University of Alberta

Development of a yogurt powder formulation that can produce a recombined product with physicochemical and rheological properties similar to those found in commercial Greek-style yogurts

by

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A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Master of Science
in
Food Science and Technology

Department of Agricultural, Food and Nutritional Science

Spring 2013
Edmonton, Alberta

Dedication

I especially want to dedicate this work to my grandparents, Osvaldo and Nélida, who are the entire inspiration of my life. Without them I would never be at this stage.

Abstract

Greek yogurt (GY) is known as strained or concentrated form with total solids (TS) of at least 22.0% and 14.3% for full and fat free, respectively. TS are increased, in traditional GY, by "draining off" whey after milk fermentation by typical mixture of yogurt bacteria. Current studies were designed to eliminate "draining off" in GY by new formulation using different combination of milk protein concentrate (MPC-85% protein), whey protein isolate (WPI-90% protein), sodium caseinate (SC) and milk permeate powder (MPP- 3.3% protein and 85.5 % lactose).

A one-block full factorial design 3*2 and response surface methodology were used to define the effect of milk ingredients and technological parameters used in new formulation on the rheological and physicochemical properties (RPP) of the experimental GY. Experimental analyses showed that GY can be formulated and manufactured from selective mixture of dry dairy ingredients with RPP similar to commercial products used as a reference in these studies.

Keywords: Yogurt, milk protein powders, rheology, microstructure, syneresis, whey separation, graininess, response surface methodology.

Acknowledgements

I would like to offer a sincere thank-you to my academic supervisor, Dr. Lech Ozimek, for providing me with the opportunity to grow as a professional and for his valuable guidance, encouragement and support throughout these two years. I will be forever grateful to him for this prodigious opportunity. Thank you to Dr. Marleny Aranda Saldana for sharing her expertise and providing insightful comments on this thesis. Thank you to Dr. Takuo Nakano, Vanessa Nieto, Jun Gao, and Susan Gibson for their support and assistance during my experimental work. We would also like to acknowledge Alistair W Johnston and Pamela Ambriz from Vitalus Nutrition Inc., Abbotsford, BC, for providing dairy ingredients used in this research.

I am especially grateful to my beloved partner Lucila, who accompanied me along and supported me in this wonderful project. Thank you to my family. Words cannot express how grateful I am to them for their love, inspiration, and belief in me. Special thanks to my mother, Patricia, for her unconditional support necessary to achieve all my goals.

Table of Contents

1. Introduction 1

1.1 References 3

2. Literature review 6

2.1 General characteristics of Greek-style yogurt 6

2.2 Compositional standards for Greek-style yogurt 8

2.3 Current importance of yogurt and Greek-style yogurt in the Canadian and U.S. markets 8

2.4 General technology of yogurt manufacturing 10

2.4.1 Milk standardization 11

2.4.2 Homogenization 12

2.4.3 Heat treatment 13

2.4.4 Incubation/fermentation 13

2.4.5 Cooling and storage 14

2.5 Greek-style yogurt manufacturing methods 15

2.5.1 Traditional method 16

2.5.2 Methods based on mechanical separators 16

2.5.3 Methods based on membrane processes 17

2.5.4 Methods based on direct recombination 18

2.6 Formation and physicochemical characteristics of acid milk gels 21

2.6.1 Casein micelle structure 22

2.6.2 Formation of acid milk gels 25

2.6.3 Effects of heat treatment on the formation of acid milk gels 27

2.7 Important factors that define yogurt quality 32

2.7.1 Rheology 32

2.7.2 Whey separation 35

2.7.3 Clusters formation 36
2.8 References 37
3. Development of the novel Greek-style yogurt powder formulation free of additives and fat 54
3.1 Introduction 54
3.2 Materials & methods 56
3.2.1 Milk Powders 56
3.2.2 Microbial Cultures 56
3.2.3 Market reference samples 57
3.2.4 Yogurt manufacturing 58
3.2.5 Dynamic rheological measurements 60
3.2.6 Whey separation measurements 60
3.2.7 Presence and size of visible clusters 61
3.2.8 Incubation time measurements 62
3.2.9 Reconstituted milk density measurements 62
3.2.10 Experimental design and statistical analysis 63
3.3 Results & discussion 65
3.3.1 Market reference samples 65
3.3.2 Experimental samples 72
3.3.3 Proposed formulation 108
3.4 Conclusion 115
3.5 References 117
4. Physicochemical and rheological stability of the recombined yogurt obtained from the dry formulation proposed, and the application of a simplified method for the hydration of the yogurt powder 127
4.1 Introduction 127
4.2 Materials & methods 128
4.2.1 Yogurt manufacture 128

4.2.2 Total solids determination 130
4.2.3 Experimental design and statistical analysis 131
4.3 Results & discussion 132
4.3.1 Rheological and physicochemical stability of the recombined yogurt throughout storage 132
4.3.2 Effects of a new simplified reconstitution method on the rheological and physicochemical aspects of the final product 140
4.4 Conclusion 145
4.5 References 147
5. Effects of storing the dry formulation on the rheological and physicochemical characteristics of the recombined yogurt 153
5.1 Introduction 153
5.2 Materials & methods 155
5.2.1 Experimental design and statistical analysis 155
5.3 Results & discussion 156
5.4 Conclusion 167
5.5 References 169
6. Summary of findings, conclusions and recommendations for future research 176
6.1 Summary of findings 176
6.1.1 Development of the novel Greek-style yogurt powder formulation free of additives and fat 176
6.1.2 Physicochemical and rheological stability of the recombined yogurt obtained from the dry formulation proposed, and the application of a simplified method for the hydration of the yogurt powder 177
6.1.3 Effects of storing the dry formulation on the rheological and physicochemical characteristics of the recombined yogurt 178
6.2 Conclusions and recommendations for future research 179
6.3 References 181

7. Appendices 185

Appendix A – Market reference samples 185

Appendix B – Development of the novel Greek-style yogurt powder formulation free of additives and fat 192

Appendix C – Physicochemical and rheological stability of the recombined yogurt obtained from the dry formulation proposed, and the application of a simplified method for the hydration of the yogurt powder 213

Appendix D – Effects of storing the dry formulation on the rheological and physicochemical characteristics of the recombined yogurt 222

List of Tables

Table 2-1: Typical chemical compositions (g 100g $^{-1}$) of industrial full and low-fat strained yogurt 6

Table 2-2: Synonyms for concentrated yogurt in different countries 7

Table 2-3: CODEX compositional standards for concentrated fermented milks 8

Table 3-1: Composition of milk powders used in this study [¥] 56

Table 3-2: Specifications of the market reference samples used in this study 57

Table 3-3: Factors and levels considered to produce experimental samples 63

Table 3-4: Composition of experimental samples according to the different factors and levels considered in the experimental design 64

Table 3-5: Rheological and physicochemical values of the market reference samples [¥] 70

Table 3-6: Physicochemical properties of market reference samples [¥, †] 71

Table 3-7: Storage modulus (G') of samples produced by methods 1 and 2 at three selected points of the stress amplitude range applied [¥, †] 75

Table 3-8: Loss tangent (tan δ = G''/G') of samples produced by methods 1 and 2 at three selected points of the stress amplitude range applied [¥, †] 88

Table 3-9: Surface whey-off present in samples before applying homogenization (SWO_{BH}) [€, Δ] 94

Table 3-10: Whey drainage present in samples [€, Δ] 100

Table 3-11: Size of visible clusters present in samples [€, Δ] 102

Table 3-12: Incubation times required for the production of samples [€, Δ] 106

Table 3-13: Physicochemical composition of the inoculated reconstituted milk base obtained by the proposed formulation [§] 113

Table 4-1: Storage modulus (G') of samples, produced by method A, after different storage times at three different points of the stress amplitude range applied [€, £, Δ] 133

Table 4-2: Loss tangent (tan δ = G''/G') of samples, produced by method A, after different storage times at three different points of the stress amplitude range applied [€, £, Δ] 134

Table 4-3: Physicochemical properties of samples, produced by method A, after different storage times [€, £, Δ] 135

Table 4-4: Storage modulus (G') of samples, produced by methods A and B, after 1 and 8 days of storage at three different points of the stress amplitude range applied [€, Δ] 142

Table 4-5: Loss tangent (tan δ = G''/G') of samples, produced by methods A and B, after 1 and 8 days of storage at three different points of the stress amplitude range applied [€, Δ] 142

Table 4-6: Physicochemical properties of samples, produced by methods A and B, after 1 day of storage [€, Δ] 144

Table 4-7: Physicochemical properties of samples, produced by methods A and B, after 8 days of storage [€, Δ] 144

Table 5-1: Factors and levels considered to produce experimental samples 155

Table 5-2: Composition of experimental samples according to the different factors and levels considered in the experimental design 156

Table 5-3: Physicochemical properties of samples produced from powders stored at different conditions [€, £, Δ] 161

List of Figures

Figure 2-1: Main processing steps in the manufacture of set and stirred yogurt 11

Figure 2-2: Dual-binding model of structure of casein micelle 24

Figure 2-3: Schematic representation of β-lactoglobulin denaturation: breakage of its tertiary structure and exposure of thiol groups 29

Figure 2-4: Schematic representation of the heating of skim milk and the subsequent acidification resulting in the formation of a protein network 29

Figure 2-5: A schematic representation of the interactions between casein micelles and whey proteins occurring in milk during heat treatment for 10 min at 80^{o}C at pH values ranging from 6.35 to 6.9 30

Figure 3-1: Methods of production considered for the manufacture of experimental samples 59

Figure 3-2: Ordinal levels used for the classification of samples according to the degree of whey drainage..... 61

Figure 3-3: Ordinal levels used for the classification of samples according to the presence and size of visible clusters 62

Figure 3-4: Storage (G') and loss (G'') modulus of reference samples at days 18 and 35 after their production 65

Figure 3-5: Loss tangent (tan δ = G''/G') values of reference samples at days 18 and 35 after their production 67

Figure 3-6: Mean storage modulus (G') of reference samples with the corresponding two-sided confidence interval limits (α = 0.05) 68

Figure 3-7: Mean loss tangent (tan δ = G''/G') of reference samples with the corresponding two-sided confidence interval limits (α = 0.05)..... 69

Figure 3-8: Storage modulus (G') of samples produced by methods 1 and 2 as a function of amplitude sweep 73

Figure 3-9: Loss modulus (G'') of samples produced by methods 1 and 2 as a function of amplitude sweep 74

Figure 3-10: Effects of MPC-85, NaCN, WPI-90, and SC concentrations on the storage modulus (G') of samples produced by production method 1 at three selected points of the stress amplitude range applied 79

Figure 3-11: Effects of MPC-85, NaCN, WPI-90, and SC concentrations on the storage modulus (G') of samples produced by production method 2 at three selected points of the stress amplitude range applied 83

Figure 3-12: Loss tangent (tan δ = G''/G') of samples produced by methods 1 and 2 as function of amplitude sweep 87

Figure 3-13: Effects of MPC-85, NaCN, WPI-90, and SC concentrations on the loss tangent (tan δ = G''/G') of samples produced by production method 1 at three selected points of the stress amplitude range applied 91

Figure 3-14: Effects of MPC-85, NaCN, WPI-90, and SC concentrations on the loss tangent (tan δ = G''/G') of samples produced by production method 2 at three selected points of the stress amplitude range applied 93

Figure 3-15: Effects of MPC-85, NaCN, WPI-90, and SC concentrations on the SWO_{BH} level of samples produced by production method 1 95

Figure 3-16: Effects of MPC-85, NaCN, WPI-90, and SC concentrations on the SWO_{BH} level of samples produced by production method 2 96

Figure 3-17: Effects of MPC-85, NaCN, WPI-90, and SC concentrations on the level of WD of samples produced by production method 1 101

Figure 3-18: Effects of MPC-85, NaCN, WPI-90, and SC concentrations on the size of clusters present in samples produced by production method 1 103

Figure 3-19: Effects of MPC-85, NaCN, WPI-90, and SC concentrations on the size of clusters present in samples produced by production method 2 104

Figure 3-20: Effects of MPC-85, NaCN, WPI-90, and SC concentrations on the incubation times of samples produced by production method 1 106

Figure 3-21: Effects of MPC-85, NaCN, WPI-90, and SC concentrations on the incubation times of samples produced by production method 2 107

Figure 3-22: Effects of MPC-85, NaCN and WPI-90 concentrations (at high SC level) on the size of clusters present in samples produced by production method 2 109

Figure 3-23: Effects of MPC-85, NaCN and WPI-90 concentrations (at high SC level) on the storage modulus (G') of samples produced by production method 2 at three selected points of the stress amplitude range applied..... 110

Figure 3-24: Effects of MPC-85, NaCN and WPI-90 concentrations (at high SC level) on the loss tangent (tan δ = G''/G') of samples produced by production method 2 at three selected points of the stress amplitude range applied.. 111

Figure 3-25: Overlaid contour plot of the effects of MPC-85, NaCN and WPI-90 concentrations (at high SC level) on the clusters' size and rheological properties of samples at three selected points of the stress amplitude range applied..... 112

Figure 3-26: Storage modulus (G') and loss tangent (tan δ = G''/G') expected for the acid milk gel obtained by the proposed formulation at three selected points of the stress amplitude range applied 114

Figure 4-1: Methods of production considered for the manufacture of experimental samples 130

Figure 4-2: Storage modulus (G') of samples, produced by method A, after different storage times as a function of amplitude sweep 132

Figure 4-3: Loss tangent (tan δ = G''/G') of samples, produced by method A, after different storage times as a function of amplitude sweep..... 133

Figure 4-4: Storage modulus (G') of samples, produced by methods A and B, after 1 and 8 days of storage as a function of amplitude sweep..... 140

Figure 4-5: Loss tangent (tan δ = G''/G') of samples, produced by methods A and B, after 1 and 8 days of storage as a function of amplitude sweep 141

Figure 5-1: Storage modulus (G') of reconstituted samples obtained from powders stored at different conditions as a function of amplitude sweep..... 157

Figure 5-2: Loss tangent (tan δ = G''/G') of reconstituted samples obtained from powders stored at different conditions as a function of amplitude sweep . 158

Figure 5-3: Effects of storage temperature and time, applied to yogurt powders, on the storage modulus (G') of the recombined samples at three selected points of the stress amplitude range applied..... 159

Figure 5-4: Effects of storage temperature and time, applied to yogurt powders, on the loss tangent (tan δ = G''/G') of recombined samples at three selected points of the stress amplitude range applied..... 160

Figure 5-5: Effects of storage temperature and time, applied to yogurt powders, on the incubation times of samples..... 161

Figure 5-6: Glass transition curve scheme which relates the glass transition temperature and moisture content..... 164

List of Abbreviations

CCP: Colloidal Calcium Phosphate

C:WP: Casein to whey protein ratio

G': Storage Modulus

G'': Loss Modulus

IT: Incubation time

MPC-85: Milk Protein Concentrate (85%)

MPP: Milk Permeate Powder

NaCN: Sodium Caseinate

RO: Reverse Osmosis

SC: Starter Culture

SWO: Surface Whey-Off

SWO_{AH}: Surface Whey-Off After Homogenization

SWO_{BH}: Surface Whey-Off Before Homogenization

tan δ: Loss Tangent

TP: Total Protein Content

TS: Total Solids Content

UF: Ultrafiltration

WD: Whey Drainage

WHC: Water Holding Capacity

WPC: Whey Protein Concentrate

WPI-90: Whey Protein Isolate (90%)

$\sigma_{fracture}$: Fracture Stress

$\gamma_{fracture}$: Fracture Strain

σ_{yield}: Yield Stress

1. Introduction

Yogurt consumption has widely increased over the past years in Canada and the United States (U.S.) (Canadian Dairy Information Centre, 2012; Chandan, 2008). This trend has been directly related to consumer awareness of the health benefits associated with yogurt (Agriculture and Agri-Food Canada, 2005; United States Department of Agriculture, 2003).

Greek-style yogurt is a concentrated type of yogurt which possesses sensory attributes that are different from and nutritional properties that are superior to regular yogurt (Nsabimana *et al*., 2005; Salji, 1991). These two distinctive features are the main reasons for consumption of this concentrated fermented milk which is nowadays driving the vast majority of the yogurt growth in the U.S. as the yogurt category has accelerated its share gains of total breakfast and meal occasions (Palmer & Sakan, 2011).

Due to the high nutritional benefits, the increased popularity, and the remarkable economic growth of concentrated yogurt in North America, the production of a Greek-style yogurt powder, which can offer a longer shelf-life and a higher thermal stability than regular strained yogurt, can potentially help to address nutritional deficiencies in regions that have a limited indigenous dairy industry or that suffer from seasonal changes in milk supply. Furthermore, the production of a concentrated yogurt powder can positively contribute to open up new markets to this highly valuable food commodity (Tong, 2002; Kneifel, 1993).

This investigation will evaluate the approach to developing a concentrated yogurt powder formulation required to produce a recombined acid milk gel with physicochemical and rheological aspects similar to those found in commercial Greek-style yogurt (0% M.F.). According to previous investigations (Avisar, 2010; Özer *et al*. 1997, 1998b, 1999a,b), the production of this dairy product by direct recombination should enhance its nutritional value (higher amount of whey proteins are retained in the final product) but may affect its quality attributes negatively (weak gels can be formed). Although numerous scientists have studied or reviewed the production of recombined concentrated yogurt (Tamime, 1993, 2003; Tamime & Robinson, 1999, 2007; Kjærgaard Jensen & Nielsen, 1982; Gilles & Lawrence, 1981; Özer, 2006; Özer *et al*., 1997, 1998a, b, 1999a, b; Tong, 2002), there is little evidence of the manufacture of a recombined, non-fat, additive-free type strained yogurt. To respond to the actual increase in consumer

demand for non-fat, additive-free products (Gould *et al*., 1994; Institute of Food Technologists, 2011), the research program has been designed with three main objectives: (1) to formulate a fat-, additive-free, Greek-style yogurt, in powder form, using a range of commercially available dairy ingredients; (2) propose a formulation that would possess similar rheological and physicochemical attributes to commercially produced Greek-style yogurt from fresh milk; (3) focused on the effects of storing the recombined and dry formulation on the rheological and physicochemical aspects of the final recombined acid milk gel.

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2. Literature review

2.1 General characteristics of Greek-style yogurt

Yogurt is defined as the "food produced by culturing one or more of the optional dairy ingredients (cream, milk, partially skimmed milk or skimmed milk) with a characterizing bacterial culture that contains the lactic acid-producing bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus"* (Hui, 2012). Yogurts differ according to their chemical composition, method of production, flavour used and the nature of post-incubation processing (Shah, 2003).

Greek-style yogurt, also known as strained yogurt, concentrated yogurt or thick yogurt, is a semisolid fermented milk product derived from yogurt by draining away part of its whey. As a result of this draining action, the final product has higher total solids and lower lactose contents than regular yogurt (**Table 2-1**). The product has a cream/white color, a soft and smooth body, good spreadability with little syneresis and a flavor that is clean and slightly acidic (Nsabimana *et al.*, 2005).

Table 2-1: Typical chemical compositions (g 100g $^{-1}$) of industrial full and low-fat strained yogurt

Composition	Full-fat	Low-fat
Total solids	22.0	14.3
Protein	4.9	9.9
Fat	10.1	0.2
Carbohydrate	6.0	3.5
Ash	1.0	0.6

Source: Tamime (2003).

Concentrated yogurt is widely consumed in the Middle East and Balkan regions (Al-Kadamany *et al.*, 2002). Evidence of its production can be found in many countries in Turkestan, the Balkans, the eastern Mediterranean, and the Indian subcontinent (Tamime & Robinson, 2007a). **Table 2-2** shows the variety of names by which this product is known in different countries.

Table 2-2: Synonyms for concentrated yogurt in different countries

Traditional names	Countries/Regions
Labneh, labaneh, lebneh, labna	Eastern Mediterranean
Ta, than	Armenia
Laban zeer	Egypt, Sudan
Stragisto, sakoulas, tzatziki	Greece
Torba, suzme	Turkey
Syuzma	Russia
Mastou, mast	Iraq, Iran
Basa, zimne, kiselo, mleko-slano	Yugoslavia, Bulgaria
Itittu	Ethiopia
Greek-style	United Kingdom
Chakka, shrikhand	India
Ymer	Denmark
Skyr	Iceland

Source: Tamime & Robinson (2007a).

Strained yogurt has a higher lactic acid concentration than normal yogurt (1.8-2.0% as lactic acid). As a result, it presents a better keeping quality than the latter form (Tamime & Robinson, 1999; Robinson, 2002; Tamime *et al.*, 1989a). High lactic acid concentrations can be expected to curtail the growth of bacterial pathogens, but yeasts, moulds and some lactic acid bacteria can still contribute to spoilage problems. At 7^{o}C, concentrated yogurt can be kept for two weeks (Nsabimana *et al.*, 2005). Any sharp taste resulting from the high lactic acid concentration will be masked by diacetyl produced during fermentation; and by the high fat content, which is typically around 10%, and (Al-Kadamany *et al.*, 2002; Robinson, 2002).

Furthermore, concentrated yogurt has superior nutritional properties to those of regular yogurt: it has higher protein (2.5x) and mineral (1.5x) concentrations; a higher number of viable lactic acid bacteria (there is a tendency for these bacteria to be retained in the crud during the concentration process); a very low lactose concentration, which makes strained yogurt even more suitable for lactose intolerant individuals than regular yogurt; and a fat content which can be varied according to consumer demand (Salji, 1991; Mahdian & Tehrani 2007; Nsabimana *et al.*, 2005).

The perceived nutritional benefits and storage characteristics of Greek-style yogurt led to its increasing popularity and economic importance during the last decade of the past century (Benezch & Maingonnat, 1994). Nowadays, concentrated yogurt is establishing as a popular nutritious product possessing a healthy image equal to or greater than that of regular yogurt (Nsabimana *et al.*, 2005).

