 2005.Impact of modifications in acid development on the insoluble calcium content and rheological properties of Cheddar cheese. J. Dairy Sci. 88:3798–3809.

Lee, S. K., M. Huss, H. Klostermeyer, and S. G. Anema. 2013. The effect of pre-denatured whey proteins on the textural and microstructural properties of model processes cheese spreads. Int. Dairy J. 32:79–88.

Lefevere, I., K. Dewettinck, and A. Huyghebaert. 2000. Cheese fat as driving force in cheese flow upon melting. Milchwissenschaft 55:563–566.

Lelièvre. J. 1995.Whey protein in cheese—An overview. Pages 359–365 in Chemistry of Structure-Function Relationships in Cheese. Advances in Experimental Medicine and Biology. Vol. 367. E. L. Malin and M. H. Tunick, ed. Plenum Press, New York, NY.

Lillford, P. J. 2011. The importance of food microstructure in fracture physics and texture perception. J. Texture Stud. 42:130–136.

Lopez, C., M.-B. Maillard, V. Briard-Bion, B. Camier, and J. A. Hannon. 2006. Lipolysis during ripening of Emmental cheese considering organization of fat and preferential localization of bacteria. J. Agric. Food Chem. 54:5855–5867.

Lopez, C., B. Camier, and J.-Y. Gassi. 2007. Development of the milk fat microstructure during the manufacture and ripening of Emmental cheese observed by confocal laser scanning microscopy. Int. Dairy J. 17:235–247.

Lopez, C., V. Briard-Bion, E. Beaucher, and M. Ollivon. 2008. Multiscale characterization of the organization of triglycerides and phospholipids in Emmental cheese: from the microscopic to the molecular level. J. Agr. Food Chem. 56:2406–2414.

Lucey, J. A. 2002. Formation and physical properties of milk protein gels. J. Dairy Sci. 85:281–294.

Lucey, J. A. 2008. Some perspectives on the use of cheese as a food ingredient. Dairy Sci. Technol. 88:573–594.

Lucey, J. A., and P. F., Fox. 1993. Importance of calcium and phosphate in cheese manufacture: a review. J. Dairy Sci. 76:1714–1724.

Lucey, J. A., B. Hauth, C. Gorry, and P. F. Fox. 1993. Acid–base buffering of milk. Milchwissenschaft 48:268–272.

Lucey, J. A., M. E. Johnson, and D. S. Horne. 2003. Invited review: Perspectives on the basis of the rheology and texture properties of cheese. J. Dairy Sci. 86:2725–2743.

Lucey, J. A., R. Mishra, A. Hassan, and M. E. Johnson. 2005. Rheological and calcium equilibrium changes during ripening of Cheddar cheese. Int. Dairy J. 15:645–653.

Luo, J., T. Pan, H. Y. Guo, and F. Z. Ren. 2013. Effect of calcium in brine on salt diffusion and water distribution of Mozzarella cheese during brining. J. Dairy Sci. 96:824–831.

Luyten, H., T. Van Vlie, and P. Walstra. 1992. Comparison of various methods to evaluate fracture phenomena in food materials. J. Texture Stud. 23:245–266.

Ma, X., B. James, L. Zhang, and E. A. C. Emanuelsson-Patterson. 2012. The stretchability of Mozzarella cheese evaluated by a temperature-controlled 3-prong hook test. J. Dairy Sci. 95:5561–5568.

Ma, X., B. James, L. Zhang, and E. A. C. Emanuelsson-Patterson. 2013a. Correlating Mozzarella cheese properties to its production processes and microstructure quantification. J. Food Eng. 115:154–163.

Ma, X., B. James, M. O. Balaban, L. Zhang, and E. A. C. Emanuelsson-Patterson. 2013b. Quantifying blistering and browning properties of Mozzarella cheese- Part II: Cheese with different salt and moisture contents. Food Res. Int. 54:917–921.

Marchesseau, S., E. Gastaldi, A. Lagaude, and J.-L. Cuq. 1997. Influence of pH on protein interactions and microstructure of process cheese. J. Dairy Sci. 80:1483–1489.

Marshall, R. T. 1992. Standard Methods for the Examination of Dairy Products. 16th ed. American Public Health Association, Washington, DC.

Masi, P., and F. Addeo. 1986. An examination of some mechanical properties of a group of Italian cheeses and their relation to structure and conditions of manufacture. J. Food Eng. 5:217–229.

McMahon, D. J., R. L. Fife, and C. J. Oberg. 1999. Water partitioning in Mozzarella cheese and its relationship to cheese meltability. J. Dairy Sci. 82:1361–1369.

McMahon, D. J., B. Paulson, and C. J. Oberg. 2005. Influence of calcium, pH, and moisture on protein matrix structure and functionality in direct-acidified nonfat Mozzarella cheese. J. Dairy Sci. 88:3754–3763.

McMahon, D. J., and B. S. Oommen. 2013. Casein micelle structure, functions, and interactions. Pages 185–209 in Advanced Dairy Chemistry: A Proteins: Basic Aspects. Vol. 1. P. L. H. McSweeney, P. F. Fox, 4th ed. Springer Science+Business Media, New York, NY.

Mead, D., and P. Roupas. 2001. Effect of incorporation of denatured whey proteins on chemical composition and functionality of pizza cheese. Aust. J. Dairy Technol. 56:19–23.

Melito, H. S., and C. R. Daubert. 2011. Rheological innovations for characterizing food material properties. Annu. Rev. Food Sci. Technol. 2:153–179.

Melito, H. S. 2012. Large amplitude oscillatory shear behavior of food systems. Thesis, North Carolina State University, Raleigh, NC.

Melito, H. S., C. R. Daubert, and E. A. Foegeding. 2012. Validation of a large amplitude oscillatory shear protocol. J. Food Eng. 113:124–135.

Melito, H. S., C. R. Daubert, and E. A. Foegeding. 2013. Relationships between nonlinear viscoelastic behavior and rheological, sensory and oral processing behavior of commercial cheese. J. Texture Stud. 44:253–288.

Mellema, M., J. W. M. Heesakkers, J. H. J. Van Opheusden, and T. Van Vliet. 2000. Structure and scaling behavior of aging rennet-induced casein gels examined by confocal microscopy and permeametry. Langmuir 16:6847–6854.

Metzger, L. E., and D. M. Barbano. 1999. Measurement of postmelt chewiness of Mozzarella cheese. J. Dairy Sci. 82:2274–2279.

Metzger, L. E., D. M. Barbano, M. A. Rudan, P. S. Kindstedt, and M. R. Guo. 2000. Whiteness change duing heating and cooling of Mozzarella cheese. J. Dairy Sci. 83:1–10.

Michalski, M.-C., V. Briard, and F. Michel. 2001. Optical parameters of milk fat globules for laser light scattering measurements. Lait 81:787–796.

Michalski, M.-C., J.-Y Gassi, M.-H. Famelar, N. Leconte, B. Camier, F. Michel, and V. Briard. 2003. The size of native milk fat globules affects physico-chemical and sensory properties of Camembert cheese. Lait 83:131–143.

Michalski, M.-C., B. Camier, J.-Y. Gassi, V. Briard-Bion, N. Leconte, M.-H. Famelar, and C. Lopez. 2007. Functionality of smaller vs control native milk fat globules in Emmental cheese manufactured with adapted technologies. Food Res. Int. 40:191–202.

Mietton, B., F. Gaucheron, and F. Salaün-Michel. 2004. Minéraux et transformations fromagères. Pages 471–563 in Minéraux et produits laitiers. F. Gaucheron. Ed., Lavoisier, Paris, France.

Mizino, R., T. Matsud, J. A. Lucey, and N. Ichihashi. 2009. Effects of changes in the distribution of soluble and insoluble calcium on Mozzarella cheese. Milchwissenschaft 64:169–172.

Monteiro, V. S., M. D. R. Costa and M. L. Gigante. 2011. Equilibrium between the protein matrix and the serum phase of Prato cheese. J. Food Process. Pres. 35:496–500.

Morell, P., I. Hernando, and S. M. Fiszman. 2014. Understanding the relevance of in-mouth food processing. A review of *in vitro* techniques. Trens Food Sci. Technol. 35:18–31.

Morris, H. A., A. C. Holt, B. E. Brooker, J. M. Banks, and W. Manson. 1988. Inorganic constituents of cheese: Analysis of juice from a one-month-old Cheddar cheese and the use of light and electron microscopy to characterize the crystalline phases. J. Dairy Res. 55:255–268.

Mosca, A. C., J. A. Rocha, G. Sala, F. Van de Velde, and M. Stieger. 2012. Inhomogeneous distribution of fat enhances the perception of fat-related sensory attributes in gelled foods. Food Hydrocolloid 27:448–455.

Moskowitz, H. R. 2001. Sensory directional for pizza: A deeper analysis. J. Sensory Studies 16:583–600.