2.2 Compositional standards for Greek-style yogurt

The CODEX ALIMENTARIUS classifies strained yogurt as a type of concentrated fermented milk and its composition and quality standards are described in: CODEX STAN 243-2003 (World Health Organization/Food and Agriculture Organization of the United Nations, 2011a).

Table 2-3: CODEX compositional standards for concentrated fermented milks

Composition	**Concentrated fermented milk**
Milk Protein (% m/m)	> 5.6
Milk Fat (% m/m)	< 10.0
Titrable acidity, expressed as % lactic acid (% m/m)	> 0.3
Sum of microorganisms constituting the starter culture (cfu/g, in total) †	$> 10^7$
Labelled microorganisms (cfu/g, total)¥	$> 10^6$

† *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. ¥Applies where a content claim is made in the labeling that refers to the presence of a specific microorganism (other than those constituting the starter culture) that has been added as a supplement to the specific starter culture.
Source: World Health Organization/Food and Agriculture Organization of the United Nations (2011a).

2.3 Current importance of yogurt and Greek-style yogurt in the Canadian and U.S. markets

Yogurt, essentially from the Eastern hemisphere, has gained considerable popularity as a wholesome and nutritious food in America. Indeed, its health properties, which extend beyond nutrition, are now being recognized (Salji, 1991). Reported health benefits associated with yogurt and probiotic cultures include growth promotion, enhancement of mineral absorption, lactose digestion (the ability to reduce symptoms of

lactose intolerance), antimicrobial function (the ability to enhance resistance to colonization by pathogenic organisms), anticholesterol effect (the ability to reduce the risk of cardiovascular disease by lowering serum cholesterol), anticarcinogenic factor (the ability to reduce risk factors for colon cancer initiation), stimulation of the host immunological system, restoration of normal balance of gastrointestinal microflora, and positive contribution to longevity (Salji, 1991; Chandan & Kilara, 2008; Chryssanthopoulos & Maridaki, 2009; Chandan & Nauth, 2012). Added to this, yogurt is commonly supplemented with various functional ingredients, such as probiotics, prebiotics, fiber, plant sterol esters, omega-3 fatty acids, minerals and vitamins to impart an even healthier image to the final product (Chandan & Kilara, 2008).

The developments of new products, along with increased consumer awareness of the health benefits associated with yogurt cultures and probiotics, had led to a sharp increase in the per capita consumption of yogurt in Canada and the U.S. during the last decades (Agriculture and Agri-Food Canada, 2005; United States Department of Agriculture, 2003).

According to the Canadian Dairy Information Centre, Canadians consumed 8.28 liters (per capita) of yogurt in their diet in 2010, almost twice as much as they had a decade ago. In Canada, yogurt consumption has been steadily increasing over the years, beginning with 0.03 liters in 1960, reaching 3.09 liters in 1990, 4.59 liters in 2000 and 8.47 liters in 2011 (Statistics Canada, 2008; Canadian Dairy Information Centre, 2012). The increased demand resulted in higher production volumes. In 2010 Canada produced 300,719 kg of yogurt, almost twice the amount produced in 2001 (Canadian Dairy Information Centre, 2011).

Similar trends in yogurt consumption were observed in the U.S. (Chandan, 2008; United States Department of Agriculture, 2003). According to Nauth (2006), from 1970 to 1997, the annual per capita yogurt consumption in the U.S. has grown six-fold, from 0.36 kg to 2.31 kg, respectively. As stated by the International Dairy Foods Association (2008), in the year 2007 the annual per capita consumption was 4.99 kg, more than twice the consumption reported by Nauth (2006) for 1997. As the consumption of yogurt increases, the yogurt industry is growing at about 3 to 4 percent every year (Nauth, 2006).

Additionally, in the U.S., the Greek yogurt segment has grown more than 100% per year from 2008 to 2010. This segment is now driving the vast majority of yogurt growth as the yogurt category has accelerated its share gains of total breakfast and meal occasions. In 2008, overall yogurt category sales were dominated by traditional (non-

Greek) yogurt, which represented 98% of category sales. Since that time Greek yogurt sales have increased at a 130% CAGR (compound annual growth rate) and now represent 19% of the overall yogurt category. Over the 52 weeks ending February 19, 2011, overall yogurt category sales increased 12% YOY (year over year), of which 85% was driven by a 146% increase in Greek yogurt sales, while a 2% increase in traditional yogurt sales accounted for only 15% of category growth (Palmer & Sakan, 2011). According to Wang (2011), from the years 2006 to 2010, U.S. Greek yogurt sales rose from $60 million a year to a staggering $1.5 billion a year. The Nielsen Company, a U.S. global information and trend measurement company, also reported a sharp increase in Greek yogurt sales during the last few years. According to Nielsen, over the past 52 weeks ending October 2, 2010, Greek yogurt dollar and unit sales went up 160% and 203% respectively, while non-Greek yogurt dollar and unit sales went up 3% and 1% (The Nielsen Company, 2010).

Limited information is available in regards to the current market trends of Greek yogurt in Canada. Although the Canadian Greek yogurt market is not as well developed as in the U.S., during the last few years Greek yogurt has gained considerable importance in the Canadian market as healthier eating remains the dominant trend in the Canadian food industry with an emphasis on lower fat and lower calorie products with nutritional benefits (Canadian Dairy Commission, 2012).

2.4 General technology of yogurt manufacturing

At present, there is a wide variety of yogurt types on the market (Yildiz, 2009; Chandan & Nauth, 2012). Yogurts are usually classified based on their fat content (full-fat, reduced-fat, and low-fat) and on the method of production and the physical structure of the coagulum (set or stirred yogurts). Set yogurt is the product formed when the fermentation of milk is carried out in a retail container, and the yogurt produced is in a continuous semisolid mass. In contrast, stirred yogurt results when the coagulum is produced from milk, and the gel structure is broken before cooling and packaging. Fluid yogurt can be considered as stirred yogurt of low viscosity (Shah, 2003). The main processing steps involved in these two types of yogurt manufacturing (**Figure 2-1**) include the standardization of milk (fat and protein content), homogenization, milk heat treatment, incubation/fermentation, cooling, and storage (Lee & Lucey, 2010).

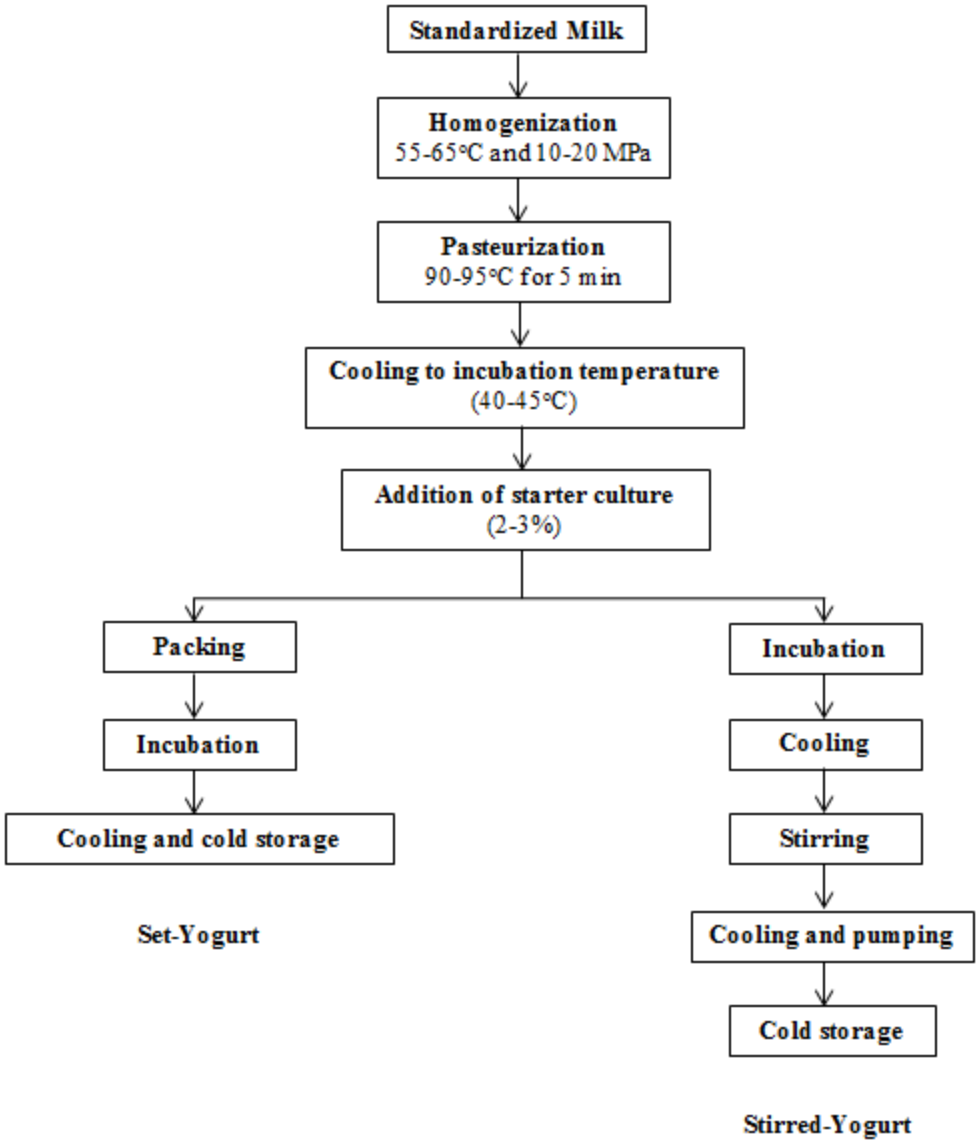

Figure 2-1: Main processing steps in the manufacture of set and stirred yogurt

Source: Adapted from Lee & Lucey (2010).

2.4.1 Milk standardization

Nowadays, three systems are available to standardize the fat and protein content of the milk base: (1) the addition of milk powders to liquid milk, (2) the evaporation of water from liquid milk under vacuum, (3) the removal of water from liquid milk by membrane processes (Robinson, 2002). Milk bases should be formulated to comply with regulations and meet consumer expectations (Nauth, 2006). Stabilizers (gelatin, starch,

pectin) and sweeteners can also be added to further impact the physical properties of the final product (Chandan & O'Rell, 2006).

Increasing the total solids increases the firmness, complex viscosity (the storage modulus and fracture stress of the gel are increased), apparent viscosity, oral viscosity, consistency index, and water holding capacity (WHC) of the resultant gel (Harwalkar & Kalab, 1986; Rohm & Schmidt, 1993; Mistry & Hassan, 1992; Lee & Lucey, 2010; Lucey, 2002; Lucey & Singh, 1998; Bhullar *et al.*, 2002; Anema, 2008; Özer, 2009; Barreto Penna *et al*., 2006; Wu *et al*., 2009; Krzeminski *et al.*, 2011; Jumah *et al*., 2001; Amatayakul *et al*., 2006). Thus, it improves the textural attributes of the gel, giving a higher sensory acceptability to the final product (Skriver *et al*., 1999; Mahdian & Tehrani, 2007; Peng *et al.*, 2009; Marafon *et al*., 2011).

2.4.2 Homogenization

Homogenization is the typical industrial process used to effect stabilization of the lipid phase against separation by gravity. During this process the average diameter of fat globules (3-4μm) is reduced to 1 or 2 μm. As a result, the fat globules do not cream during the incubation of the yogurt. Because of the size reduction, there is usually a four-to-six-fold increase in the surface area (Shah, 2003). Upon homogenization, the fat-globule membrane is destroyed, and caseins and whey proteins form the new surface layer of fat globules, which increases the number of possible structure-building components in yogurt made from homogenized milk. Homogenized milk fat globules act like protein particles due to the presence of protein on the fat surface (Lee & Lucey, 2010). Therefore, homogenization also improves gel strength upon fermentation due to greater protein–protein interaction (Chandan & Nauth, 2012). As the fat globule membrane is destroyed during homogenization, lipids are vulnerable to attack by lipase. To prevent lipolysis, milk must be pasteurized immediately after homogenization (Shah, 2003). Homogenization pressures used are usually between 10 and 20 MPa and, since the efficiency of homogenization is much better when the fat phase is in a liquid state, the process is usually carried out at high temperatures (55^{o}C to 80^{o}C) (Chandan & O'Rell, 2006).

2.4.3 Heat treatment

Heat treatments, which are much more severe than fluid milk pasteurization, are necessary to:

(1) Generate a yogurt with the desired textural properties. Thus, the heating/holding regime both alters the physicochemical properties of the caseins and denatures the whey proteins, so that β-lactoglobulin, in particular, may become attached to the casein micelles; this linkage improves the texture (set yogurt) or viscosity (stirred yogurt) of the final product (See section 2.6.3).

(2) Cause some breakdown of the whey proteins to liberate free amino acids that stimulate the activity of the starter culture.

(3) Expel oxygen from the processed milk because, as the starter bacteria are microaerophilic, deaeration provides the correct environment for rapid growth.

(4) Kill any non-sporing pathogens that may be present, helping to ensure that yogurt retains its image as a "safe" product (Robinson, 2002).

To meet these requirements, milk is generally heated, using a continuous plate heat exchanger, at 85 to 95^{o}C for 10 to 30 minutes (Yildiz, 2009). According to Chandan & O'Rell (2006), optimum results are obtained by using a heat treatment of 90-95^{o}C and a holding time of 5-10 minutes.

2.4.4 Incubation/fermentation

After heat treatment, the milk base is cooled to the incubation temperature used for growth of the starter culture. An optimum temperature of the thermophilic lactic acid bacteria, i.e., *Streptococcus* ssp. *thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, is around 40- 45^{o}C. Bacterial fermentation converts lactose into lactic acid, which reduces the pH of milk. During the acidification of milk, the pH decreases from 6.7 to ≤4.6. In unheated milk gels, gelation occurs at around pH 4.9, while in heated milks gelation occurs at pH 5.2-5.4 (because denatured β-lactoglobulin has a higher isoelectric point than casein) (Lucey, 2009; Lee & Lucey, 2010; Sodini *et al.*, 2004).

The essential flora of yogurt (*Sc. thermophilus* and *Lb. delbrueckii* ssp. *bulgaricus*) displays an obligate symbiotic relationship during their growth in a milk medium. The rates of acid and flavor production by mixed yogurt cultures are

considerably higher than by either of the two organisms grown separately (Özer, 2009; Chandan & O'Rell 2006). *Lb. delbrueckii* ssp. *bulgaricus* hydrolyzes milk proteins, the caseins, thus releasing essential amino acids, including valine, which stimulate the growth of *Sc. thermophilus*. Initially, *Sc. thermophilus* grows rapidly, reducing the pH to around 5.4, which stimulates the growth *of Lb. delbrueckii* ssp. *bulgaricus*, which is acid-tolerant and produces large amounts of lactic acid, which reduces the pH. *Sc. thermophilus* uses oxygen during its growth, which makes oxidation–reduction potential more favorable for *Lb. delbrueckii* ssp. *bulgaricus*; it also produces purine, pyrimidine, CO_2, formic acid, oxaloacetic acid, and fumaric acid that stimulate the growth of the lactobacillus (Shah, 2003; Özer *et al*., 2009; Chandan & O'Rell 2006). During the growth in milk, *L. delbrueckii* ssp. *bulgaricus* apparently exhibits a preference for utilizing β-casein over other proteins as a nitrogen source, indicating that the type of protein is also an important factor influencing the growth of this culture (Özer, 2009). Starter bacteria can continue to produce acid until a very low pH (e.g. ~4.0) is attained when bacteria become inhibited by the low pH; in practice bacterial gels are cooled when sufficient acidity has been attained (pH ~4.6). The rate of pH change during fermentation or addition of acid is controlled by the acid-base buffering properties of milk (Lucey & Singh, 1998).

2.4.5 Cooling and storage

Since the yogurt organisms show limited growth activity around 10^oC, the primary objective of cooling is to drop the temperature of the coagulum from 30-45^oC to $<10^oC$ as quickly as possible so as to control the final acidity of the product. The process of cooling yogurt may be carried out using one-phase or two-phase cooling (Tamime & Robinson, 2007b). In single-phase cooling, the temperature of fermenting milk is directly reduced from 43°C to <10°C. This model is more appropriate for plain set-type yogurt production. Two-phase cooling is widely employed for stirred-type yogurt production. In the first phase, fermenting milk is stirred gently in a tank to obtain a homogeneous body, and cooled to 20–24°C. At this stage, fruit is added and the yogurt cups are filled. The filled cups are then cooled to <10°C over a period of 10–12 hours (Özer, 2009). To improve yogurt quality, the second stage of cooling should be carried out as slowly as possible over a 12-hour period (Shah, 2003). The rate of cooling is of critical importance

in obtaining a product with the desired textural quality. Cooling too quickly can cause a weak body and stimulate whey separation during cold storage (Özer, 2009).

Storing yogurt for 1-2 days improves the viscosity. During the first 24–48 hours of cold storage, an improvement in the physical characteristics of the coagulum is observed, mainly because of hydration and/or stabilization of casein micelles. Proper hydration is required to avoid syneresis. It is therefore important to delay the sale or distribution of yogurt for 24–48 hours (Shah, 2003).

2.5 Greek-style yogurt manufacturing methods

Much of the concentrated yogurt consumer acceptability is dependent on its sensory properties, which in turn, seem to be heavily dependent on the method of processing of the material (Özer *et al.*, 1998b; Abu-Jdayil *et al.*, 2002). Concentrated yogurt is traditionally manufactured by straining the natural set yogurt in cloth bags (Yamani & Abujaber, 1994). However, nowadays there are other methods available to manufacture this product in large volumes. The current methods available for manufacturing concentrated yogurt have been widely reviewed by Tamime & Robinson (1988,1999, 2007), Robinson & Tamime (1993), Özer (2006), Salji (1991), Nsabimana *et al.* (2005), Tamime (1993, 2003), Tamime & Marshall (1997), Tamime *et al.* (2001) and can be classified as follows:

- Traditional method (cloth bag) (Tamime *et al.*, 1989a,b, 1991b,c; Özer *et al.*, 1997, 1998a,b, 1999a,b; El-Samragy *et al.*, 1997; Tamime & Robinson, 1978; Abou-Donia, 2004).
- Methods based on mechanical separators (Dagher & Ali-Ghariebeh, 1985; Rasic, 1987; Lehmann *et al.*, 1991).
- Methods based on membrane processes (Tamime *et al.*, 1989a,b, 1991a,b,c; Özer *et al.*,1997, 1998a,b, 1999a,b; Özer & Robinson, 1999; El-Samragy *et al.*, 1997; El-Samragy & Zall, 1988; Hofi, 1988).
- Methods based on direct recombination (Gilles & Lawrence, 1981; Özer *et al.*, 1997, 1998a,b, 1999a,b; Kjærgaard Jensen & Nielsen, 1982).

2.5.1 Traditional method

The basic principle of using the traditional cloth bag method is to extract water from plain yogurt until the desired total solids level has been reached. The duration of drainage for yogurt in cloth bags takes about 15-20 hours at <10^{o}C. The whey separation can be achieved either by gravity drainage (small scale production) or by pressing (large scale production, i.e., by piling 25-kg bags on top of each other); however, the drainage time can be shortened by up to 6 hours by applying pressure of 2 kg kg^{-1} on the yogurt (Özer, 2006).

The sensory properties of the product made with this traditional system are excellent (Robinson, 2002). However, this method could be described as slow, labour intensive and unhygienic by the nature of the process, and the yield obtain is rather low due to residues left in the bag (Zayan *et al*., 2010**;** Tamime *et al.*, 1989a,b; Robinson, 2002; Gilles & Lawrence, 1981). Consequently, this system is not suitable for large-scale processing (Özer *et al.*, 1998b; Salji, 1991).

Despite this, the traditional production method is still preferred in some countries in the Middle East, as the investment in mechanised systems of production is rather high (Özer, 2006).

2.5.2 Methods based on mechanical separators

Mechanical separators have been used successfully for the industrial-scale production of strained yogurt (Tamime & Marshall, 1997). Salji *et al.* (1987a,b) reported the use of this method for factory-scale production in Saudi Arabia.

This method requires the use of a nozzle or Quarg separator. Only, skimmed milk should be used when manufacturing yogurt in this way; if whole milk is used, the fat globules will clog the separator nozzles. However, recent developments in the design of centrifugal separators have made it feasible to use fermented whole milk to produce strained yogurt (Tamime, 2003).

Producing concentrated yogurt by centrifugation is a two-step procedure. First, milk is fermented until it achieves the desired level of acidification (pH 4.6-4.8). After acidification, fermented skimmed milk is stirred vigorously, heated up to 55-60^{o}C to inactivate the culture and control the level of acidity, and cooled to 40^{o}C. Next, any large clots or clumps are removed by passing the fermentate through a metal sieve before it

enters the separator. The fermented milk is also de-aerated for 15-20 minutes before entering the centrifuge to assist the separation of whey in the separator. Once in the separator, the fermented milk is concentrated to the desired total solids level. The concentrated product leaving the separator is blended with any source of fat or cream, to provide the desired fat level in the final product. Then it is cooled and packaged (Özer, 2006; Tamime, 2003, 1993; Nsabimana *et al.* 2005; Tamime & Robinson, 1999, 2007). Capacities of such separators are up to 6.5 tonnes h^{-1}, depending on the composition of the milk used and the acidity of the fermented milk prior to concentration (Tamime, 2003; Tamime & Marshall, 1997).

According to Dagher & Ali-Ghariebeh (1985), strained yogurt, produced from heated yogurt by centrifugation for 5 minutes at different speeds between 4000 and 11 700g, had organoleptic characteristics similar to those of control samples made by the traditional method.

2.5.3 Methods based on membrane processes

Membrane techniques, especially ultrafiltration (UF), have been successfully used in the yogurt industry for the last 20-25 years (Özer, 2006). Production of strained yogurt by reverse osmosis (RO) has also been studied. However, previous scientific works revealed that using RO to produce concentrated yogurt created weaker structures which did not give gel properties close to those of concentrated yogurt made by the traditional method (Özer *et al.*, 1997, 1998a,b, 1999a,b; Özer & Robinson, 1999).

Two different systems of UF have been used to produce concentrated yogurt: (a) the fermentation of UF retentate that has the solids content desired in the final product (El-Samragy & Zall, 1988; Hofi, 1988; El-Samragy *et al.*, 1997), and (b) UF of yogurt at 40-50^{o}C (Tamime *et al.*, 1989a, 1991a,c) to produce a concentrated product with the desired total solids content (Tamime & Robinson, 2007).

Several scientific works studied the microstructures and rheological properties of concentrated yogurt obtained by these two UF methods (Tamime *et al.*, 1989a,b; Özer *et al.*, 1997, 1998a,b, 1999a,b; Özer & Robinson, 1999). Although the final chemical compositions of both products are similar to each other, the physical and organoleptic properties are considerably different (Özer, 2006). Researchers concluded that the concentrated yogurt made from UF milk retentate had much greater firmness than the products manufactured using the traditional method or UF of yogurt (Tamime &

Robinson, 2007; Nsabimana *et al.*, 2005; Tamime, 2003; Tamime *et al.*, 1989a,b). Moreover, the concentration of milk by UF before yogurt-making carries a risk of bitterness in the final product since the calcium content will be higher (Özer, 2006). On the other hand, the quality of strained yogurt made by UF of warm yogurt closely resembles the traditional product in terms of elasticity, firmness, and structure (Tamime, 2003, Tamime *et al.*, 1989a).

The manufacturing process is as follows: after the fermentation period, the warm yogurt is heated to 58-60^{o}C for 3 minutes in the plate heater exchanger, to inactivate the culture and control the level of acidity, cooled to 40^{o}C, concentrated in a two-to-four stage UF plant (depending on the desired degree of concentration), cooled in a plate cooler to about 20^{o}C and finally packaged (Nsabimana *et al.*, 2005).

According to Özer *et al.* (1998b) and Tamime *et al.* (1989a,b), UF applications can be used as an industrial alternative to the traditional strained yogurt-making process. Several studies which have investigated the rheology of concentrated yogurt produced by a range of techniques for increasing total solids have concluded that compared to other techniques (such as RO and direct recombination), UF of yogurt gives the gel properties that are closest to those of the traditional product (Özer *et al.*, 1997, 1998b,1999a). Other advantages of UF as compared with other conventional methods are: higher yield (10% increase), shortening of processing time (e.g., by 25%), reduced wheying-off, and easy automation and process control (Nsabimana *et al*., 2005; Özer, 2006). In addition, when using UF instead of the traditional method, the volumes of milk and starter cultures are reduced by around 10% and 80%, respectively (Özer, 2006). Due to all these advantages, a wide range of UF plants are now available on the market for the production of strained yogurt on a large scale (Robinson & Tamime, 1993; Tamime, 1993, 2003).

2.5.4 Methods based on direct recombination

According to the Food and Agriculture Organization of the United Nations, a recombined milk product is a product resulting from the combining of milk-fat and milk-solids-non-fat in their preserved forms with or without the addition of water to achieve the appropriate milk product composition (World Health Organization/Food and Agriculture Organization of the United Nations, 2011b).

In order to eliminate the drainage stage during the manufacture of concentrated yogurt, it is feasible to manufacture this product from recombined dairy ingredients

(Özer, 2006; Tamime, 2003, 1993). The process involves reconstituting powders in water, up to the total solids level required in the final product, and blending the reconstituted milk with anhydrous milk fat and stabilisers (Tamime, 2003). After the recombination is complete, the recombined milk is handled and processed in a similar way to the production of traditional yogurt (Tamime & Robinson, 1999).

The quality of recombined dairy products is directly related to the composition, properties, and microbiological standards of the ingredients used (Kjærgaard Jensen & Nielsen, 1982). According to Gilles & Lawrence (1981), good quality yogurt can be obtained from milk powders as long as the powders are free of off-flavours. Odet (1990) stated that there were no organoleptic differences between yogurt produced from recombined and fresh milks.

The introduction of membrane techniques to the dairy industry has enabled the production of different types of milk powders containing diverse protein to lactose ratios and altered whey protein to casein ratios (e.g., milk retentate, milk permeate, whey retentate, and whey permeate powders) (Avisar, 2010; Caric, 2002). The use of these latter powders has enabled the production of recombined dairy products containing high protein and low lactose contents, such as concentrated yogurt. Several authors recommended using these types of powders to fortify the milk base during yogurt production and/or to produce concentrated yogurt using recombination technology (Mistry & Hassan, 1992; Gonzalez-Martinez *et al*., 2002; Guzman-Gonzales *et al.*, 1999; Guzman-Gonzales *et al.*, 2000; Tamime, 2003; Guilles & Lawrence, 1981). In order to obtain a recombined strained yogurt with good textural and physicochemical properties, experts recommend using heat-treated high protein dairy powders (with reduced lactose content) free of inhibitory substances that can slow or restrain the growth of lactic bacteria (Guilles & Lawrence, 1981; Kjærgaard Jensen, 1990; Tong, 2002). However, if processing steps include a high heat treatment, low-heated milk powders can also be used effectively to produce a good quality product (See section 2.6.3) (Tong, 2002).

Since recombined products generally contain high amounts of water, it is important to have a high quality water source. Excessively hard water can lead to problems with powder solubility and stability (Tong, 2002). According to the recommendations from the International Dairy Federation (IDF), water used to recombine dairy products should not exceed the following maximum salt concentrations: total hardness, 100μg of calcium carbonate g^{-1}; chloride, 100μg g^{-1}; sulfate, 100 μg g^{-1};

nitrate, 45 μg g^{-1} (Kneifel, 1993). Nichols & Kozak (1990) discussed in depth the importance of water used for recombining milk and milk products.

Milk powders used for recombination are very stable and have a shelf-life of 12 months at ambient temperatures without refrigeration, although storage at 20°C or below is recommended (Christensen & Colding, 1988). The long durability and good thermal stability of ingredients makes direct recombination a suitable option to provide a nutritious and high-quality source of dairy products in areas where a fresh raw milk supply is not readily available or is in short supply. Because refrigeration and transportation may not be readily available in some regions, utilization of preserved milk ingredients may be the only viable means of producing dairy products (Tong, 2002). Several authors (Mottaleb, 1990; Schulthess, 1990; Borland, 1990; Ketulo, 1990; Ezzat Jaafar & Seppala, 1990) reported the use of recombination technology to produce milk and dairy products in developing countries where, as a result of geographic/climatic/economic conditions, setting up a conventional dairy industry base using local milk production is impractical (Staal, 1990). On the other hand, in industrialized countries where there is a milk surplus, milk recombination offers the opportunity to transfer raw materials (milk powders, anhydrous milk fat, etc.) from surplus production areas to deficiency areas, in order to compensate for the abovementioned problems and to open up new markets (Kneifel, 1993). Therefore, it is believed that a widespread use of this technique to produce concentrated yogurt will potentially increase the international trade of powders high in protein and low in lactose (Avisar, 2010). However, it is important to point out that indiscriminate distribution of dairy ingredients for recombining purposes can, under certain circumstances, be detrimental to local milk producers (Staal, 1990).

The production of concentrated yogurt by direct recombination offers important advantages over other industrial production methods. Direct recombination does not involve whey disposal problems (there is less environmental damage, and the yogurt produced is more nutritious because all whey proteins are retained in the final product) and requires low investment and production costs (depending on the local market) (Christensen & Colding, 1988; Avisar, 2010). However, several scientific publications stated that the rheological properties of recombined concentrated yogurt were different from those of strained yogurt produced by the traditional method or by UF (Tamime & Robinson, 1999, 2007). Özer *et al.* (1997, 1998b, 1999a,b) concluded that strained yogurt

made by directly recombining full-cream milk powder to 23% (w/v) total solids formed weaker gels than those made by traditional or UF methods.

Although numerous scientists have studied or reviewed the production of recombined concentrated yogurt (Tamime, 1993, 2003; Tamime & Robinson, 1999, 2007a; Kjærgaard Jensen & Nielsen, 1982; Gilles & Lawrence, 1981; Özer, 2006; Özer *et al.*, 1997, 1998a, b, 1999a, b; Tong, 2002), there is little evidence of the manufacture of recombined non-fat strained yogurt. The present investigation was intended to find an effective formulation for producing a recombined non-fat, additive-free type of Greek-style yogurt.

2.6 Formation and physicochemical characteristics of acid milk gels

Acid-induced milk gels are formed by aggregation of casein particles as the pH of milk decreases and the isoelectric point (pH 4.6) of casein is approached (Lucey, 2001). Acid casein gels have a particulate, heterogeneous structure, consisting of fairly large conglomerates and holes (void spaces where the aqueous phase is confined). These conglomerates are thought to be built of smaller ones, which, in turn, consist of casein particles aggregated in strands and nodes. This heterogeneity, which depends, e.g., on the temperature during gel formation, largely determines the mechanical properties of the gel (Roefs & van Vliet, 1990; Lucey & Singh, 1998).

Casein gels are very dynamic and rearrangements of the clusters and particles forming the network may occur before or during gel formation (Lucey, 2001). The physical characteristics of these particulate gels are determined by both strong permanent bonds (covalent bonds: SH/S-S exchange) formed during the aggregation, and subsequent rearrangements of protein particles (noncovalent bonds: electrostatic, hydrophobic interactions and, probably, the ever-present Van der Waals attraction, as well as steric and entropic effects related to protein conformation). The balance between these strong and weak bonds controls the rheology of yogurt gels (Özer *el at.*, 1999b; Özer *et al.*, 1998b; Roefs & van Vliet, 1990).

Milk protein gels are irreversible, in contrast to many other food gels. Although milk gels are usually classified as particle gels, it is now recognized that they are not

simple particle gels because the internal structure of the casein particle plays an important role in the rheological properties of milk gels (Lucey, 2002).

2.6.1 Casein micelle structure

In bovine milk, there are 4 major types of caseins: αs_1-casein, αs_2-casein, β-casein, and κ-casein (McMahon & Oommen, 2008). The amphipathic nature of these phosphoproteins is preserved across species with each of these caseins showing their own pattern of segregation into hydrophobic and hydrophilic regions. Caseins are normally divided, according to their calcium-binding capacity, into two groups: the calcium-sensitive and the non-calcium-sensitive. κ-Casein is calcium-insensitive and αs_1-, αs_2- and β-caseins are calcium-sensitive (Horne, 2006). The extent of calcium-binding is directly related to the number of phosphoserine residues in the molecules. κ-Casein, with only one phosphoserine, binds little calcium, while αs_1-, αs_2- and β-caseins reveal high binding capacities for calcium as they present high numbers of phosphoserine residues in their structures (the latter caseins precipitate in the presence of calcium due to the reduction of their negative charge when binding with calcium cations) (Horne, 2002).

In fresh milk, the different types of caseins are joined together forming essentially spherical particles ranging from 15 to about 1000 nm in diameter. These particles are known as casein micelles (Creamer, 2002). At least three types of models for the structure of casein micelles have been proposed. Schmidt (1982) and Walstra (1990) suggested a model that proposes that the micelle core is divided into discrete sub-units (sub-micelles) with distinctly different properties (Lucey, 2002). In this model, the individual caseins come together in their appropriate portions to form internal sub-micelles, if depleted in κ-casein, or external sub-units rich in κ-casein, colloidal calcium phosphate (CCP) is regarded as the cement which links these discrete sub-units together. Another model, proposed by Holt (1992), regards the micelle as a mineralized, cross-linked protein gel in which the CCP nanoclusters are the agents responsible for cross-linking the proteins and holding the network together (Horne, 1998). A major failing of these two models is their lack of a plausible mechanism for assembly, growth and, more importantly, termination of growth of the casein micelles. All such elements are in place in a recent model, proposed by Horne (1998), which suggests a dual-binding (polycondensation-type) mechanism for gel assembly (Horne, 2002; Lucey, 2002).

In the dual-binding model, micellar assembly and growth take place by a polymerization process involving, as the name suggests, two distinct forms of bonding: crosslinking through hydrophobic regions of the caseins or bridging across CCP nanoclusters. Central to the model is the concept that micellar integrity and hence stability is maintained by a localized excess of hydrophobic attraction over electrostatic repulsion (Horne, 2002). The energy of interaction between molecules present inside the micelle is calculated as the sum of electrostatic repulsion and hydrophobic attraction as

$$IE = ER + HI \qquad \text{[Eq. 2-1]}$$

where, IE: interaction Energy; ER: electrostatic repulsion; HI: hydrophobic interaction (Horne, 1998).

This model sees the micellar CCP not just as cross-links but also as neutralizing agents which, being positively charged, bind to negatively charged phosphoserine clusters to reduce the protein charge to the level where the attractive interactions between the hydrophobic regions of the caseins can be allowed to dominate (Horne, 1998).

Figure 2-2 illustrates the structure of the casein micelle according to the dual-binding model and can be used to explain the two types of linkage postulated between protein molecules. The first linkage is hydrophobic, where two or more hydrophobic regions from different molecules form a bonded cluster. The growth of these polymers is inhibited by the protein-charged residues whose repulsion pushes up the interaction free energy. Neutralization of the phosphoserine clusters by incorporation into the CCP diminishes that free energy as well as producing the second type of cross-linking bridge, since it is considered that up to four or more phosphoserine clusters from different casein molecules can be accommodated at each CCP nanocluster (Horne, 1998).

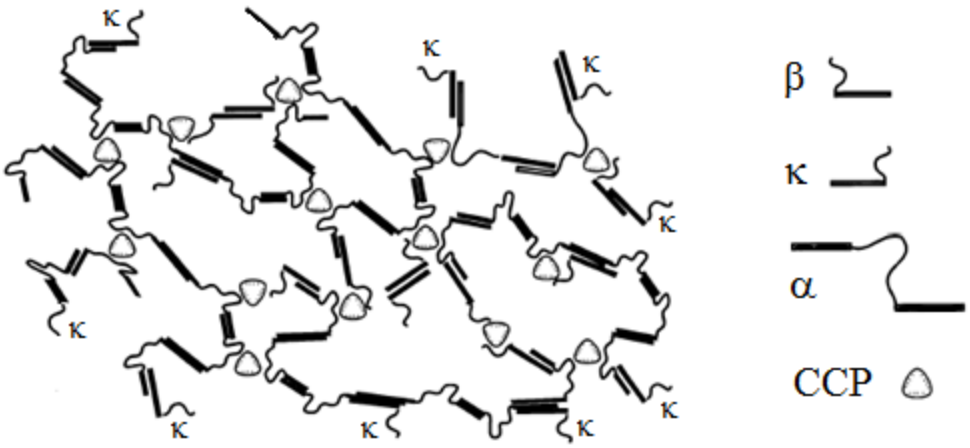

Figure 2-2: Dual-binding model of structure of casein micelle

Bonding occurs between the hydrophobic regions, shown as rectangular bars, and by linkage of hydrophilic regions containing phosphoserine clusters to CCP clusters. Molecules of κ-casein limit further growth and are labeled with the letter 'κ'. *Source:* Adapted from Horne (1998).

Although the κ-casein molecules can interact via their hydrophobic domains with the hydrophobic regions of the other caseins, further growth beyond the κ-casein is not possible because it possesses neither a phosphoserine cluster for linkage via CCP (the only phosphoserine residue in κ-casein lies in the macropeptide which forms the putative hairy layer deemed essential for micellar stability in all accepted models, thus, this residue cannot be involved in any cross-linking via CCP), nor another hydrophobic anchor point to extend the chain via this route. κ-Casein acts as a terminator for both types of growth. Unless circumvented by the growing network, it will become part of the surface structure of the micelle. Hence its surface location, a prime requirement for any structural model, arises naturally in this model (Horne, 1998).

This concept of a localized excess of hydrophobic attraction over electrostatic repulsion allows the visualization of micellar growth and successfully accommodates the response of the micelles to changes in pH, temperature, urea addition or removal of CCP by sequestrants, all in accordance with experimental observations (Horne, 2009). Urea does not rupture the CCP linkages but disrupts the hydrophobic bonds, bringing about micellar disintegration. Further, micellar integrity is largely maintained when the CCP is dissolved out by acidification because the phosphoserine negative charges are neutralized by the acid medium. If the milk is dialyzed and the pH is then restored to that of the original milk, dissociation of the micelle complex is observed as the negative charges of

the phosphoserine residues are not neutralized and the electrostatic repulsion effect predominates over the hydrophobic attraction. The same dissociation is observed at natural pH when the CCP is removed by sequestration with EDTA. Increasing pH from the natural value in milk leads to dissociation of the micelles. Whether this is due to conversion of the phosphoserine residues from singly to doubly negatively charged units which are no longer capable of linking to the CCP nanoclusters, or whether the increase in charge itself is sufficient to upset the balance of electrostatic repulsion and hydrophobic attraction in favour of electrostatic repulsion and the micelles dissociate. Decreasing the temperature decreases the level of hydrophobic attraction and any β-casein not linked through its phosphoserine cluster could then be released into the serum phase (Horne, 1998; Horne, 2002; Horne, 2009). These facts suggests that CCP does not cement the micelle together, as described by the earlier models, but rather it helps to control and modulate the effects of calcium and charged groups on caseins. It is also clear that hydrophobic interactions and hydrogen bonding are important for micelle integrity (Lucey, 2002).

2.6.2 Formation of acid milk gels

As the pH of milk is reduced, CCP is dissolved, the micelle structure is altered (the charge on individual caseins is altered and the ionic strength of the solution increased) and caseins are liberated into the serum phase (Lucey & Singh, 1998; Ozcan *et al*., 2011). The extent of liberation of caseins depends on the temperature at acidification (low temperatures results in a decrease in the level of hydrophobic attractions inside the casein micelle); at fermentation temperatures commonly used for yogurt manufacture (>30^{o}C), no dissociation of casein likely occurs (Ozcan *et al*., 2011). When the isoelectric point of caseins (pH ≈ 4.6) is approached, aggregation occurs and low-energy bonds, mainly hydrophobic, are progressively established between proteins (Remeuf *et al*., 2003). Three pH regions in the acidification of milk from pH 6.7 to 4.6 can be distinguished:

(a) *pH from 6.7 to 6.* The decrease in pH causes a decrease in the net negative charge on the casein micelles, thereby reducing electrostatic repulsion. Only a relatively small amount of CCP is dissolved above pH 6.0, so the structural features of the micelles are relatively unchanged (e.g., size) (Lucey, 2004).

(b) *pH from 6 to 5.* As the pH of milk decreases further from pH 6.0 to 5.0, the net negative charge on casein micelles greatly decreases and the charged "hairs" of κ-casein may shrink (or curl up). This results in a decrease in electrostatic repulsion and steric stabilization, which are both responsible for the stability of casein micelles in the original milk. At pH ≤6.0 the rate of solubilization of CCP increases, which weakens the internal structure of casein micelles and increases the electrostatic repulsion between the exposed phosphoserine residues. In milk, CCP is completely solubilized in casein micelles by pH ~5.0 (Lee & Lucey, 2010).

(c) *pH ≤ 5.* When the pH of milk becomes close to the isoelectric point of casein (pH 4.6), there is a decrease in the net negative charge on casein, which leads to a decrease in electrostatic repulsion between casein molecules. On the other hand, casein-casein attractions increase due to increased hydrophobic and electrostatic charge interactions (and van der Waals' forces) (Lee & Lucey, 2010; Lucey, 2009). In unheated milk, gels gelation occurs at around pH 4.9, while in heated milks, gelation occurs at pH 5.2-5.4 (because denatured β-lactoglobulin has a higher isoelectric point than casein) (Lucey, 2009; Lee & Lucey, 2010; Sodini *et al*., 2004). Casein particles aggregate as a result of (mainly) charge neutralization (Lucey, 2009). The acidification process results in the formation of a three-dimensional network consisting of clusters and chains of caseins (Lee & Lucey, 2010).

Solubilization of CCP during the acidification process undoubtedly changes the structural integrity of the casein micelles (Peng *et al*., 2009). When CCP is depleted from the micelle, the casein molecules will have more dispersed structures with a higher number of interaction sites (Horne, 2009). Therefore, the loss of CCP from casein micelles dramatically influences the properties of casein gels (Lucey, 2004). If the acidification is proceeding slowly, then this may allow equilibration and rearrangement into localized denser structures with few linkages between, giving rise to weaker gels. More rapid drops in pH may lock the protein into a more dispersed structure with greater density of possibly stronger strands (Horne, 2009). These statements are verified by the experimental work done by Lee & Lucey (2004a). These authors reported that higher inoculation rates resulted in lower fermentation times and stiffer gel networks. They

support their results by stating that the solubilization of CCP in milk during acidification is a slow process, and may require a slightly lower pH to completely dissolve CCP under conditions of fast acidification. When CCP dissolves at a lower pH, caseins at this lower pH value may be less sensitive to excessive rearrangements (due to the fact that at lower pH values there will be lower electrostatic repulsion and higher hydrophobic interactions between casein particles); thus, stiffer gel networks are obtained. Consequently, the solubilization of CCP appears to alter the balance between viscous and elastic components in the gel network (Lucey, 2002).

Hydrophobic interactions are unlikely to play a direct role in the strength of acid gels as the stiffness of acid gels increases as the measurement temperature decreases. Cooling results in an increase in the stiffness of the gel, probably as a result of the swelling of casein particles (caused by the weaker hydrophobic interactions) and an increase in the contact area between particles. A similar trend occurs when lower incubation temperatures are used. The use of lower incubation temperatures leads to longer incubation times, but firmer and more viscous gels that are less prone to whey syneresis are formed. At a lower incubation temperature, there is an increase in the size of the casein particles because of a reduction in hydrophobic interactions which, in turn, leads to an increased contact area between the casein particles (Lucey, 2009). Higher incubation temperatures (i.e., higher gelation pH) also make the gel network more prone to rearrangements during gelation, and these changes can lead to greater whey separation (Lucey, 2009; Lee & Lucey, 2004a).