Moynihan, A. C., S. Govindasamy-Lucey, J. J. Jaeggi, M. E. Johnson, J. A. Lucey, and P. L. H. McSweeney. 2014. Effect of camel chymosin on the texture, functionality, and sensory properties of low-moisture, part-skim Mozzarella cheese. J. Dairy Sci. 97:85–96.

Muliawan, E. B., and S. G. Hatzikiriakos. 2007. Rheology of Mozzarella cheese. Int. Dairy J. 17:1063–1072.

Muliawan, E. B., and S. G. Hatzikiriakos. 2008. Rheology of Mozzarella cheese: extrusion and rolling. Int. Dairy J. 18:615–623.

Mulvaney, S., S. Rong, D. M. Barbano, and J. J. Yun. 1997. Systems analysis of the plasticization and extrusion processing of Mozzarella cheese. J. Dairy. Sci. 80:3030–3039.

Muthukumarappan, K., Y.-C. Wang, and S. Gunasekaran. 1999. Short communication: modified Schreiber Test for evaluation of Mozzarella Cheese meltability. J. Dairy Sci. 82:1068–1071.

My Research Dashboard. Author' personal account. Elsevier ed. Online. www.myresearchdashboard.com, consulted on October 11th, 2015.

Ni, H., and S. Gunasekaran. 2004. Image processing algorithm for cheese shred evaluation. J. Food Eng. 61:37–45.

Noronha, N., E. Duggan, G. R. Ziegler, E. D. O'Riordan, and M. O'Sullivan. 2008. Investigation of imitation cheese matrix development using light microscopy and NMR relaxometry. Int. Dairy J. 18:641–648.

Oberg, C. J., W. R. McManus, and D. J. McMahon. 1993. Microstructure of Mozzarella cheese during manufacture. J. Food Struct. 12:251–258.

Olivares, M. L., S. E. Zorrilla, and A. C. Rubiolo. 2009. Rheological properties of Mozzarella cheese determined by creep/recovery tests: effect of sampling direction, test temperature and ripening time. J. Texture Stud. 40: 300–318.

O'Mahony, J. A., J. A. Lucey, and P. L. H. McSweeney. 2005. Chymosin-mediated proteolysis, calcium solubilization, and texture development during the ripening of Cheddar cheese. J. Dairy Sci. 88:3101–3114.

O'Mahony, J. A., P. L. H. McSweeney, and J. A. Lucey. 2006. A model system for studying the effects of colloidal calcium phosphate concentration on the rheological properties of Cheddar cheese. J. Dairy Sci. 89:892–904.

Ong, L., K. Soodam, S. E. Kentish, I. B. Powell, and S. L. Gras. 2015. The addition of calcium chloride in combination with a lower draining pH to change the microstructure and improve fat retention in Cheddar cheese. Int. Dairy J. 46:53–62.

Paquet, A., and M. Kaláb. 1988. Amino acid composition and structure of cheese baked as pizza ingredient in conventional and microwave ovens. Food Microstruct. 7:93–103.

Pascua, Y., H. Koç, and E. A. Foegeding. 2013. Food structure: roles of mechanical properties and oral processing in determining sensory texture of soft materials. Curr. Opin. Colloid In. 18:324–333.

Pastorino, A. J., R. I. Dave, C. J. Oberg, and D. J. McMahon. 2002. Temperature effect on structure-opacity relationships of nonfat Mozzarella cheese. J. Dairy Sci. 85:2106–2113.

Pastorino, A. J., N. P. Ricks, C. L. Hansen, and D. J. McMahon. 2003a. Effect of calcium and water injection on structure-function relationships of cheese. J. Dairy Sci. 86:105–113.

Pastorino, A. J., C. L. Hansen, and D. J. McMahon. 2003b. Effect of salt on structure-function relationships of cheese. J. Dairy Sci. 86:60–69.

Patarin, J., H. Galliard, A. Magning, and B. Goldschmidt. 2014. Vane and plate-plate rheometry of cheeses under oscillations and large strains: a comparative study and experimental conditions analysis. Int. Dairy J. 38:24–30.

Paulson, B. M., D. J. McMahon, and C. J. Oberg. 1998. Influence of sodium chloride on appearance, functionality, and protein arrangements in nonfat Mozzarella cheese. J. Dairy Sci. 81:2053–2064.

Pechak, D. G., and A. K. Smith, 2007. Instrumental techniques for sample preparation. Pages 17–58 in Structure of dairy products. A. Y. Tamine . Ed. Blackwell publishing Ltd, Oxford, UK.