2.6.3 Effects of heat treatment on the formation of acid milk gels

With the exception of proteose-peptone, whey proteins are very sensitive to heat treatment. Unlike caseins, whey proteins have three-dimensional structures or configurations. Each configuration is stabilized by hydrogen and hydrophobic bonds, and other forces. Secondary and tertiary structures of whey proteins tend to be broken down by heat treatment because heating weakens hydrogen and hydrophobic bonds (Özer, 2009). Denaturation of whey proteins occur above 60^{o}C. At temperatures up to 90^{o}C, unfolding of the protein is rate-limiting but further increases in the heating temperature result in only small increases in the rate of denaturation as aggregation of the proteins becomes rate-limiting (Augustin & Udabage, 2007). Below 65°C, at least in theory, denaturation or functional changes of whey proteins (mainly β-lactoglobulin) are

reversible, but above 70°C irreversible functional changes in whey proteins occur (Özer, 2009).

The most abundant whey protein is β-lactoglobulin in which a heat-induced conformational change results in the exposure of a reactive thiol group (**Figure 2-3**). This thiol group can form disulfide bonds with other cysteine-containing proteins, such as β-lactoglobulin or bovine serum albumin, or with proteins having disulfide bridges, such as α-lactalbumin, κ- and αs_2-casein. The latter process occurs through thiol group-disulfide bridge exchange reactions, resembling a polymerization process in which heat-denatured β-lactoglobulin is the initiator. Interaction of β-lactoglobulin with κ-casein, present at the exterior of the casein micelle, leads to coating of the casein micelles with β-lactoglobulin. Interactions of β-lactoglobulin with cysteine-containing serum caseins might lead to casein-whey protein aggregates. Additionally, interactions of β-lactoglobulin with cysteine-containing whey proteins, such as α-lactalbumin and β-lactoglobulin molecules, result in the formation of whey protein aggregates (Vasbinder *et al*., 2003a). Hydrogen bonding and electrostatic and hydrophobic interactions have also been suggested as major forces in whey protein aggregation (Britten & Giroux, 2001). To summarize, heat treatment of milk results in a complex mixture of native whey proteins and denatured whey proteins present as whey protein aggregates, casein-whey protein aggregates and whey protein coated casein micelles (Vasbinder *et al*., 2003a). The association of denatured whey proteins to casein micelles significantly increases the casein micelle size (Anema & Li, 2003; Remeuf *et al*., 2003). According to Pesic *et al.* (2012), after exposing bovine milk to a severe heat treatment (90^{o}C; 10 minutes) at natural pH (6.71), about 30% of denatured whey proteins were involved in soluble complexes. **Figure 2-4** shows a schematic representation of the effects of heat treatment and subsequent acidification on casein micelles and whey proteins present in skim milk.

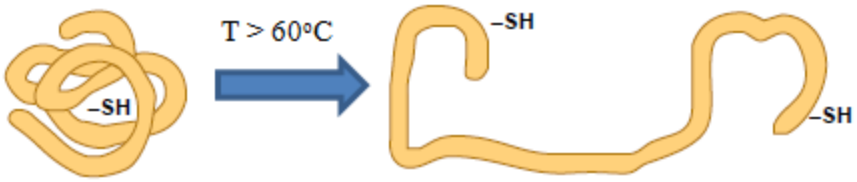

Figure 2-3: Schematic representation of β-lactoglobulin denaturation: breakage of its tertiary structure and exposure of thiol groups

Source: Adapted from Bylund (1995).

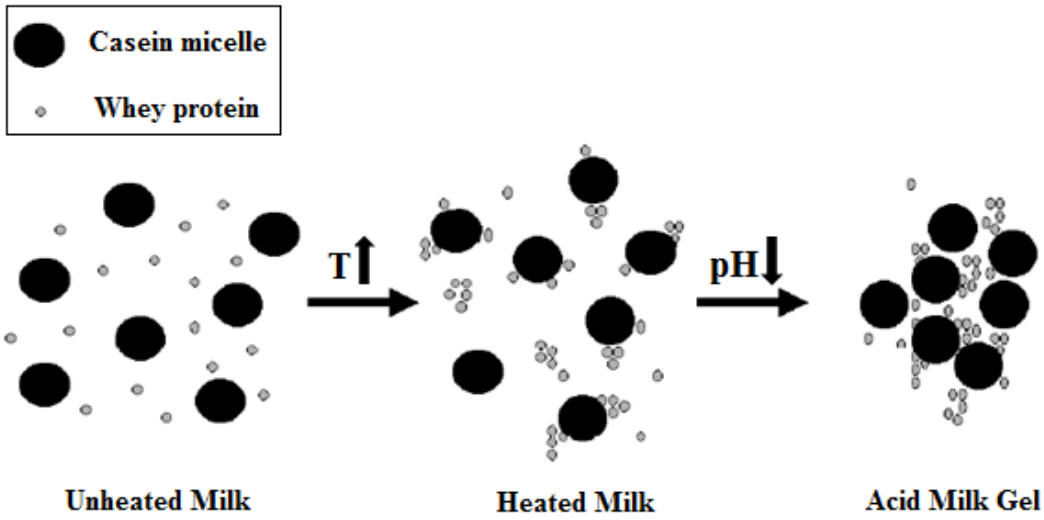

Figure 2-4: Schematic representation of the heating of skim milk and the subsequent acidification resulting in the formation of a protein network

Source: Adapted from Vasbinder *et al.* (2003b).

The extent and rate of denaturation of whey proteins are determined by a number of factors. Amongst these are the pH value, the ionic strength and the ionic composition, the protein concentration and casein to whey protein ratio of the heat treated whey protein solution, and the duration and temperature of the heat treatment (Kessler & Beyer, 1991).

Increasing the pH above the natural pH of milk markedly accelerates the rate of denaturation of β-lactoglobulin. Generally a decrease in the pH of milk systems prior to heating results in an increased association between the denatured whey proteins and the casein micelle. Even small changes in pH can shift the distribution of the association of the denatured whey proteins with the casein micelle. For example, at a level of 95% whey

protein denaturation, approximately 70% of denatured whey proteins are associated with the casein micelle at pH 6.55. This decrease to approximately 30% when the pH of milk prior to heating is 6.7. The difference in association level is reflected in the increase in the casein micelle size when milk is heated at the lower pH (Augustin & Udabage, 2007). According to Vasbinder *et al.* (2003a), the denatured whey protein aggregates that form contain a ratio of α-lactalbumin to β-lactoglobulin which is representative of the ratio of total denatured whey proteins in milk. α-lactalbumin is more easily incorporated in aggregates than it is involved in coating of micelles, while the whey protein coating of the casein micelles clearly contains more β-lactoglobulin. Vasbinder *et al*. (2003c) stated that at high pH, β-lactoglobulin-β-lactoglobulin interactions causing whey protein aggregates are favoured over κ-casein-β-lactoglobulin interactions, while κ-casein-β-lactoglobulin-β-lactoglobulin reactions hardly take place. At lower pH, formation of separate whey protein aggregates hardly occurs, but clusters of whey proteins are formed on the surface of the casein micelle. Apparently, at these conditions κ-casein-(β-lactoglobulin)$_n$ interactions are favoured over κ-casein-β-lactoglobulin interactions. **Figure 2-5** summarizes the different interactions that take place between casein micelles and denatured whey proteins when different pH mediums are considered prior to heating.

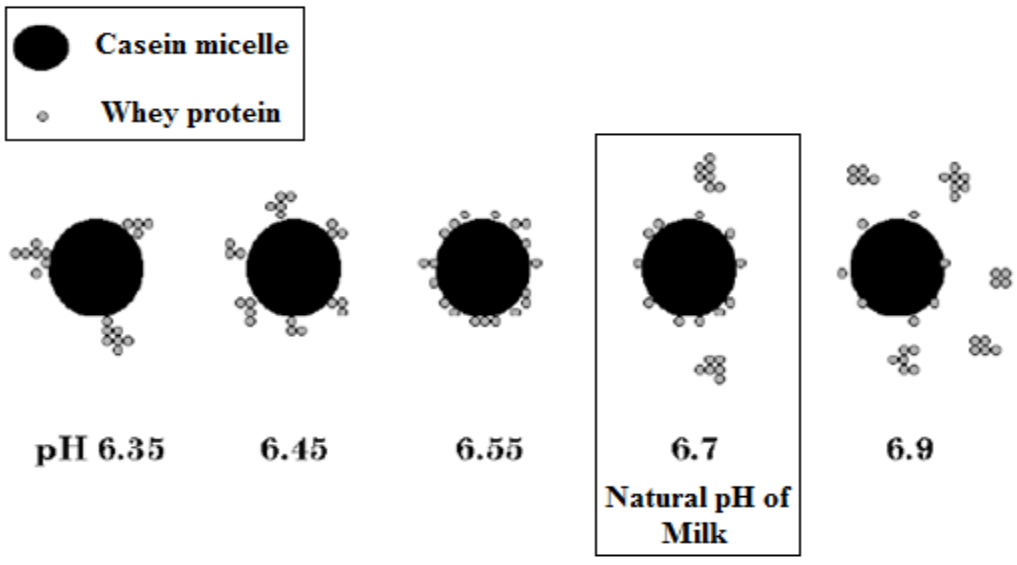

Figure 2-5: A schematic representation of the interactions between casein micelles and whey proteins occurring in milk during heat treatment for 10 min at 80^{o}C at pH values ranging from 6.35 to 6.9

Native whey proteins are not included in the figure. *Source:* Adapted from Vasbinder *et al.* (2003c).

Anema (2008) explains this phenomenon by stating that as the pH of the milk is increased from about pH 6.5 to pH 7.1 before heating, κ-casein progressively dissociates from the casein micelles so that, at pH 6.5, the majority of the κ-casein is associated with the casein micelles, whereas at pH 7.1, about 60–70% of the κ-casein is found in the milk serum. As the denatured whey proteins interact with the casein micelles via disulfide bonding with the κ-casein, this dissociation of κ-casein probably explains why the association of the whey proteins with the casein micelles is pH-dependent.

It is important to note that a more severe heat treatment at a constant pH will cause more denaturation of whey proteins, but the ratio of denatured whey proteins associated with the casein micelle and present in aggregates will remain constant (Vasbinder *et al.*, 2003a).

Heat-induced interactions of casein micelles and whey proteins are also affected by the casein to whey protein ratio of the milk base. It is believed that κ-casein presents limited number of available binding sites for β-lactoglobulin association. Thus, when these sites are saturated, denatured whey proteins will interact with each other, increasing the amounts of whey protein aggregates in the system. According to Cho *et al*. (2003), after a heat treatment at pH 6.7, a maximum number of disulfide bonds between κ-casein and whey proteins is formed when using a casein to whey protein ratio of 4:1 (Gunasekaran & Solar, 2012).

Calcium ions promote the association of β-lactoglobulin with casein micelles, perhaps due to the ability of ions to influence the degree of electrostatic attraction or repulsion between β-lactoglobulin and κ-casein by providing an ionic environment around the interacting molecules. Additionally, salts could be affecting the reactivity of thiol groups. Furthermore, lactose concentration is a limiting factor for the whey protein denaturation. The glucosyl residues are bound to β-lactoglobulin via gluconic acid or melibionic acid, making this whey protein fraction stable against heat treatment. Lactose concentrations of milk with normal chemical composition do not have any negative effect on the rate of whey protein denaturation. However, if the lactose level of yogurt milk is increased during standardization, the rate of whey protein denaturation is likely reduced. In order to overcome this handicap, milk should exposed to a higher heat treatment (at >90°C for 10–15 min) (Özer, 2009).

The association of denatured whey proteins with micellar caseins on heating gives improved yogurt texture and gel strength (Law, 1996). On the other hand, it is thought that native whey proteins do not interact with casein micelles during the

acidification of unheated milk and act as a destructive filler, or a structure breaker, in acid milk gels (Sodini *et al*., 2004). Lee & Lucey (2004b) reported that yogurt gels made from milk heated at high temperatures (>80°C) presented a higher cross-linked and branched protein structure with smaller pores than gels made from milk heated at low temperatures (Lee & Lucey, 2010). This branched microstructure increases the elasticity, gel strength and water binding capacity of the final gel (Lucey *et al.*, 1999; Lucey *et al.*, 1998a; Kessler & Beyer, 1991; Lee & Lucey, 2004b; Lucey & Singh, 1998). Therefore, yogurts produced from heated milks will have greater firmness and lower susceptibility to syneresis. According to Sodini *et al*., (2004), a heating that ensures 60 to 90% of β-lactoglobulin denaturation generally optimizes both the WHC and the rheological properties of the final gel. On the other hand, a too severe heating generally (above 90% β-lactoglobulin denaturation) has a slightly detrimental effect on yogurt's physical properties.

2.7 Important factors that define yogurt quality

Two of the most important parameters that define yogurt quality and determine consumer acceptance are, unquestionably, the textural attributes and the WHC of the gel network (Abu-Jdayil *et al*., 2000; Abu-Jdayil *et al*., 2002; Lee & Lucey, 2006; Sodini *et al*., 2004; Lucey, 2002; Lucey *et al*., 1998b; Lee & Lucey, 2010; Lucey & Singh, 1998).

2.7.1 Rheology

Textural attributes, including the desired oral viscosity, are very important criteria that determine the identity, quality and consumer acceptance of yogurt (Lee & Lucey, 2006; Abu-Jdayil *et al*., 2000). Although texture is related to the sensory perception of a food product, rheology and structure of a product evaluated by instrumental methods also provide relevant information on its textural properties (Sodini *et al*., 2004). Skriver *et al.* (1999), Richardson *et al.* (1989), and Stanley & Taylor (1993) reported that sensory texture analyses are highly correlated with the rheological properties of stirred yogurt and other semi-solid foods. Due to this fact, rheological properties of milk gels are important physical attributes which contribute to the overall sensory perception and functionality of these products (Lucey, 2002).

Yogurt is defined as a weak viscoelastic gel system which is unable to keep its structural integrity during high shear (Özer *et al*., 1997, 1998b; Lee & Lucey, 2010). Several authors reported the advantages of using oscillatory dynamic tests over other destructive rheological techniques (e.g., penetrometer, rotational viscometers) to evaluate the rheological characteristics of viscoelastic semisolid foods (Özer *et al*., 1997; Özer *et al*, 1998b; Özer *et al*, 1999a; Bylund, 1995; Lee & Lucey, 2010; Gunasekaran & Ak, 2000). The principal advantage of dynamic tests is that they enable measurements to be made without incurring structural damage to the samples. Therefore, this type of tests can be used to relate dynamic rheological parameters to molecular structures (Gunasekaran & Ak, 2000). On the other hand, each penetration into or rotation in a gel network causes a breakdown in the elastically effective bonds, and the procedure thus fails to measure the actual physical characteristics of the gel. Once the gel structure is disturbed, it is rarely possible to re-form the gel structure in the same way, because yogurt is a metastable gel and any change in its enthalpic/entropic nature creates irreversible deformation. Thus, any kind of destructive effect may lead to atypical physical properties in the yogurt, and provide erroneous results. Due to this fact, dynamic studies are much more reliable than destructive rheological techniques for studying the physical properties of concentrate yogurt (Özer *et al*., 1997). Consequently, during the last decades, dynamic tests have been widely used to investigate the rheological aspects of acid milk gels (Damin *et al*., 2009; Marafon *et al.* 2011a,b; Wu *et al*., 2009; Oliveira *et al*., 2001; Krzeminski *et al*., 2011; Sodini *et al*., 2005**;** Sodini *et al*., 2006; Remeuf *et al*., 2003; Ozcan *et al*., 2011; Peng *et al*., 2009; Vlahopoulou *et al*., 2001; Vlahopoulou & Bell, 1993; Lee & Lucey, 2004a,b; Lucey *et al*.,1998a; Puvanenthiran *et al*., 2002; Lucey *et al*., 1999**;** Lucey *et al*., 1997a,b; Cho *et al*., 1999).

In the present study, small amplitude oscillatory tests have been used to compare the rheological aspects of experimental and commercial samples of concentrate yogurt. Small deformation is defined as a small relative deformation which, when applied, does not disrupt the gel network structure, i.e., within the linear viscoelastic region. This type of test involves applying an oscillatory (sinusoidal) stress or strain to the material and measuring the strain or stress responses (Lee & Lucey, 2010). The magnitude and phase shift of the transmission depend on the material's viscoelastic nature. Much of the stress is transmitted in highly elastic materials while it is dissipated in frictional losses in highly viscous ones. The phase shift is large for highly viscous materials but small for highly elastic materials (Sahin & Sumnu, 2006).

Several rheological parameters are determined in a small amplitude oscillatory rheology test. The storage modulus (G') expresses the magnitude of the energy that is stored in the material or recoverable per cycle of deformation (indicates the solid-like properties). The loss modulus (G") is a measure of the energy which is lost as viscous dissipation per cycle of deformation (reflects the liquid-like properties). Therefore, for a perfectly elastic solid, all the energy is stored, that is, G" is zero and the stress and the strain will be in phase. In contrast, for a liquid with no elastic properties, all the energy is dissipated as heat, that is, G' is zero and the stress and the strain will be out of phase by 90°. For a specific food, magnitudes of G' and G" are influenced by frequency, temperature, and strain. For strain values within the linear range of deformation, G' and G" are independent of strain. The loss tangent (tan δ) is the ratio of the energy dissipated to that stored per cycle of deformation and indicates the type of viscoelastic properties in a material. A high tan δ value (i.e., G'' >G') means that the material has liquid-like behavior (Rao, 2007; Lee & Lucey, 2010). These parameters are defined as follows:

$$G' = \left[\frac{\sigma_0}{\gamma_0}\right] \cos\delta \qquad \text{[Eq. 2-2]}$$

$$G'' = \left[\frac{\sigma_0}{\gamma_0}\right] \sin\delta \qquad \text{[Eq. 2-3]}$$

$$\tan\delta = \left[\frac{G''}{G'}\right] \qquad \text{[Eq. 2-4]}$$

where σ_0 is the amplitude of the share stress, γ_0 is the amplitude of the strain and δ is the phase angle difference between the stress and the strain (Rao, 2007; Lucey & Singh, 1998; Roefs *et al*., 1990). In acid milk gels, the G' is determined by the number and/or strength of non-relaxing protein bonds (covalent bonds), whereas the G'' is determined by rapidly relaxing bonds (non-covalent bonds) (Özer *et al*., 1998b). The G' and G'' are similarly related to the spatial distribution and the number of protein-protein bonds, which, therefore, suggests that tan δ is related to the nature of the protein bonds (Özer *et al*., 1999a).

2.7.2 Whey separation

Whey separation, i.e., the appearance of whey on the surface of a milk gel, is a common defect in fermented milk products such as yogurt (Lucey & Singh, 1998; Lucey *et al*., 1998b). Whey separation negatively affects consumer perceptions of yogurt, as consumers think there is something microbiologically wrong with the product (Lee & Lucey, 2010). Due to this fact, manufacturers try to prevent whey separation by increasing the total solids content of milk, subjecting the milk to a severe heat treatment (to increase whey protein denaturation) or by adding stabilizers such as gelatin, pectin, starches, or gums (Lucey, 2002).

Spontaneous syneresis is the usual cause of whey separation (Lee & Lucey, 2010). Syneresis is defined as shrinkage of a gel and this occurs concomitantly with expulsion of liquid or whey separation. Spontaneous syneresis is contraction of a gel without the application of any external forces (e.g., centrifugation) and is related to instability of the gel network (i.e., large scale rearrangements) resulting in the loss of the ability to entrap all the serum phase (Lucey *et al*., 1998b). Hence, excessive rearrangements of particles in the gel network are responsible for high levels of whey separation (Lucey, 2001). Previous studies showed that several manufacturing conditions, such as low total solids content (protein content) of the mix, very low acid production ($pH \geq 4.8$), excessive heat treatment of the mix, and very high incubation temperatures, promote whey separation (Lucey & Singh, 1998; Lucey *et al*., 2001).

Whey separation is intimately related to the gel network's microstructure. Extensive rearrangements of protein particles in the gel network may be associated with increased local breakage of weak protein strands that make up the junctions in the network. This may result in the formation of weak spots and a less stable gel network (Lee & Lucey, 2006). Several authors reported that a high number of relaxing (non-covalent) protein bonds present in the gel favor rearrangements in the network and results in greater whey separation (van Vliet *et al*., 1991; Lucey, 2001; Lee & Lucey, 2004a; Lee & Lucey, 2004b). As the number of non-relaxing (covalent) protein bonds increases, the level of rearrangements in the gel network decreases and a lower level of whey separation is obtained (Lucey, 2001; Lee & Lucey, 2004a; Lee & Lucey, 2004b; Weidendorfer *et al*., 2008). Hence, high tan δ values together with low G' values can be correlated with high levels of whey separation (Lucey, 2001). On the other hand, whey separation is also related to the permeability of the gel network. Finer networks with a higher level of

cross-links and smaller pores will have less of a tendency to exhibit whey drainage under the force of gravity than coarser, more open structures (Puvanenthiran *et al*., 2002).

2.7.3 Clusters formation

Undesired clusters can have a negative effect on a yogurt's texture. Numerous manufacturing parameters, such as severe heat treatments, excessive whey protein to casein ratios, high incubation temperatures, certain types of starter cultures and the use of excessive amounts of starter culture, are associated with textural defects of stirred yogurt like graininess (particles) or surface roughness (irregularities in the yogurt matrix) (Kucukcetin, 2008; Sodini *et al*., 2004). Remeuf *et al*. (2003) reported that graininess can be related to an increase in the casein micelles size caused by the interaction of micelles with denatured whey proteins. Puvanenthiran *et al.* (2002) associated the observed granny texture with the formation of big whey protein aggregates.