Perrie, J. 2012. Development of methods for assessing the effect of moisture and aging on sliceability of cheese. Thesis. Utah State University, Logan, UT.

Punidadas, P., J. Feirtag, and M. A. Tung. 1999. Incorporating whey protein into Mozzarella cheese. Int. J. Dairy Technol. 52:51–55.

Rankin, S. A., C. M. Chen, D. Sommer, and A. Esposito. 2006. Mozzarella and Scamorza cheese. Pages 1-13 in Chap.150 of Handbook of Food Science, Technology and Engineering. Vol. 4. Y. H. Hui. ed. CRC Press, Boca Raton, FL.

Rehman, S-U., N. Y. Farkye, and B. Yim. 2003. Use of dry milk protein concentrate in pizza cheese manufactured by culture or direct acidification. J. Dairy Sci. 86:3841–3848.

Reid, D. S., and H. Yan, 2004. Rheological, melting and microstructural properties of Cheddar and Mozzarella cheeses affected by different freezing methods. J. Food Qual. 27:436–458.

Rémillard, N., and M. Britten. 2011. Quantitative determination of micellar calcium in milk and cheese using acid-base titration. Milchwissenschaft 66:137–140.

Renda, A., D. M. Barbano, J. J. Yun, P. S. Kindstedt, and S. J. Mulvaney. 1997. Influence of screw speed of the mixer at low temperature on characteristics of Mozzarella cheese. J. Dairy Sci., 80:1901–1907.

Reparet, J.-M., and Y. Noël. 2003. Relation between a temperature-sweep dynamic shear test and functional properties of cheeses. Lait 83:321–333.

Rogers, N. R., D. J. McMahon, C. R. Daubert, T. K. Berry, and E. A. Foegeding. 2010. Rheological properties and microstructure of Cheddar cheese made with different fat contents. J. Dairy Sci. 93:4565–4576.

Rowney, M. K., P. Roupas, M. W. Hickey, and D. W. Everett. 2003. The effect of compression, stretching, and cooking temperature on free oil formation in Mozzarella curd. J. Dairy Sci. 86:449–456.

Rowney, M. K., P. Roupas, P., M. W. Hichey, and D. W. Everett. 2004. Salt-induced structural changes in 1-day old Mozzarella cheese and the impact upon free oil formation. Int. Dairy J. 14:809–816.

Rudan, M. A., and D. M. Barbano. 1998. A model of Mozzarella cheese melting and browning during pizza baking. J. Dairy Sci. 81:2312–2319.

Rudan, M. A., D. M. Barbano, J. J. Yun, and P. S. Kindstedt. 1999. Effect of fat reduction on chemical composition, proteolysis, functionality, and yield of Mozzarella cheese. J. Dairy Sci. 82:661–672.

Sakia, R. M. 1992. The Box–Cox transformation technique: A review. Statistician 41:169–178.

Salaün, F., B. Mietton, and F. Gaucheron. 2005. Buffering capacity of dairy products. Int. Dairy J. 15:95–109.

Savage, A. A., and W. M. Mullan. 2000. Evaluation of helical viscometry for assessing the functional properties of Mozzarella cheese. Int. J. Dairy Technol. 53:57–62.

Schenkel, P., R. Samudrala, and J. Hinrichs. 2011. Fat-reduced semi-hard cheese enriched with a microparticulated whey protein concentrate: impact on cheese-making properties and rheological characteristics. Milchwissenschaft 66:43–47.

Schenkel, P., R. Samudrala, and J. Hinrichs. 2013a. Thermo-physical properties of semi-hard cheese made with different fat fraction: influence of melting point and fat globule size. Int. Dairy J. 30:79–87.

Schenkel, P., R. Samudrala, and J. Hinrichs. 2013b. Effect of adding whey protein particles as inert filler on thermophysical properties of fat-reduced semihard cheese type Gouda. Int. J. Dairy Technol. 66:220–230.

Schenkel, P., K. I. Hartmann, R. Samudrala, and J. Hinrichs. 2014. Characterization of commercial cheese regarding thermophysical properties and application of multivariate statistical analysis to elaborate product mapping. J. Texture Stud. 45:440–451.

Serrano, J., G. Velazquez, D. Lopetcharat, J. A. Ramirez, and J. A. Torres. 2005. Moderately high hydrostatic pressure processing to reduce production cost of shredded cheese: microstructure, texture, and sensory properties of shredded milled curd cheddar. J. Food Sci. 70:286–293.