Although manufacturing parameters have a direct influence on the formation of clusters, according to Lee & Lucey (2010), stirred yogurts are likely to have clusters of protein aggregates which are presumably created by the collisions and shearing during the mixing process involved in their production.

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3. Development of the novel Greek-style yogurt powder formulation free of additives and fat

3.1 Introduction

Greek-style yogurt (strained yogurt) is a semisolid dairy product obtained by removing part of the whey from regular yogurt (Yazici & Akgun, 2004; Mihyar *et al*., 1999). As a result of this action, the total solids content and lactic acid concentration of the initial yogurt are increased (concentrated yogurt typically contains 22-23g/100g total solids and has an acidity of around 1.60-1.80g/100g lactic acid), giving the final product a much thicker consistency and a distinctive, slightly tangy taste (Yıldız, 2009; Robinson, 2002). In addition, the product obtained has nutritional properties superior to those of regular yogurt, with higher protein and mineral contents and very low lactose content. It also has better keeping qualities due to the increased lactic acid concentration (Mahdian & Tehrani 2007; Nsabimana *et al*., 2005; Salji, 1991).

Health benefits associated with yogurt cultures and probiotics led to a sharp increase in the per capita consumption of yogurt in Canada and the United States during the last decades (Agriculture and Agri-Food Canada, 2005; USDA, 2001). According to Chandan (2008), yogurt sales in the U.S. have been spectacular, increasing from 1,837 million pounds in the year 2000 to 2,990 million pounds in 2005, and they continue to show remarkable growth. Palmer & Sakan (2011) affirmed that over a 52-week period (ending February 19, 2011), overall yogurt category sales increased 12% year after year. Of those sales, 85% was driven by a 146% increase in Greek-style yogurt sales, while a 2% increase in traditional yogurt sales accounted for only 15% of category growth.

To take advantage of the current remarkable economic growth of Greek-style yogurt, this investigation aims to develop an efficient formulation for the production of strained yogurt powder. It is believed that a dried type of concentrated yogurt will help to expand the economic boom of Greek-style yogurt in areas that have a limited indigenous dairy industry, or regions that suffer from seasonal deficiencies in milk supply (Kneifel,1993).Thus, this type of product is intended to open new markets to this highly valuable food commodity.

The pronounced economic growth of Greek-style yogurt has led to a noticeable diversification of the traditional product. Many mechanized systems based on modern

techniques, such as membrane processes, centrifugation, and direct reconstitution, have been developed to manufacture strained yogurt in large volumes (Özer & Robinson, 1999; Tamime & Robinson, 1999; Tamime *et al.*, 1991). Because the overall characteristics of concentrated yogurt depend on the method of production, the use of different manufacturing methods has led to the production of diverse varieties of commercial Greek-style yogurt which significantly differ in their composition (Abu-Jdayil *et al.*, 2002). Tamime (2003) and Tamime & Robinson (2007) have reported about the difference in composition of various types of commercial concentrated yogurt that exist around the world. In order to respond to the increasing consumer preference for reduced fat and additive-free products, the current study will emphasize the production of a non-fat, additive-free type of dried, concentrated yogurt (Gould *et al.*, 1994; Institute of Food Technologists, 2011).

Regardless of the production method and composition of the final product, one of the major concerns facing the Greek-style yogurt industry is the production and maintenance of a product with optimum consistency, stability and texture properties (Abu-Jdayil *et al*., 2000). The overall visual appearance, microstructure, and rheological properties of acid milk gels are important physical attributes which contribute to the overall sensory perception and functionality of these products (Lucey, 2002). Textural attributes, including the desired oral viscosity, are very important criteria for quality and for consumer acceptance of yogurt (Lee & Lucey, 2006). Skriver *et al.* (1999), Richardson *et al*. (1989), and Stanley & Taylor (1993) reported that sensory texture analyses are highly correlated with the rheological properties of stirred yogurt and other semi-solid foods. Due to this fact, when proposing a new formulation for producing a dried type of Greek-style yogurt, it is critical to evaluate the final product's rheological properties t. Thus, the objective of this experiment was to design an effective formulation for the production of a recombined, non-fat, additive-free type of acid milk gel with rheological and physicochemical attributes similar to those of plain, Greek-style yogurts (0% M.F.) commercialized in Edmonton, AB, Canada.

3.2 Materials & methods

3.2.1 Milk Powders

Four types of milk ingredients, in powder form, were used. Milk protein concentrate (MPC-85), whey protein isolate (WPI-90), and milk permeate powder (MPP) were provided by Vitalus Nutrition Inc., Vancouver, Canada. Sodium caseinate (NaCN) was purchased from the American Casein Company, Burlington, U.S. All powders were stored at 5^{o}C until used in experiments. The composition of these powders is shown in **Table 3-1**.

Table 3-1: Composition of milk powders used in this study [¥]

Component	MPC-85	WPI-90	NaCN	MPP
Protein[†] (% min.)	85.0	90.0	88.0	3.3
Lactose (% max.)	7.0	3.0	1.0	85.5
Moisture (% max.)	5.5	5.0	6.0	3.1
Fat (% max.)	4.0	1.0	1.8	0.1
Ash (% max.)	8.0	3.5	4.2	9.3

[¥] Specifications obtained from the manufacturers. [†] On wet basis.

3.2.2 Microbial Cultures

A commercial, concentrated, freeze-dried starter culture (SC) (i.e., direct-to-vat inoculation), YO-MIX™ 215 LYO (Danlac Canada Inc., AB, Canada), was used to ferment the recombined milk. The starter was a thermophilic multiple-species culture composed of *Streptococcus thermophilus, Lactobacillus delbrueckii subsp. lactis, Lactobacillus acidophilus and Bifidobacterium lactis*. For the production of stirred yogurt, the manufacturer recommends using this culture at a rate of 10-50 direct culture units (DCU) 100L^{-1} and at an incubation temperature of 42^{o}C. According to the producer's recommendations, the starter culture was stored at -25^{o}C. The starter culture was defrosted at room temperature before use.

3.2.3 Market reference samples

All types of commercial, plain Greek-style yogurts (0% M.F.) produced from natural dairy ingredients without added preservatives, emulsifiers or stabilizers (according to their labeling), that were commercialized in Edmonton by three supermarket chains (Walmart, Superstore, and Safeway) during September-December 2011 were used as reference samples in this study. Two products that met these requirements were labeled as "A" and "B". Reference samples were purchased between day 10 and 17 after their production and were stored at 5^{o}C until completion of analyses. Specifications of these samples are shown in **Table 3-2**.

Table 3-2: Specifications of the market reference samples used in this study

	A	B
Ingredients[¥]	Skim milk, live active cultures	Skim milk, live active cultures
Production method[†]	Traditional/Stirred	Traditional/Stirred
Shelf life (Days)[†]	35	35
Fat content (%)[¥]	0.0	0.0
Protein content (%)[¥]	10.3	10.3
Carbohydrates content (%)[¥]	3.4	6.9
Total Solids content (%)[§]	13.7	17.1

[¥] Specifications obtained from products labels on September 2011. [†] Specifications obtained from customer services on September 2011. [§] Total solids content was calculated based on the carbohydrates, fat and protein contents.

3.2.4 Yogurt manufacturing

Experimental samples were produced in batch mode using direct recombination technology. Milli-Q water (de-ionized and distilled water) was used to recombine milk powders. All reconstituted milks had the same amount of total solids (TS) and total protein (TP), 13.7% and 10.3%, respectively. Samples differed in their total protein composition, casein to whey protein ratios and starter culture contents. MPC-85, WPI-90 and NaCN were used as protein sources and MPP was used to standardize the concentration of total solids in the different samples. Starter culture was used at a rate of 10 to 50 DCU $100L^{-1}$, according to the type of sample produced. A mass of 900 grams of recombined and inoculated milk was produced per batch of production but only 500 grams of this milk was incubated at $42 \pm 1^{o}C$ until the desired acidity was reached. This was done in order to reduce experimental errors caused by mass loses during the recombination process. Samples were produced by two different methods. **Figure 3-1** illustrates in detail the different manufacturing procedures applied.

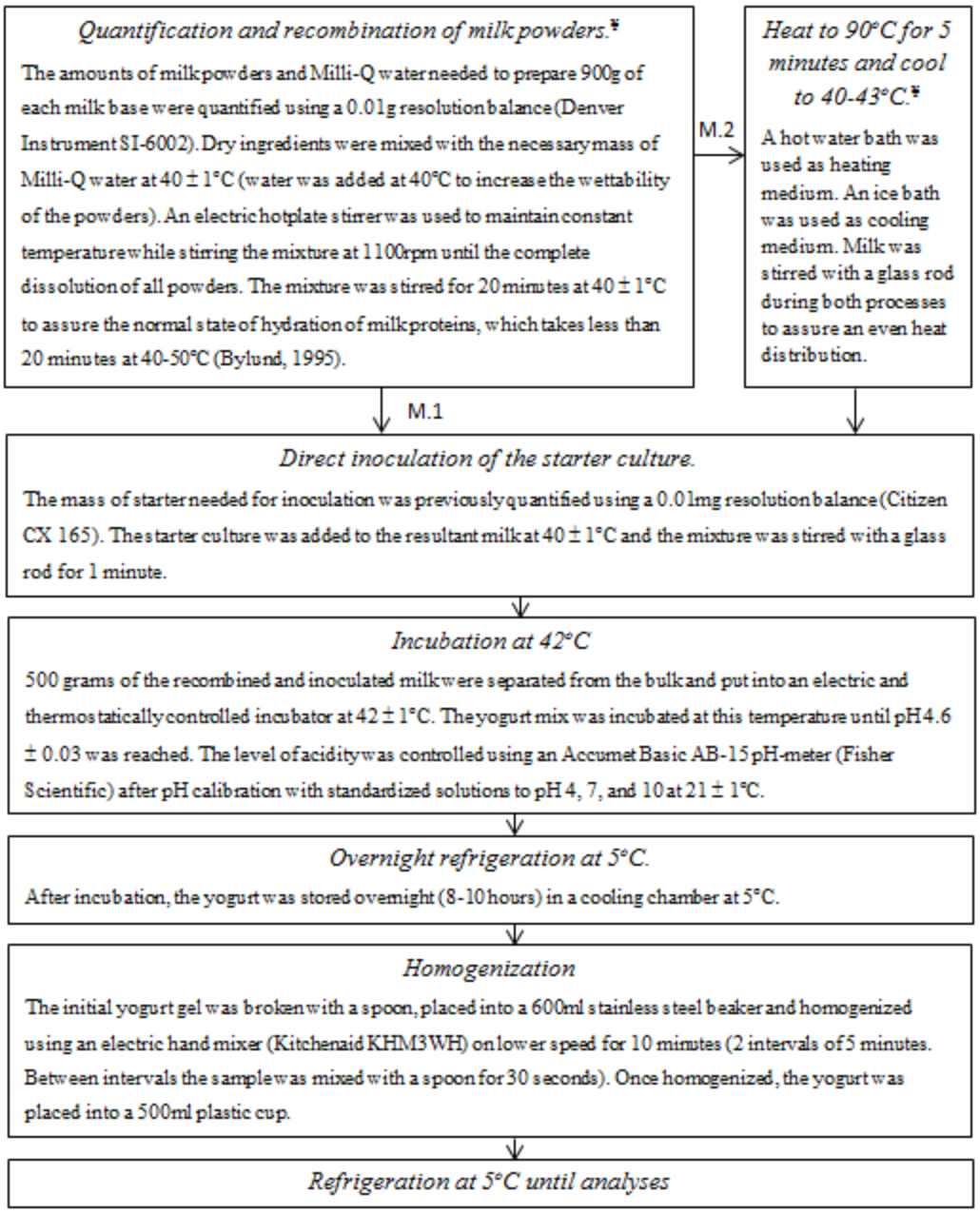


Figure 3-1: Methods of production considered for the manufacture of experimental samples

¥ The average mass losses caused by powders losses or water evaporation during these two steps represented less than 2% of the total mass of the final recombined milk.

M1: Production method 1; M2: Production method 2.

3.2.5 Dynamic rheological measurements

Small amplitude oscillatory rheology (SAOR) tests were performed with a Paar Physica UDS200 MCR Rheometer. The evaluation method was adapted from Özer *et al.* (1997, 1998a, 1998b, 1999a).The rheometer was set up with a parallel-plate geometry (10 mm plate radius and 1 mm gap setting). All samples were gently stirred with a spoon for 30 seconds before measurement in order to mix the potential free whey with the resultant gel. Each sample was loaded into the rheometer and allowed to relax and equilibrate to measuring temperature ($25 \pm 0.1^{o}C$) for 2 minutes prior to testing. The temperature of the samples inside the rheometer was maintained by a circulating cooling system. Rheological aspects of all samples were evaluated by conducting stress amplitude sweep tests. A sweeping amplitude from $1.5x10^{-2}$ to $1.5x10^{-1}$ mNm at 0.25 Hz was used and 25 measuring points were performed through the sweeping range. Storage (G') and loss (G'') moduli were recorded. Two replications were conducted for each sample.

3.2.6 Whey separation measurements

I. Surface whey-off (SWO).

The method used to quantify the amount of free whey present on top of the resultant gel was adapted from Lucey *et al.* (1998a). Experimental samples were evaluated before and after applying homogenization during their production. Any free whey expelled on top or around the sides of the gel was gently sucked with a polyethylene transfer pipet and weighted. Once all the free whey was sucked from the surface, the gel was allowed to rest for 1 minute and any further surface whey was sucked and weighted. The degree of whey separation was expressed as a percentage of the total sample weight (% m/m). After quantification, free-whey was reintroduced into the samples.

II. Whey drainage (WD).

To evaluate the degree of whey drainage present in the final samples, the resultant gels were broken with a spoon and the level of whey drainage was quantified using an ordinal numerical scale from 0 (no visible whey drainage) to 2 (high amount of whey drainage). **Figure 3-2** illustrates the different levels that were used to classify the samples according to whey drainage.

Level 0 *Level 1* *Level 2*

Figure 3-2: Ordinal levels used for the classification of samples according to the degree of whey drainage

3.2.7 Presence and size of visible clusters

An ordinal numerical scale from 0 (no visible clusters) to 3 (big visible clusters) was used in order to evaluate the presence and size of visible clusters in the final products. All samples were gently stirred with a spoon for 30 seconds before the evaluation in order to mix the potential free whey with the resultant gel. **Figure 3-3** shows the different levels that were used to classify the samples according to the presence and size of the clusters.

Level 1 *Level 2* *Level 3*

Figure 3-3: Ordinal levels used for the classification of samples according to the presence and size of visible clusters

Level 0 could not be assigned to any of the evaluated samples. All samples presented visible clusters.

3.2.8 Incubation time measurements

The incubation time of each sample was registered using a chronometer. The stopwatch was turned on when the samples were put inside the incubator and stopped when the desired level of acidity (pH = 4.60 ± 0.03) was reached.

3.2.9 Reconstituted milk density measurements

Density measurements were carried out in order to calculate the exact amount of SC that was needed to inoculate 900g of the reconstituted milk. A volume of 100 ml of the reconstituted milk was measured using a previously weighed PIREX volumetric flask 100 ± 0.08 ml [TC = 20^{o}C] (Manufacture No.: 5660). The mass of the volumetric flask containing 100 ml of milk was registered using a 0.01mg resolution balance (Citizen CX 165) at 20 ± 1^{o}C. Triplicate measurements and two replications were used. The density of the reconstituted milk was calculated using the following equation:

$$Density\ of\ milk = \frac{Milk\ with\ vol.flask\ weight\ (g) - Empty\ vol.flask\ weight(g)}{100\ ml} \quad \text{[Eq. 3-1]}$$

3.2.10 Experimental design and statistical analysis

The market reference samples were evaluated for their rheological and physicochemical properties at day 18 and 35 after their production. All measurements were carried out in triplicate. According to the data collected from these analyses, a mean reference value and a two-sided confidence interval ($\alpha = 0.05$) was established for each parameter tested. Reference confidence intervals at $P < 0.05$ were used for comparisons with experimental data.

Experimental samples were produced using two manufacturing methods (See **Figure 3-1**). A one-block full factorial design 3*2 was used to investigate the effect of NaCN, WPI-90 and SC concentrations on the rheological and physicochemical properties of recombined yogurt gels obtained by each manufacturing method. **Table 3-3** illustrates the different factors (3) and levels (2) considered in the experiment. **Table 3-4** shows the composition of the different samples used in this experiment according to the combination of factors and levels detailed in **Table 3-3**.

Table 3-3: Factors and levels considered to produce experimental samples

Factors	Low Level	High Level
WPI-90 Content (% of TP)	0	15
NaCN Content (% of TP)	0	25
SC Content (DCU/100L)	10	50

Table 3-4: Composition of experimental samples according to the different factors and levels considered in the experimental design

Sample No.	WPI-90 (% of TP)	NaCN (% of TP)	SC (DCU $100L^{-1}$)	C:WP [¥]
1	0	0	10	4.6:1
2	15	0	10	2.2:1
3	0	25	10	6.6:1
4	15	25	10	2.8:1
5	0	0	50	4.6:1
6	15	0	50	2.2:1
7	0	25	50	6.6:1
8	15	25	50	2.8:1

[¥] C:WP = casein to whey protein ratio.

Yogurt samples were made in triplicate, resulting in a total of 48 batches (24 batches per production method). All measurements were carried out at day 1 after production.

Response surface methodology was applied, using Minitab 16 software (Inc., State College, PA, USA) version 16.1.1, in order to evaluate the effects of factors on the rheological and physicochemical parameters tested. Statistical analysis of data was performed using SPSS (Inc., Chicago, IL, U.S.) version 19.0. Significant means of main effects between different samples produced by the same method of production were differentiated by the Duncan test at $\alpha = 0.05$. Significant differences between the mean values of the same type of samples produced by different manufacturing methods were detected by independent samples T tests at $\alpha = 0.05$.

Based on the results, a final formulation with desired rheological and physicochemical properties was proposed and compared to the reference two-sided confidence intervals ($\alpha = 0.05$) to detect significant differences.

3.3 Results & discussion

3.3.1 Market reference samples

3.3.1.1 Dynamic rheological analyses

In particular, the weak viscoelastic nature of yogurt gel is well established and the rheological properties of yogurt can be explained by measuring its viscous (G'') and elastic (G') moduli (Özer *et al*., 1997). **Figure 3-4** shows the dynamic moduli (G' and G'') of the reference samples when applying a stress amplitude sweep test. Consistent with the data presented in **Figure 3-4**, it can be stated that Greek-style yogurt is a typical weak viscoelastic gel whose elastic properties predominate over its viscous properties over the measured range.

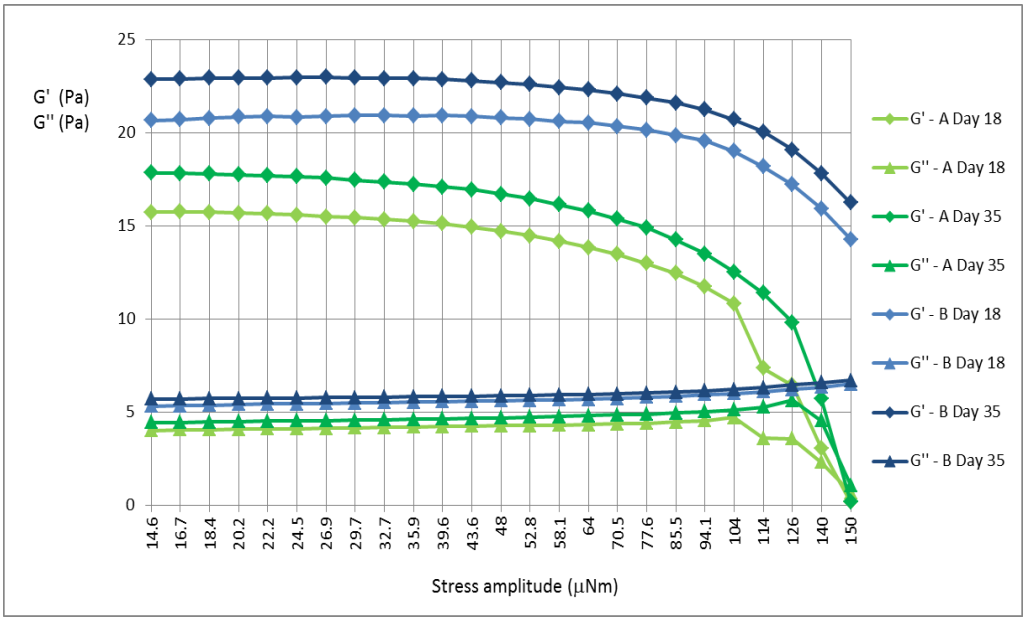


Figure 3-4: Storage (G') and loss (G'') modulus of reference samples at days 18 and 35 after their production

Presented values are the means of triplicate measurements. Appendix A (Tables A-2 to A-5) provides the experimental data used to construct this graph.

According to the previous figure, both fundamental dynamic parameters (G' and G'') showed a stress-amplitude dependence. Also, a linear viscoelastic region was evident for all samples. A structural degradation was observed in all samples at some point during the stress-amplitude range applied. These breakdown as a function of amplitude persisted during storage of the materials, suggesting that mechanical changes produced in the gels

during the manufacturing process were essentially permanent and nonreversible (Özer *et al*., 1998a; Lucey, 2002). These findings agreed with Özer *et al.* (1997) who stated that yogurt is a metastable gel and any change in its enthalpic/entropic nature creates irreversible deformation.

Although both types of reference samples had the same protein contents (**Table 3-2**), B samples had higher G' and G'' moduli than A samples. As a result of more protein-protein interactions at higher protein levels, a much denser and stronger gel structure can be expected; however, the rheological properties of yogurt are not only dependent on the protein content, but are also highly dependent on total solids content and on the type of protein present in the gel matrix (Özer *et al*., 1998a; Oliveira *et al*., 2001; Jumah *et al*., 2001a; Barretto Penna *et al*., 2006). B samples had a higher total solids content than A samples (**Table 3-2**); therefore, this might be the reason that B samples presented higher dynamic moduli than A samples. Even though the spatial distribution of the protein-protein bonds over the gel network, the strength of the interaction forces between protein molecules and the structure of the protein particles themselves also defined the mechanical properties of a gel network (Özer *et al*., 1999b; Bremer *et al.*, 1990).

Both reference samples presented a significant increase in their G' and G'' upon storage at 5^{o}C. This matches the findings of Marafon *et al.* (2011b), Serra *et al.* (2009) and Weidendorfer *et al.* (2008), who reported an increase in G' in stirred yogurts within storage. This fact suggests that casein gels are dynamic by nature and that further development of the gel structure occurs during storage (van Vliet *et al*., 1997). Özer *et al.* (1998b) affirms this point by stating that the number and/or strength of nonrelaxing and relaxing protein bonds in a protein gel matrix increases during storage. Upon storage, casein particles experience several large-scale rearrangements which result in the formation of new linkages to decrease the total free energy of the system and move to a more thermodynamically stable state (Lucey, 2002; van Vliet *et al*., 1997; Serra *et al.* 2009).

Figure 3-5 presents the loss tangent (tan δ = G''/G') values of the reference samples in days 18 and 35 after their production. Although B samples had higher G' and G'' values than A samples, within the linear viscoelastic region, the tan δ values of these two samples were similar.

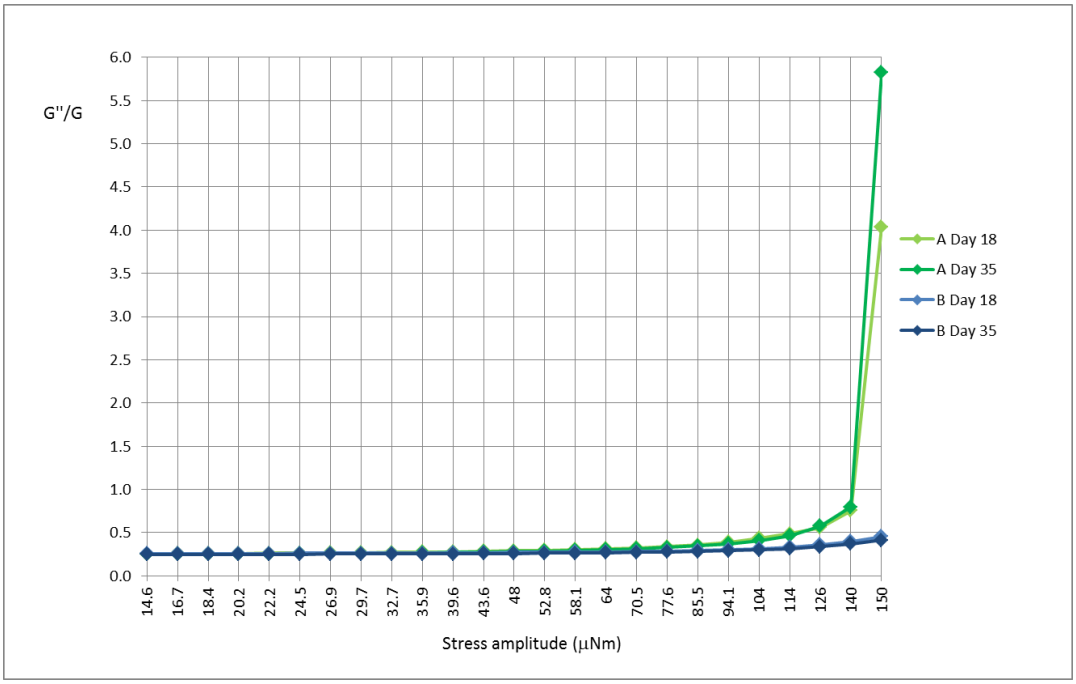


Figure 3-5: Loss tangent (tan δ = G''/G') values of reference samples at days 18 and 35 after their production

Presented values are the means of triplicate measurements. Appendix A (Tables A-2 to A-5) provides the experimental data used to construct this graph.

Loss tangent values are highly dependent on the nature of bonds between the particles integrating the gel network (Özer *et al*., 1997; Özer *et al*., 1998a; Özer *et al*., 1999a). Thus, it can be stated that at low amplitudes, the nature of bonds between the particles integrating both gels was similar. However, at high amplitudes, A samples presented a significant increase in their tan δ values due to the rupture of their gel structures, which resulted in a non-proportional decrease in the number and/or strength of non-relaxing protein bonds and relaxing bonds. After the breakage point, the number and/or strength of non-relaxing bonds declined more rapidly than the number and/or strength of relaxing bonds; therefore, G' decreased more pronouncedly than G'', indicating a partial breakdown of the elastic structure and a change to a relatively more viscous behavior. Due to this fact, at high amplitudes, A samples had a higher liquid-like behavior than B samples, and a significant difference in tan δ values was observed between both samples (Biliaderis, 2009; Lee & Lucey, 2010).

Even though the G' and G'' of both reference samples increased upon storage time (the number and/or strength of non-relaxing protein bonds and rapidly relaxing

bonds increase with storage time), the tan δ remained almost unchanged for each sample throughout the storage period, suggesting the formation of essentially similar network structures throughout storage time (Özer *et al*., 1998b). This fact led to a proportional increase in G' and G'' during storage; hence, the tan δ values (tan δ = G''/G') for the same types of samples, after 18 and 35 days of storage, were similar.

Figure 3-6 illustrates the mean G' of all the reference samples tested with the corresponding two-sided confidence interval limits (α=0.05) for the entire sweeping stress amplitude range considered. The mean tan δ of all the reference samples tested was also calculated and presented together with the corresponding two-sided confidence interval limits (α=0.05) in **Figure 3-7**.

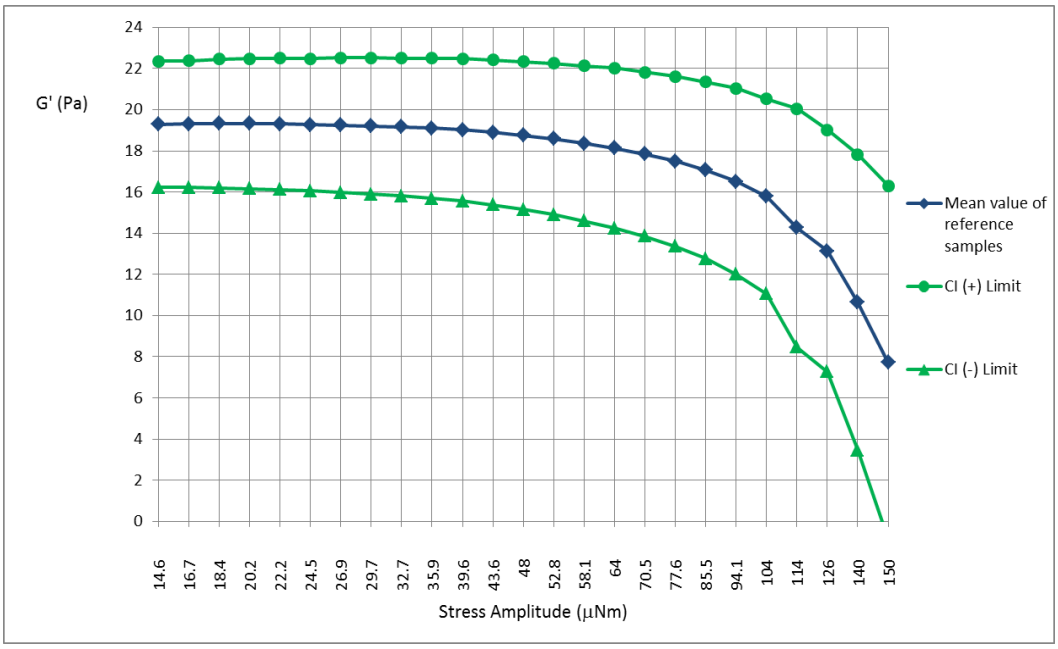


Figure 3-6: Mean storage modulus (G') of reference samples with the corresponding two-sided confidence interval limits (α = 0.05)

CI (+) Limit: Confidence Interval Positive Limit. CI (-) Limit: Confidence Interval Negative Limit. Data used to construct this graph can be found in Appendix A (Table A-6).

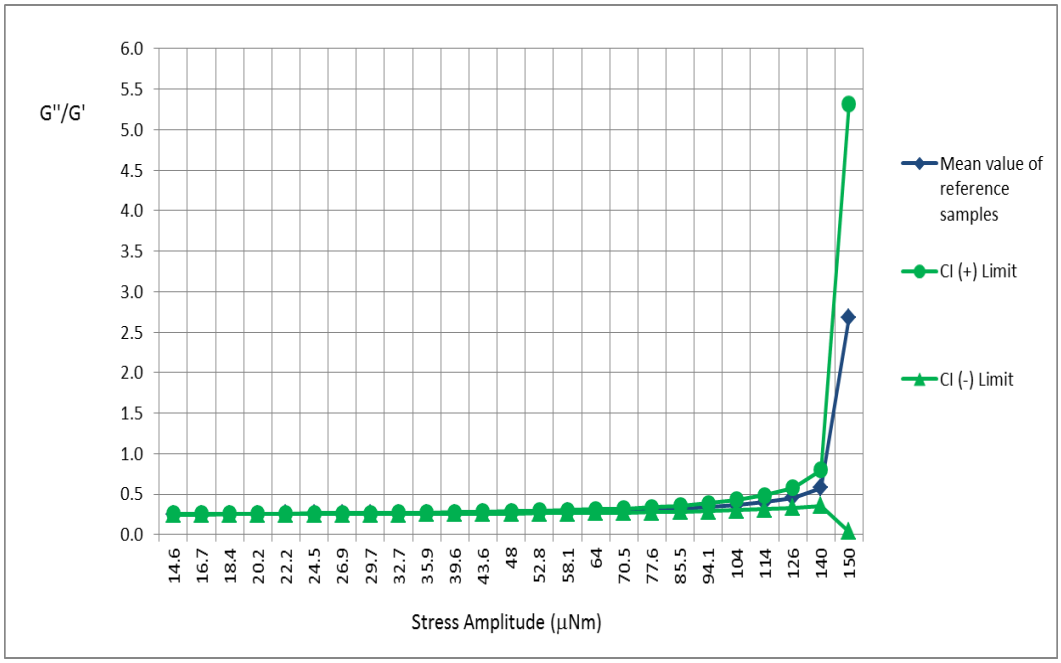


Figure 3-7: Mean loss tangent (tan δ = G''/G') of reference samples with the corresponding two-sided confidence interval limits (α = 0.05)

CI (+) Limit: Confidence Interval Positive Limit. CI (-) Limit: Confidence Interval Negative Limit. Data used to construct this graph can be found in Appendix A (Table A-7).

For comparative purposes, **Table 3-5** provides the two-sided confidence interval limits (α = 0.05) for the mean G' and tan δ values of the reference samples at points 1, 12 and 25 of the sweeping amplitude range applied. This table also presents the corresponding confidence interval limits (α = 0.05) for the mean SWO value of the reference samples and the mean values of the ordinal measurements (WD; presence and size of clusters) that were carried out on these samples.

Table 3-5: Rheological and physicochemical values of the market reference samples ¥

Parameters tested	Market Reference Values
G' (Pa) [P1: 14.6 μNm]†	22.358 – 16.226 [a]
G''/G' [P1: 14.6 μNm] †	0.257 – 0.249 [a]
G' (Pa) [P12: 43.6 μNm] †	22.418 – 15.374 [a]
G''/G' [P12: 43.6 μNm] †	0.283 – 0.260 [a]
G' (Pa) [P25: 150 μNm] †	16.304 – 0.000 [a]
G''/G' [P25: 150 μNm] †	5.324 – 0.040 [a]
SWO (%m/m) †	0.190 – 0.028 [a]
WD§	0.000 ± 0.000 [b]
Presence and size of clusters§	1.000 ± 0.000 [b]

¥ Data and calculations used to construct this table can be found in Appendix A (Tables A-1, A-6, A-7).
†Scale measures. §Ordinal measures. [a] 95% two-sided confidence intervals. [b] Mean values of reference samples ± SD.

As the level of WD and the size of visible clusters were measured using ordinal scales, confidence intervals could not be determined for this type of data. Therefore, the total mean values for these two measurements were considered as reference values for comparison with experimental data.

3.3.1.2 Physicochemical analyses

Table 3-6 shows the physicochemical results obtained from market reference samples at days 18 and 35 after their production. Samples did not differ in levels of WD and in the size of visible clusters. All tested samples presented significant levels of SWO. Both market samples presented higher amounts of SWO as storage time increased. Due to this fact, it can be stated that, during storage, large scale rearrangements occurred in the gel network which increased the level of instability of the gel, resulting in the loss of the ability to entrap all the serum phase (Lucey *et al.*, 1998a). This observation agrees with Al-Kadamany *et al.* (2002) who reported that the level of free whey in concentrated yogurt produced by the traditional method increases upon storage. Additionally, Salvador

& Fiszman (2004) reported that the level of syneresis in whole and skimmed set types of yogurt increases with storage time.

Table 3-6: Physicochemical properties of market reference samples [¥, †]

Reference samples	SWO (%m/m)	WD	Size of visible clusters
A – Day 18	0.050 ± 0.087	0.000 ± 0.000	1.000 ± 0.000
A – Day 35	0.203 ± 0.021	0.000 ± 0.000	1.000 ± 0.000
B – Day 18	0.030 ± 0.052	0.000 ± 0.000	1.000 ± 0.000
B – Day 35	0.153 ± 0.150	0.000 ± 0.000	1.000 ± 0.000
Total mean value	0.109 ± 0.083	0.000 ± 0.000	1.000 ± 0.000

[¥] Presented values are the means of 3 replicate trials ± SD. [†]Appendix A (Table A-1) provides the experimental data used to construct this table.

Although none of the reference samples had visible WD, all of them presented small visible clusters. According to Lee & Lucey (2006), stirred yogurts are likely to have clusters of protein aggregates which are presumably created by the collisions and shearing during the mixing process involved in their production. Due to this mechanical process, the characteristic three-dimensional gel matrix of set yogurt is no longer visible in stirred products (Lee & lucey, 2010). Lee & Lucey (2010) stated that stirred yogurt is a weak gel system and although "particle size" is sometimes reported for stirred yogurt it should be recognized that there are no individual particles; rather, there are weakly associated clusters of proteins that make up the network. The stirring action associated with the production of stirred yogurts disrupts the weak protein network and creates "particles". It is important to remark that the damage done to the coagulum during the production of concentrated stirred yogurts has a major impact on the viscosity of the final products. The larger the undisturbed aggregations of casein, and the smaller the whey-filled spaces, the higher the viscosity of the final product (Özer *et al*., 1999b).

Several researchers, such as Weidendorfer *et al.* (2008), studied and continue to study the way to avoid or minimize visual particles in stirred yogurt. Kucukcetin (2008) stated that numerous manufacturing parameters, such as high incubation temperatures, excessive whey protein to casein ratios, certain types of starter cultures and the use of excessive amounts of starter culture, are associated with textural defects of stirred yogurt,

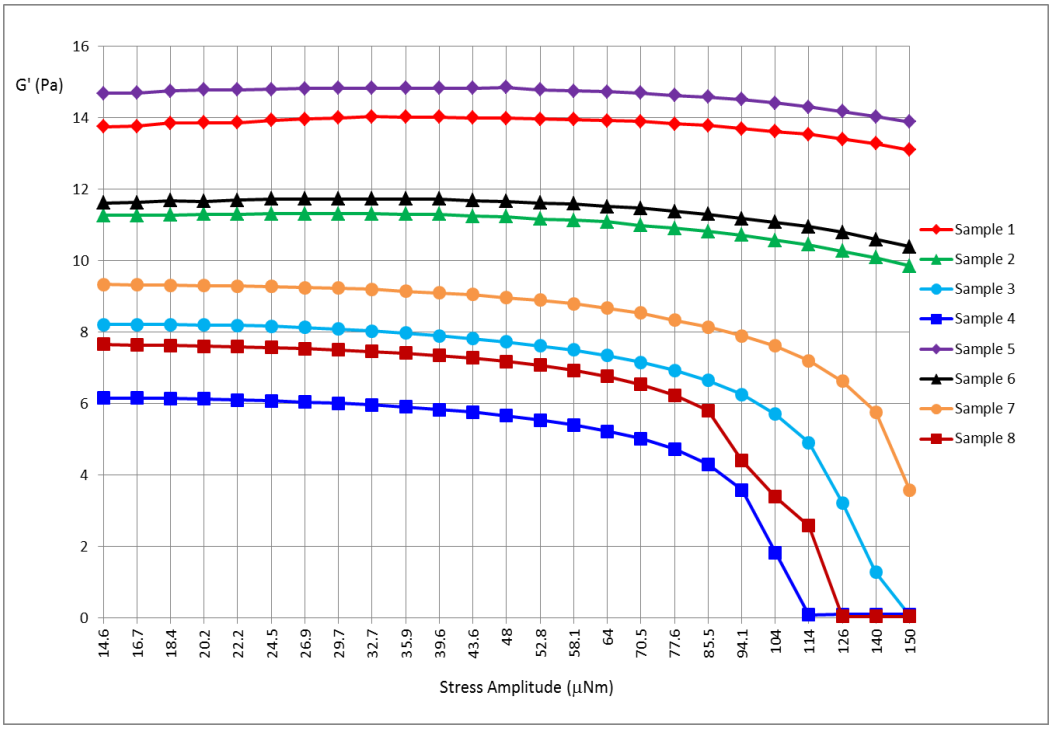
including graininess (particles) and surface roughness (irregularities in the yogurt matrix). Thus, to minimize visual clusters in the final product, it is very important to control these production parameters.

3.3.2 Experimental samples

3.3.2.1 Dynamic rheological analyses

Figures 3-8 and **3-9** illustrate the G' and G'' of experimental samples, produced by both manufacturing methods considered, as a function of amplitude sweep. All samples showed a predominant elastic character over their viscous behavior (G'>G''), indicating that non-relaxing protein bonds dominated over rapidly breaking and reforming weak bonds (Özer *et al*., 1998a).

Method 1



Method 2

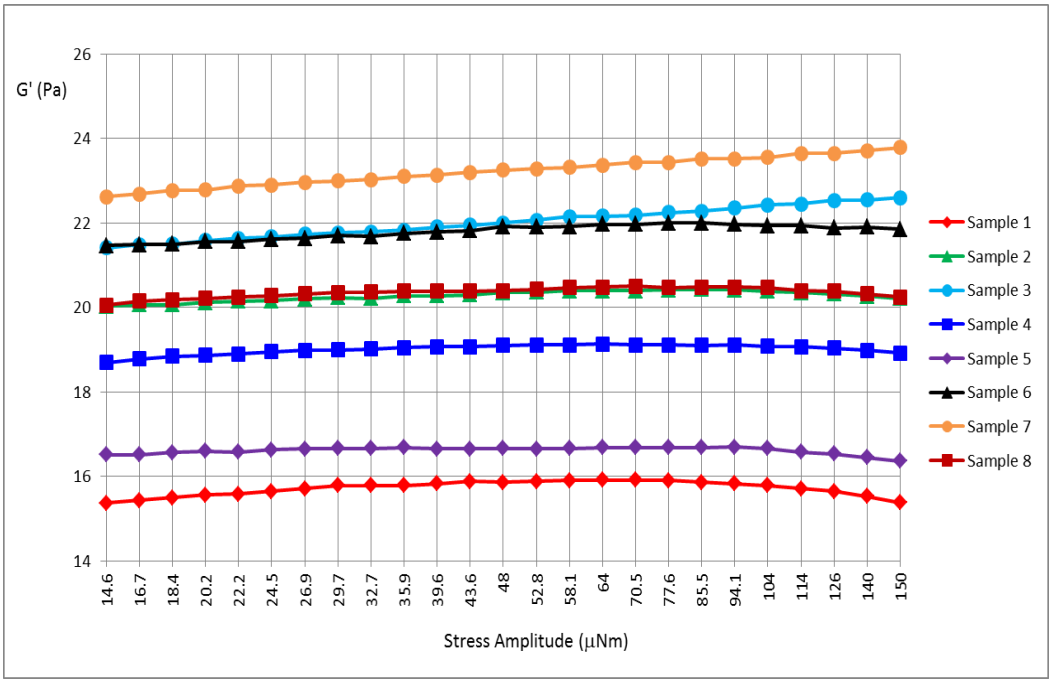
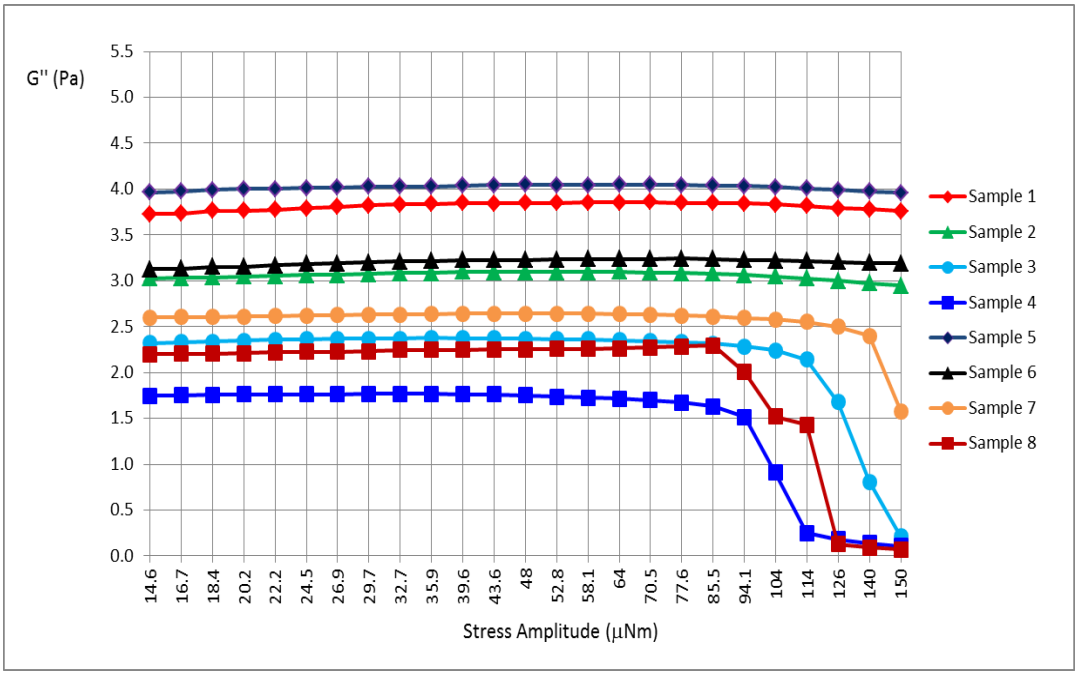


Figure 3-8: Storage modulus (G') of samples produced by methods 1 and 2 as a function of amplitude sweep

Presented values are the means of triplicate measurements. Appendix B (Tables B-4 to B-19) provides the experimental data used to construct these graphs.