Shakeel-Ur-Rehman, and N. Y. Farkye. 2006. Effect of setting pH on the properties of Mozzarella cheese made by direct acidification of whole milk standardized with dry milk protein concentrate. Aust. J. Dairy Technol.61:8–12.

Sheehan, J. J., and T. P. Guinee. 2004. Effect of pH and calcium level on the biochemical, textural and functional properties of reduced-fat Mozzarella cheese. Int. Dairy J. 14:161–172.

Sheehan, J. J., K. O'Sullivan, and T. P. Guinee. 2004. Effect of coagulant type and storage temperature on the functionality of reduced-fat Mozzarella cheese. Lait 84:551–566.

Shirodkar, P., Y. Mehta, A. Nolan, K. Dahm, R. Dusseau, and L. McCarthy. Characterization of creep and recovery curve of polymer modified binder. Constr. Build. Mater. 34:504–511.

Singh, M. P., S. K. Kanawjia, A. Giri, and Y. Khetra. 2015. Effect of temperature and microcrystalline cellulose on moisture sorption characteristics of shredded mozzarella cheese. J. Food Process. Pres. 39:521–529.

Skeie, S., G. M. Alseth, H. Østlie, R. K. Abrahamsen, A. G. Johansen, and J. Øyaas. 2013. Improvement of the quality of low-fat cheese using a two-step strategy. Int. Dairy J. 33:153–162.

Solar, O., R. K. Connelly, and S. Gunasekaran. 2009. Interactions between rennet casein and whey protein isolate during cooking in a torque rheometer. J. Food Eng. 95:119–125.

Steffl, A., M. Hafenmaier, A. Hechler, and J. Hinrichs. 1999. Influence of whey protein particle on the renneting properties of milk. Milchwissenschaft 54:510–513.

Stenz, B., A. Hoeglmeier, U. Fischer, S. Roeck, and U. Kulozik. 2006. Comparison of the melting properties of cheese by means of meltability tests and rheological measurements. Milchwissenschaft 61:304–307.

Subramanian, R. and S. Gunasekaran. 1997. Small amplitude oscillatory shear studies on Mozzarella cheese Part I: Region of linear viscoelasticity. J. Texture Stud. 28:633–642.

Subramanian, R., K. Muthukumarappan, and S. Gunasekaran. 2003. Effect of methocel as water binder on the linear viscoelastic properties of Mozzarella cheese during early stages of maturation. J. Texture Stud. 34:361–380.

Subramanian, R., K. Muthukumarappan, and S. Gunasekaran. 2006. Linear viscoelastic properties of regular- and reduced-fat pasteurized process cheese during heating and cooling. Int. J. Food Prop. 9:377–393.

Taneya, S., T. Izutsu, T. Kimura, and T. Shioya. 1992. Structure and rheology of string cheese. Food Structure 11:61–71.

Tariq, S., A. J. Giacomin, and S. Gunasekaran. 1998. Nonlinear viscoelasticity of cheese. Biorheology 35:171–191.

Thibaudeau, E., D. Roy, and D. St-Gelais. 2015. Production of brine-salted Mozzarella cheese with different ratios of NaCl/KCl. Int. Dairy J. 40:54–61.

Truong, V. D., and C. R. Daubert. 2000. Comparative study of large strain methods for assessing failure characteristics of selected gels. J. Texture Stud. 31:335–353.

Truong, V. D., and C. R. Daubert. 2001. Textural characterization of cheeses using vane rheometry and torsion analysis. J. Food Sci. 66:716–721.

Truong, V. D., C. R. Daubert, M. A. Drake, and S. R. Baxter. 2002. Vane rheometry for textural characterization of Cheddar cheeses: correlation with other instrumental and sensory measurements. LWT-Food Sci. Technol. 35:305–314.

Tunick, M. H. 2010. Activation energy measurements in rheological analysis of cheese. Int. Dairy J. 20:680–685.

Tunick, M. H. 2011. Small-strain dynamic rheology of food protein networks. J. Agric. Food Chem. 59:1481–1486.

Udayarajan, C. T. 2007. Relating physicochemical characteristics of cheese to its functional performance. Thesis. University of Wisconsin-Madison, Madison, WI.

Udyarajan, C. T., D. S. Horne, and J. A. Lucey. 2007. Use of time-temperature superposition to study the rheological properties of cheese during heating and cooling. Int. J. Food Sci. Tech. 42:686–698.