Method 1



Method 2

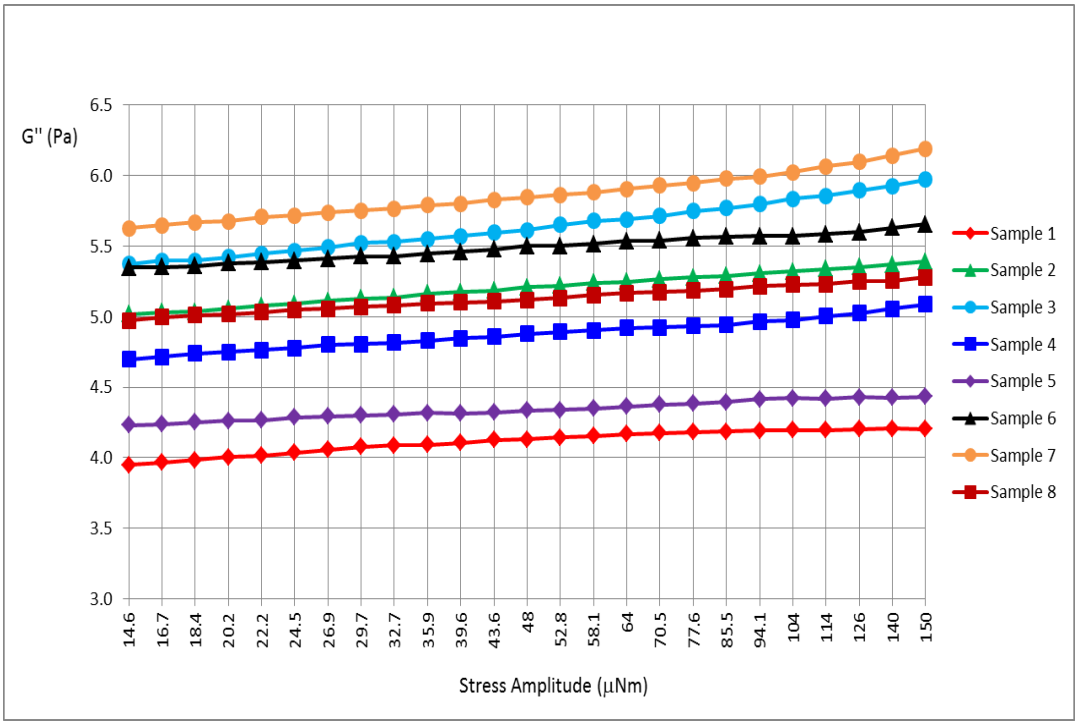


Figure 3-9: Loss modulus (G'') of samples produced by methods 1 and 2 as a function of amplitude sweep

Presented values are the means of triplicate measurements. Appendix B (Tables B-4 to B-19) provides the experimental data used to construct these graphs.

Both moduli (G' and G'') showed a stress-amplitude dependence, and a linear viscoelastic region was evident for all samples. Samples 3, 4, 7, and 8 produced by method 1 were the only ones that presented a structural breakdown at some point within the range of amplitude applied. Samples produced by method 2 presented higher dynamic moduli (G' and G'') throughout all the stress amplitude range applied. On the other hand, the SC content had a notable effect on the G' and G'' of the samples. Samples, produced by the same manufacturing method that contained equal casein to whey protein ratios but higher SC concentrations, presented higher G' and G'' values. This last statement agrees with the results obtained by Lee & Lucey (2004a) and Wu *et al.* (2009) who affirmed that high SC concentrations have a positive significant effect on the G' and apparent viscosity of the final gels. Lee & Lucey (2004a) also reported a positive correlation between SC concentrations and the final's gel strength.

Data presented in **Figures 3-8** and **3-9** affirm the findings of Peng *et al.* (2009), Isleten & Karagul-Yuceer (2008), Damin *et al.* (2009), and Amatayakul *et al.* (2006),

who state that the type of milk protein used to standardized the protein content of the milk base had a significant impact on the physical properties of yogurt. On the other hand, presented results also support the statements of Cho *et al*. (1999), Lucey *et al*. (1997), Lucey *et al.* (1998b), and Lucey *et al*. (1999) who reported that viscoelastic properties of acid milk gels depend significantly on the degree to which the milk base is heated.

3.3.2.1.1 Storage Modulus (G')

Table 3-7 illustrates statistical differences between the G' of different samples made by the same method of production and between the same samples made by different methods of production at three selected points of the stress amplitude range applied.

Table 3-7: Storage modulus (G') of samples produced by methods 1 and 2 at three selected points of the stress amplitude range applied [¥, †]

Sample #	G' (Pa) at Point 1 (Stress Amplitude = 14.6μNm) (mean ± SD)		P-value
	Method 1[£]	Method 2[£]	
1	13.750 ± 0.427^{A}	15.367 ± 0.153^{A}	.003
2	11.267 ± 0.306^{B}	20.033 ± 0.425^{B}	.000
3	8.217 ± 0.457^{C}	21.417 ± 0.425^{C}	.000
4	6.163 ± 0.207^{D}	18.700 ± 0.312^{D}	.000
5	14.683 ± 0.388^{E}	16.517 ± 0.451^{E}	.006
6	11.617 ± 0.306^{B}	21.467 ± 1.127^{C}	.000
7	9.333 ± 0.468^{F}	22.617 ± 0.729^{F}	.000
8	7.663 ± 0.313^{C}	20.050 ± 0.737^{B}	.000

Sample #	G' (Pa) at Point 12 (Stress Amplitude = 43.6μNm) (mean ± SD)		P-value
	Method 1[£]	Method 2[£]	
1	14.000 ± 0.346^{A}	15.883 ± 0.247^{A}	.002
2	11.250 ± 0.350^{B}	20.300 ± 0.391^{B}	.000
3	7.823 ± 0.534^{C}	21.950 ± 0.427^{C}	.000
4	5.767 ± 0.275^{D}	19.067 ± 0.144^{D}	.000
5	14.833 ± 0.293^{E}	16.650 ± 0.450^{A}	.004
6	11.683 ± 0.284^{B}	21.817 ± 1.156^{C}	.000
7	9.060 ± 0.610^{F}	23.200 ± 0.889^{E}	.000
8	7.278 ± 0.357^{C}	20.383 ± 0.765^{B}	.000

Sample #	G' (Pa) at Point 25 (Stress Amplitude = 150μNm) (mean ± SD)		P-value
	Method 1[£]	Method 2[£]	
1	13.100 ± 0.726^{A}	15.383 ± 0.202^{A}	.006
2	9.858 ± 0.469^{B}	20.217 ± 0.493^{B}	.000
3	0.057 ± 0.009^{C}	$22.600 \pm 0.527^{C,E}$	.000
4	0.101 ± 0.084^{C}	18.917 ± 0.058^{D}	.000
5	13.883 ± 0.275^{A}	16.367 ± 0.475^{A}	.001
6	10.390 ± 0.355^{B}	21.850 ± 1.253^{C}	.000
7	3.573 ± 2.699^{D}	23.783 ± 0.929^{E}	.000
8	0.051 ± 0.001^{C}	20.250 ± 0.826^{B}	.000

[¥] Presented values are the means of 3 replications. [†]Appendix B (Tables B-4 to B-19) provides the experimental data used to construct this table. [£] Numbers with different letters within the same column are significantly different ($P < 0.05$).

The same samples produced by different manufacturing methods presented significantly different G' values ($P < 0.05$) at the three stress amplitude points selected. This dissimilarity can be attributed to the difference in the amount of denatured whey proteins present in the same type of samples produced by the different methods.

Production method 2 included a heat treatment step (90°C for 5 minutes) before inoculation and further incubation of the milk base. Upon heat treatment of milk above 60°C, several processes take place, of which denaturation of whey proteins is the most obvious. The most abundant whey protein is β-lactoglobulin (β-lg) in which a heat-induced conformational change results in the exposure of a reactive thiol group. This thiol group can form disulfide bonds with other cysteine-containing proteins, like β-lg or bovine serum albumin, or with proteins having disulfide bridges, like α-lactalbumin (α-lac), κ- and αs_2-casein. The latter process occurs through thiol group-disulfide bridge exchange reactions, resembling a polymerisation process in which heat-denatured β-lg is the initiator (Vasbinder *et al*., 2003). Denatured whey proteins that are associated with casein micelles during heat treatment may act as bridging material by interacting with other denatured whey proteins, forming a branched microstructure (Lucey *et al.,* 1999). Lee & Lucey (2004b) reported that yogurt gels made from milk heated at high temperatures (>80°C) had a more cross-linked and branched protein structure with small pores compared with milk heated at low temperatures (Lee & Lucey, 2010). This branched microstructure contributes to an increase in G' value (due to high levels of non-relaxing protein bonds) and increases the water binding capacity of the final gel (Lucey *et*

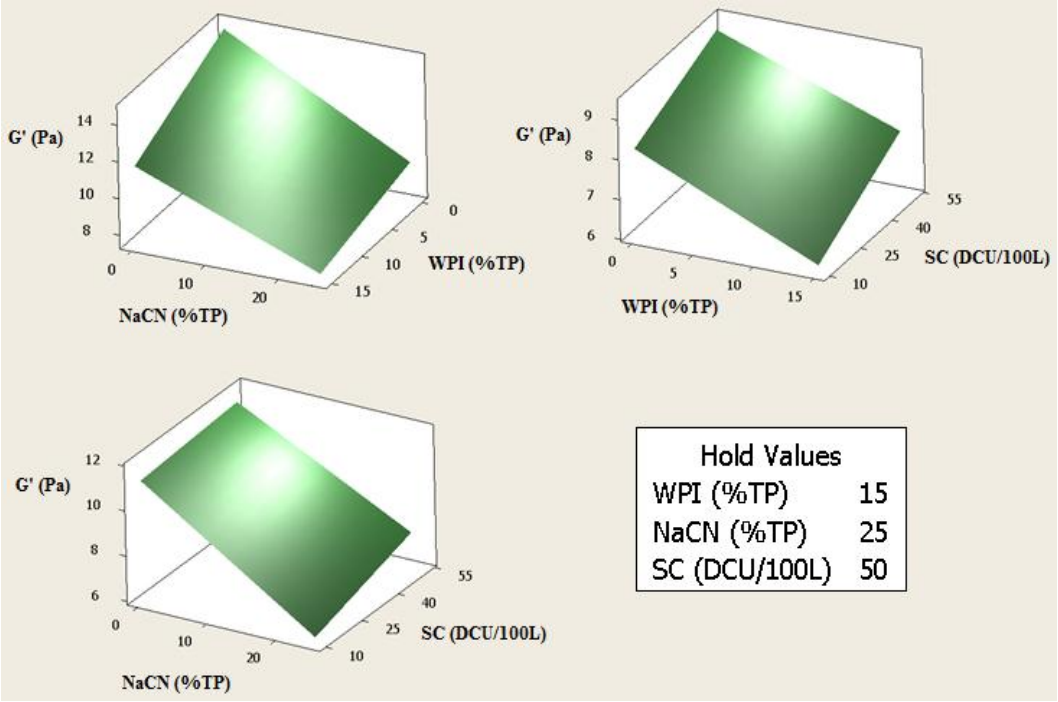
al., 1999; Lucey *et al.*, 1998b; Kessler & Beyer, 1991; Lee & Lucey, 2004b; Lucey & Singh, 1998). Thus, the final product obtained by heat treatment will be much more firm and less susceptible to syneresis. Parnell-Clunies *et al.* (1986) reported a correlation coefficient between yogurt firmness and whey protein denaturation of .83 and between apparent viscosity and whey protein denaturation of .89. Kucukcetin (2008) stated that the syneresis of yogurt decreases progressively with an increase in the degree of whey protein denaturation, but above 90% denaturation this trend was less pronounced than in yogurt obtained from milk containing $\geq$ 90% of denatured whey protein. However, these results are different than those reported by Sodini *et al.* (2006). These authors affirmed that the G' and water-holding capacity of yogurts increases when the whey protein denaturation level decreases.

According to Özer *et al.* (1998a) and Özer *et al.* (1999a), the differences in the overall gel strength at low amplitudes suggest that, although the type of protein-protein interactions (mainly whey protein-κ-casein interactions) in each case may be similar, there are differences in the degree of interaction. As a consequence of the heat treatment, gel networks produced by method 2 had a higher degree of protein interactions. Bremer *et al.* (1990) and Roefs *et al.* (1990) stated that the mechanical strength of a heterogeneous casein network will be largely determined by the number, length and thickness of the stress carrying strands formed per unit volume and their rheological properties which, in turn, depend on the number of protein-protein bonds per cross section and on their strength and relaxation time. Bremer *et al.* (1990) also stated that the nature and position of these strands in the network will also determine the rheological properties of the gel. Gels with a high number of short and thick strands have a high strength, and the elasticity of the networks (G') decrease as the number of stress-carrying strands decrease. Özer *et al.* (1999b) agreed with these statements by affirming that as the chains of casein particles become shorter, the dimensions of the whey filled spaces diminish and the density of the matrix increases, resulting in much denser structures. Consequently, as gels produced by method 2 had a higher degree of protein interactions than gels produced by method 1, higher dynamic moduli are expected for yogurts produced by method 2.

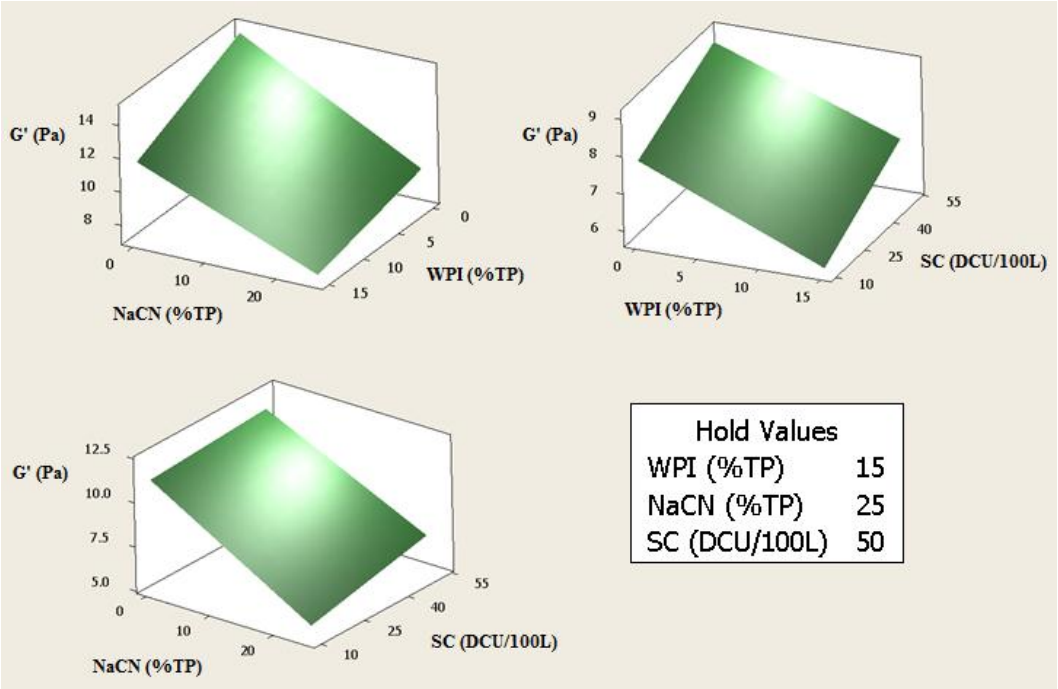
According to **Table 3-7**, almost all method 1 samples that contained different amounts of protein sources differed significantly ($P < 0.05$) in their G' values at the three stress amplitude points selected. The storage modulus of samples produced by method 1 was strongly influenced by the concentration of MPC-85 present in the samples. Samples

with higher MPC-85 contents (samples 1, 2, 5, and 6) presented higher dynamic moduli and kept their structural integrities against increasing shear. As the concentration of MPC-85 diminished, the G' was reduced (See **Figure 3-10**). Furthermore, as stated previously, almost all samples that contained the same casein to whey protein ratio but had higher SC levels presented significantly higher G' values ($P < 0.05$).

A. Point 1 (Stress Amplitude = 14.6μNm)



B. Point 12 (Stress Amplitude = 43.6μNm)



C. Point 25 (Stress Amplitude = 150μNm)

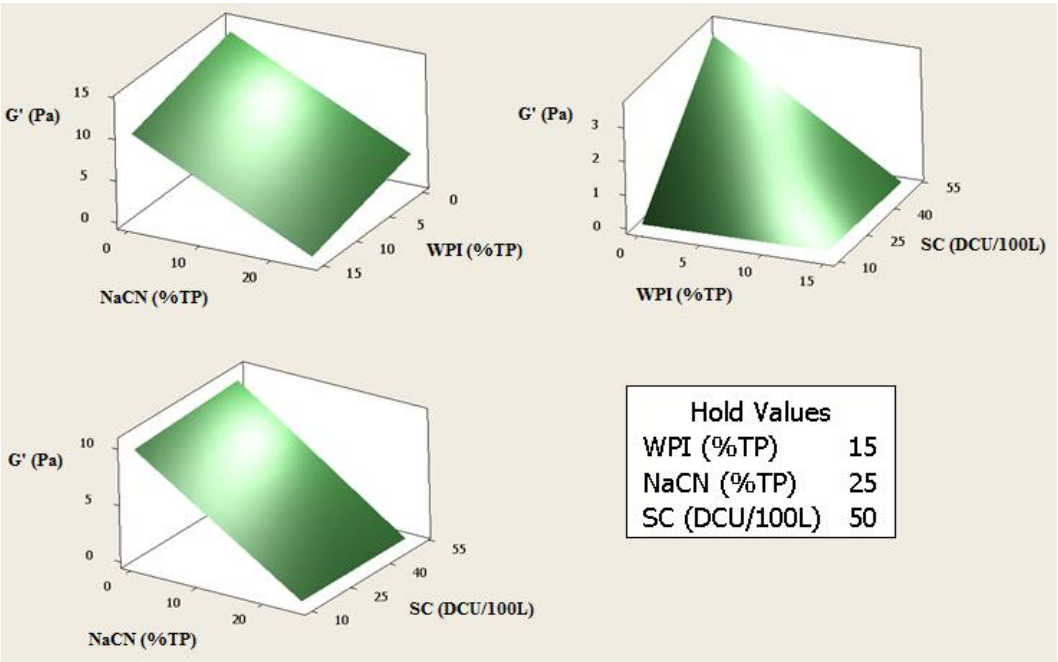


Figure 3-10: Effects of MPC-85, NaCN, WPI-90, and SC concentrations on the storage modulus (G') of samples produced by production method 1 at three selected points of the stress amplitude range applied

Surface plots were constructed using the means of triplicate measurements. Appendix B (Tables B-4 to B-11) provides the experimental data used to construct these graphs.

According to the previous figure, the effects of the different protein sources on the G' were very similar for the three stress amplitude points considered. However, there were some dissimilarities between the surface plots obtained for points 1 and 12 and those obtained for point 25. These differences can be attributed to diverse rheological behaviors of samples after their breakage (once outside their viscoelastic region). Samples that suffered a structural breakdown throughout the stress amplitude range applied presented very low G' at point 25, regardless of their SC content.

Is believed that the MPC-85 used for the production of samples contained a significant amount of denatured whey proteins. Due to this fact, samples, produced by method 1, that contained high amounts of MPC-85 presented high G' and G'' values and big visible clusters (See section 3.3.2.2.3). As the experimental samples produced by method 1 were not exposed to any heat treatment above 60^{o}C during their manufacture, almost no whey protein denaturation occurred during the process. Hence, samples, produced by method 1, that contained high levels of WPI-90 presented high amount of undenatured whey proteins in the final gels. These undenatured whey proteins could not interact with κ-casein; thus, a lower degree of protein interactions was obtained inside the gel networks. As a result, high levels of WPI-90 did not contribute to an increase in the water-binding capacity or in the dynamic moduli of the final gels. In fact, the added whey proteins remained undenatured and probably acted as a filter in the gel matrix (increasing the permeability of the resultant gel), causing a reduction in G' and G'' and reducing the water-binding capacity of the gel networks (Lucey *et al.*, 1999; Lucey, 2001).

Consequently, samples that contained high levels of WPI-90 presented the highest level of WD (See Section 3.3.2.2.2) and lower dynamic moduli values than samples that contained high levels of MPC-85. These results agree with those of Özer (2009), who stated that an increased addition of whey protein concentrate to the milk base without further heat treatment resulted in a weak gel development. Lucey *et al.* (1999) also reported that the addition of whey protein concentrate to unheated milk resulted in a reduction in the G' and shear stress. These results show that native whey proteins do not contribute to the gel matrix (Lucey *et al*., 1999).