Upreti, P., and L. E. Metzger. 2007. Influence of calcium and phosphorus, lactose, and salt-to-moisture ratio on cheddar cheese quality: pH changes during ripening. J. Dairy Sci. 90:1–12.

Ustunol, Z., K. Kawachi, and J. Steffe. 1995. Rheological properties of Cheddar cheese as influenced by the fat reduction and ripening time. J. Food Sci. 60:1208–1210.

Vithanage, C. R., M. J. Grimson, and B. G. Smith. 2009. The effect of temperature on the rheology of butter, a spreadable blend and spreads. J. Texture Stud. 40:346–369.

Vogt, S. J., J. R. Smith, J. D. Seymour, A. J. Carr, M. D. Golding, and S. L. Codd. 2015. Assessment of the changes in the structure and component mobility of Mozzarella and Cheddar cheese during heating. J. Food Eng. 150:35–43.

Wadhwani, R., W. R. McManus, and D. J. McMahon. 2011. Improvement in melting and baking properties of low-fat Mozzarella cheese. J. Dairy Sci. 94:1713–1723.

Wang, Y-C., K. Muthukumarappan, and S. Gunasekaran. 1998. A device for evaluating melt/flow characteristics of cheeses. J. Texture Stud. 29:43–55.

Wang, H.-H., and D.-W. Sun. 2002a. Melting characteristics of cheese: analysis of effects of cooking conditions using computer vision technology. J. Food Eng. 51:305–310.

Wang, H.-H., and D.-W. Sun. 2002b. Correlation between cheese meltability determined with a computer vision method and with Arnott and Schreiber tests. J. Food Sci. 67:745–749.

Wang, H.-H., and D.-W. Sun. 2003. Assessment of cheese browning affected by baking conditions using computer vision. J. Food Eng. 56:339–345.

Wang H.-H., and D. W.-Sun. 2004. Evaluation of the oiling off property of cheese with computer vision: correlation with fat ring test. J. Food Eng. 61:47–55.

Wang, W., P. S. Kindstedt, J. A. Gilmore, and M. R. Guo. 1998. Changes in the composition and meltability of Mozzarella cheese during contact with pizza sauce. J. Dairy Sci. 81:609–614.

Watkinson, P., C. Coker, R. Crawford, C. Dodds, K. Johnston, A. McKenna, and N. White. 2001. Effect of cheese pH and ripening time on model cheese textural properties and proteolysis. Int. Dairy J. 11:455–464.

Wehr, H. M., and J. F. Frank. 2004. Standard Methods for Examination of Dairy Products, 17th Ed., American Public Health Association, Washington, DC.

Yang, X., N. R. Rogers, T. K. Berry, and E. A. Foegeding. 2011. Modeling the rheological properties of Cheddar cheese with different fat contents at various temperatures. J. Texture stud. 42:331–348.

Ye, A., and S. Hewitt. 2009. Phase structures impact the rheological properties of rennet-casein based imitation cheese containing starch. Food Hydrocolloid 23:867-873.

Yu, C., and S. Gunasekaran. 2004. Modeling of melt conveying in a deep-channel single-screw cheese stretche. J. Food Eng. 61:241–251.

Yu, C., and S. Gunasekaran. 2005a. A system analysis of pasta filata process during Mozzarella cheese making. J. Food Eng. 69:399–408.

Yu, C., and S. Gunasekaran. 2005b. Modeling of melt conveying and heat transfer in a twin-screw cheese stretcher. J. Food Eng. 70:245–252.

Yun, J. J., D. M. Barbano, P. S. Kindstedt, and K. L. Larose. 1995. Mozzarella cheese: impact of whey pH draining on chemical composition, proteolysis, and functional properties. J. Dairy Sci. 78:1–7.

Yven, C., J. Patarin, A. Magnin, H. Labouré, M. Repoux, E. Guichard, and G. Feron. 2012. Consequences of individual chewing strategies on bolus rheological properties at the swallowing threshold. J. Texture Stud. 43:309–318.

Zisu, B., and N. P. Shah. 2005. Textural and functional changes in low-fat Mozzarella cheeses in relation to proteolysis and microstructure as influenced by the use of fat replacer, pre-acidification and EPS starter. Int. Dairy J. 15:957–972.

Zisu, B., and N. P. Shah. 2007. Texture characteristics and pizza bake properties of low-fat Mozzarella cheese as influenced by pre-acidification with citric acid and use of encapsulated and ropy exopolysaccharide producing cultures. Int. Dairy J. 17:985–997.