As the samples produced by method 1 contained a low amount of denatured whey proteins, the addition of NaCN did not result in an increase in the dynamic moduli of the final gels. Not all the casein present in NaCN could interact with the few denatured whey proteins present in the milk base. Thus, caseins which could not form bonds with denatured whey proteins did not strongly contribute to the gel strength and probably

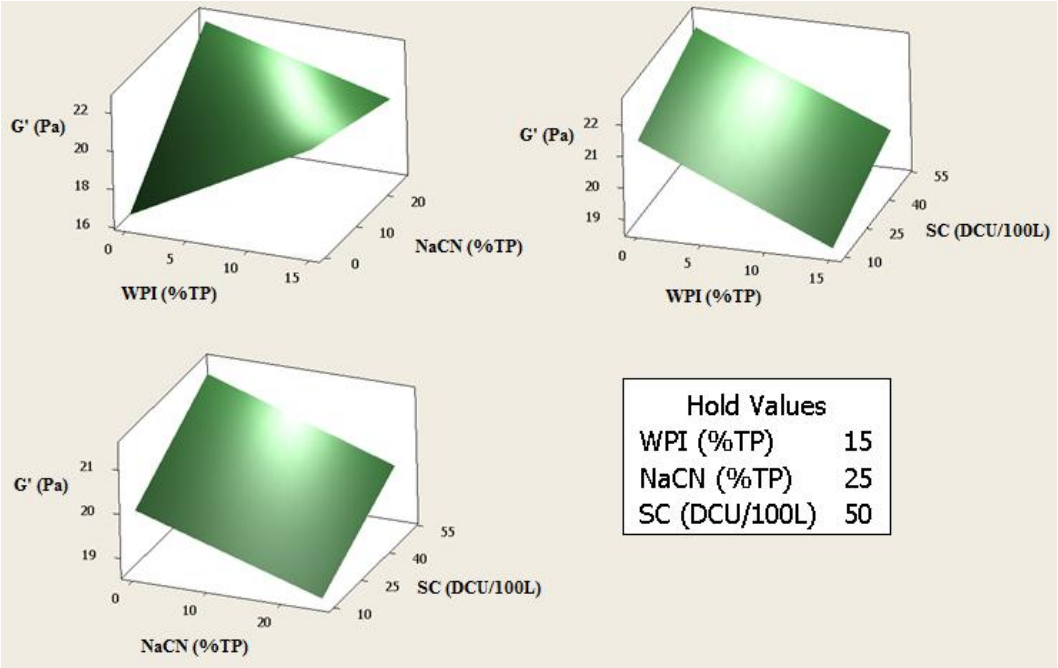
acted as a filter in the gel matrix such as the undenatured whey proteins. As a result, method 1 samples that contained high levels of NaCN had lower G' values than samples that contained high levels of MPC-85.

Samples containing high levels of NaCN (25%TP) had lower G' than samples containing high levels of WPI-90 (15%TP). This is because samples containing higher amounts of NaCN presented lower levels of MPC-85 and higher quantities of molecules which did not strongly contribute to the final gel strength. In addition, samples that contained high levels of NaCN and WPI-90 (25%TP and 15%TP, respectively) presented the lowest G' values.

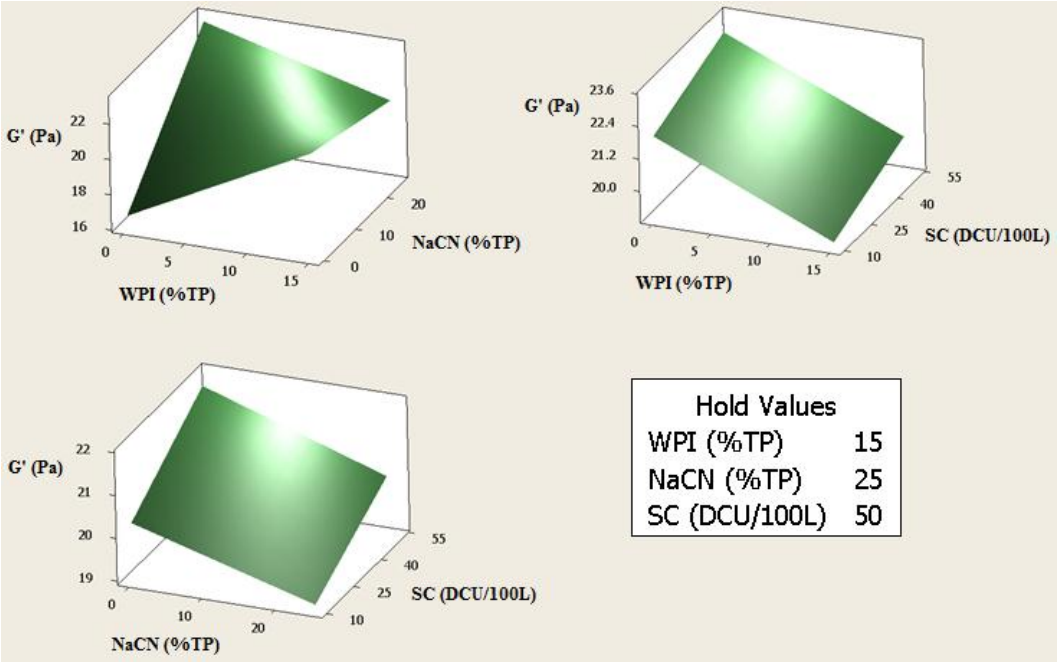
Based on the previous cited statements and the results observed in **Figures 3-8**, **3-9**, and **3-10**, it can be stated that, for production method 1, samples that contained high concentrations of MPC-85 presented a higher number of short strands per unit volume (higher degree of non-relaxing interactions between protein molecules) and smaller whey filled spaces than samples that contained high levels of WPI-90 and NaCN. Due to this fact, samples produced by method 1 that contained high amounts of MPC-85 presented the highest dynamic moduli and kept their structural integrities against increasing shear.

The results obtained from samples produced by production method 2 strongly contrast those obtained from samples manufactured by method 1**. Figure 3-11** illustrates the effects of the different protein sources on the G' of samples produced by production method 2.

A. Point 1 (Stress Amplitude = 14.6μNm)



B. Point 12 (Stress Amplitude = 43.6μNm)



C. Point 25 (Stress Amplitude = 150μNm)

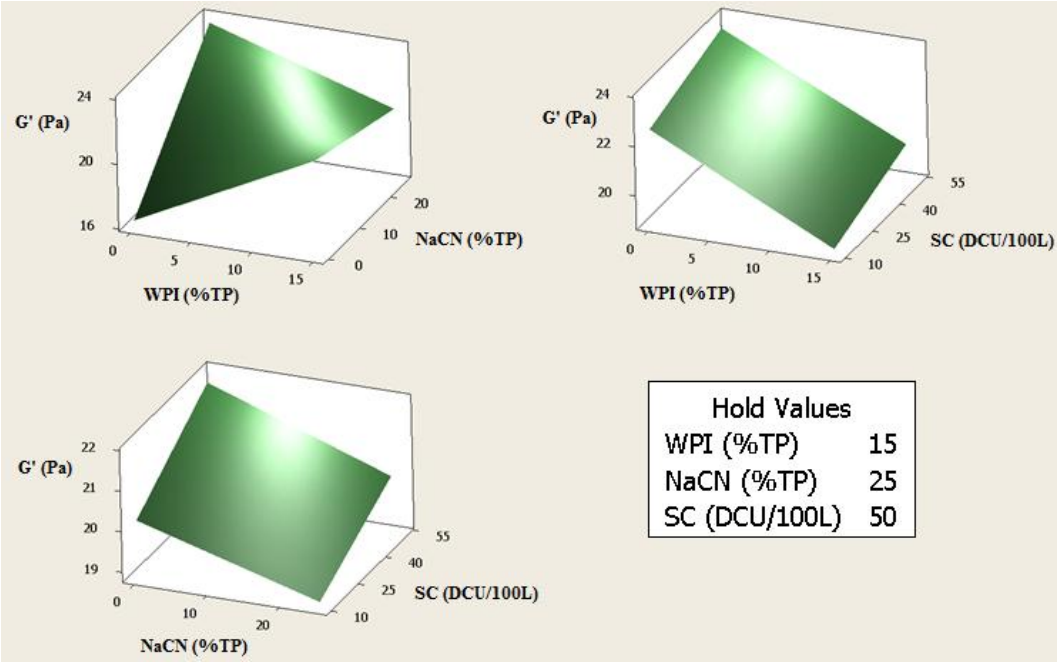


Figure 3-11: Effects of MPC-85, NaCN, WPI-90, and SC concentrations on the storage modulus (G') of samples produced by production method 2 at three selected points of the stress amplitude range applied

Surface plots were constructed using the means of triplicate measurements. Appendix B (Tables B-12 to B-19) provides the experimental data used to construct these graphs.

According to **Table 3-7**, at the three stress amplitude points selected, samples produced by method 2 that contained different amounts of protein sources significantly differed ($P < 0.05$) in their G' values. Production method 2 yogurts that contained high levels of NaCN (25%TP) and no WPI-90 presented the highest G' values. Samples that contained high levels of WPI-90 (15%TP) and no NaCN exhibited the second highest G' values, while samples containing high amounts of NaCN and WPI-90 presented lower G' than the first ones. In contrast to the results obtained for method 1, yogurts produced by method 2 that contained the highest levels of MPC-85 presented the lowest G' values. Additionally, as stated before, almost all samples containing the same casein to whey protein ratios but higher SC concentrations presented significantly higher G' ($P < 0.05$).

Results obtained are consistent with the works done by Molder *et al*. (1983), Tamime *et al*. (1984), Guinee *et al.* (1995), Damin *et al.* (2009), Peng *et al.* (2009), Bremer *et al* (1990), and Guzman-Gonzalez *et al.* (2000), who studied the effect of NaCN, whey protein powders and milk powders on yogurt's physical properties and

concluded that, at similar protein levels, yogurts fortified with NaCN presented higher viscosity and gel strength than those enriched with other dairy powders. Krzeminski *et al.* (2011) stated that the resistance towards shear-induced disruption of yogurt gels increased with an increasing proportion of casein protein in the protein mixture, whereas products with a high whey protein level revealed lower resistance behaviour towards shear forces. This finding matches the results of Damin *et al.* (2009), Peng *et al.* (2009), and Bremer *et al* (1990), who reported that samples with high NaCN contents presented high levels of yield stress (σ_{yield}) and fracture stress ($\sigma_{fracture}$), respectively. Furthermore, recently, Alkalin *et al.* (2012) studied the influence of sodium calcium caseinate (NaCaCN) and whey protein concentrate (WPC) on the textural characteristics of yogurt and concluded that higher values of viscosity were obtained in yogurts fortified with NaCaCN. However, Isleten & Karagul-Yuceer (2006), Isleten & Karagul-Yuceer (2008) and Cho *et al*. (1999) reported that, at similar protein levels, yogurts fortified with WPC or WPI had higher hardness or viscosity than those fortified with NaCN.

Peng *et al*. (2009) explained that adding NaCN significantly reduces the buffering capacity of the yogurt mix by apparently solubilizing part of the indigenous colloidal calcium phosphate (CCP) present in the initial milk base. Partial removal of CCP from casein micelles affects the internal structure of casein micelles, increases the mobility of casein particles, and disperses the casein micelles, which may increase the contact area of casein particles and result in an increase in G' values at a pH approaching 4.6. The increased molecular flexibility of the caseins may enhance the formation of cross-links between casein particles and strands. These findings match the results of Ozcan-Yilsay *et al.*, (2007) who studied the addition of low concentrations of trisodium citrate to milk to reduce the level of CCP cross-linking between caseins inside the micelle and concluded that removing CCP from micelles facilitated greater rearrangements and molecular mobility of casein particles inside the micelles, which may have helped increase the formation of cross-links between strands in yogurt gel networks. Furthermore, the added soluble casein molecules in NaCN-fortified milks may have helped to increase the G' of yogurt gels by increasing the number of cross-links between strands. Due to this fact, a higher degree in the number and/or strength of non-relaxing protein bonds (mainly denatured whey protein–κ casein) is expected in yogurts fortified with NaCN. However, the number and/or strength of non-relaxing protein interactions also depends on the amounts of denatured whey proteins present in the milk base.

In the absence of NaCN, samples with lower casein to whey protein ratios presented the highest G' values. These results coincide with those reported by Puvanenthiran *et al*. (2002), Lucey *et al*. (1999), Kucukcetin (2008), Oliveira *et al*. (2001), and Remeuf *et al*. (2003), although several authors reported different results. Amatayakul *et al*. (2006) affirmed that the firmness of yogurt gels decreased as the casein to whey protein ratio was reduced. Damin *et al.* (2009) stated that samples supplemented with skim milk powder (SMP) and WPC resulted in a decrease in G'. Marafon *et al.* (2011a), Guzman-Gonzalez *et al.* (1999) and Cho *et al.* (1999) argued that lower viscosities were obtained when enriching the milk with WPC instead of SMP. According to Puvanenthiran *et al*. (2002), this discrepancy between results from literature could be related to a variation in the WPI or WPC preparation, which strongly influences their functionalities. Guinee *et al.* (1995) indicated that for a given protein level, the influence of WPC on rheological and syneretic properties of yogurt was very much dependent on the protein level of the WPC. Cho *et al*. (1999) reported an increase in G' from 13 to 80 Pa for yogurt gels enriched with two kinds of WPC, having low or high level of denaturation (Remeuf *et al*., 2003). Vasbinder *et al.* (2003) stated that altered properties of heated milk are related to the total degree of whey protein denaturation. Remeuf *et al*. (2003) applied the same heat treatment as the one involved in this investigation (production method 2) and stated that with that thermal treatment a level of denaturation of whey protein of >50% was obtained. They also reported that this level of denaturation should induce a high bridging capacity of whey proteins, resulting in a viscosity-increasing effect similar to that of caseinate.

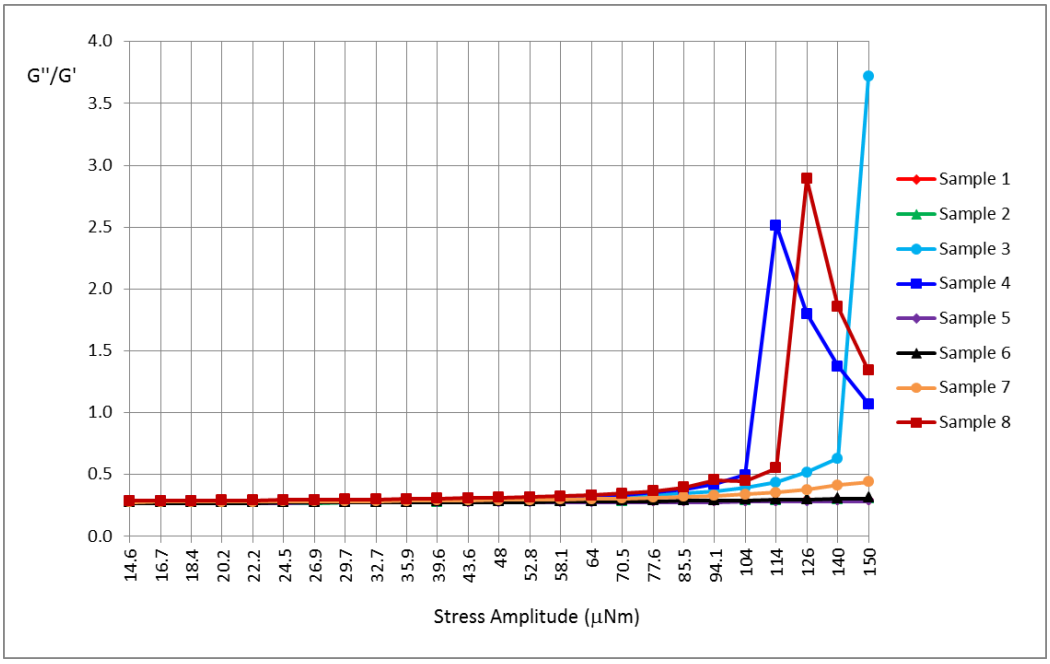
Unexpectedly, samples that contained high levels of NaCN and WPI-90 (casein:whey protein = 2.8:1) presented lower G' than samples containing only high levels of NaCN or WPI-90. These results are consistent with Remeuf *et al.* (2003), who studied the effect of two types of caseinate and WPC blends on yogurt's physical properties and reported that, for a fixed amount of total protein, samples that were made using a blend containing caseinate to whey protein ratios of 2:1 had lower complex viscosity than samples supplemented with NaCN and WPC. However, they also stated that yogurt made with a blend containing caseinate to whey protein ratios of 1:2 had higher complex viscosity than samples supplemented with NaCN and WPC. They explained these results by suggesting a synergistic effect of casein and whey protein on yogurt viscosity, and the probable occurrence of one or more optimum values for the casein to whey protein ratio.

Finally, samples produced by method 2 that contained MPC-85 as the only protein source presented the lowest G' values. These samples presented the second highest casein to whey protein ratio (4.6:1) after the samples containing only high levels of NaCN (6.6:1). Due to this fact, our results confirmed the hypothesis suggested by Remeuf *et al.* (2003) and Peng *et al.* (2009), showing that although the casein to whey protein ratio is a good parameter to consider during the manufacture of yogurt, this ratio is not directly correlated with the rheological aspect of the final gels, because samples with close casein to whey protein ratios can have very different rheological properties. Therefore, it is of great importance to consider the composition of the samples beyond their casein to whey protein ratios. Depending on the composition of the final sample, more than one optimum value for the casein to whey protein ratio can be used to obtain the desired rheological properties of a final product (Remeuf *et al.*, 2003).

3.3.2.1.2 Loss Tangent (tan δ = G''/G')

Figure 3-12 illustrates the tan δ of all the experimental samples when applying the stress amplitude sweep tests. The changes in G' as function of amplitude were almost proportional to the changes in G'' within the viscoelastic region. At low amplitudes (from ≈ 14.6 to 43.6 μNm), the tanδ values of all samples ranged from 0.248 to 0.310. Thus, δ range from 13.93° to 17.22°. According to Velez-Ruiz & Barbosa Canovas (1997), viscoelastic materials present $0° < \delta < 90°$. Therefore, these results confirmed the viscoelastic behavior of the samples (Oliveira *et al*., 2001).

Method 1



Method 2

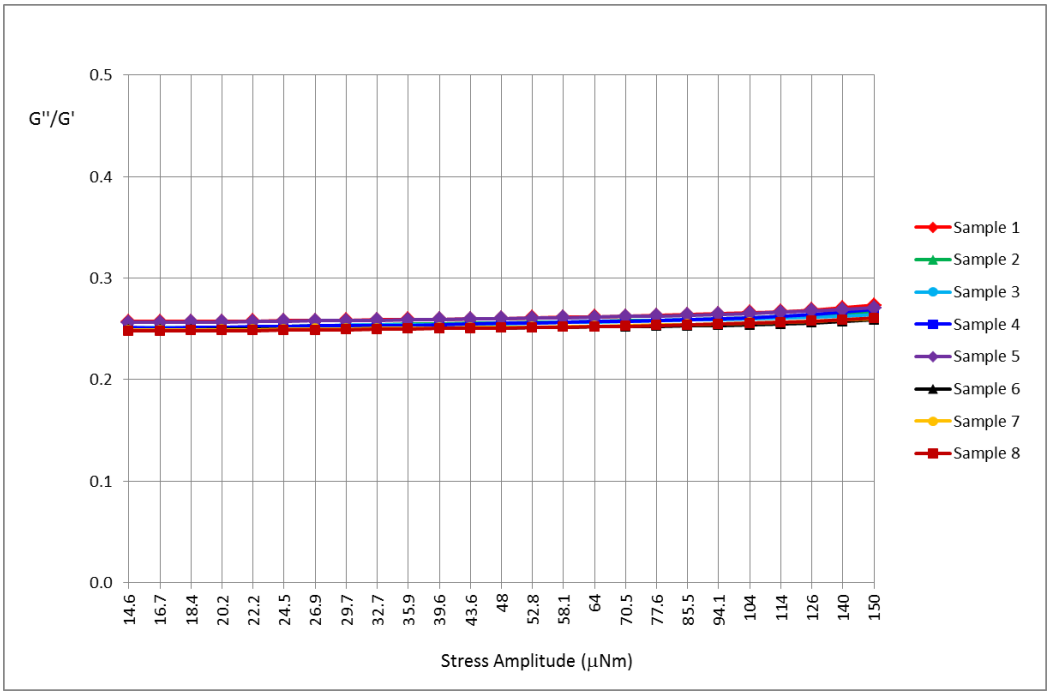


Figure 3-12: Loss tangent (tan δ = G''/G') of samples produced by methods 1 and 2 as function of amplitude sweep

Presented values are the means of triplicate measurements. Appendix B (Tables B-4 to B-19) provides the experimental data used to construct these graphs.

According to the results obtained for production method 1, at high amplitudes, outside the viscoelastic region, changes in G' (attributed to gel rupture) were not proportional to changes in G''. This gave rise to significant differences ($P < 0.05$) in the tan δ values of the different samples at high amplitudes. Outside the viscoelastic region, G' and tan δ were inversely related. On the other hand, samples produced by method 2 maintained their structural integrities throughout all the stress amplitude range applied. Due to this fact, none of them experienced a marked increase in their tan δ value at high amplitudes.

Table 3-8 illustrates statistical differences between tan δ values of different samples made by the same method of production and between same samples made by different methods of production.

Table 3-8: Loss tangent (tan δ = G''/G') of samples produced by methods 1 and 2 at three selected points of the stress amplitude range applied [¥, †]

Sample #	tan δ at Point 1 (Stress Amplitude = 14.6μNm) (mean ± SD)		P-value
	Method 1[£]	Method 2[£]	
1	0.271 ± 0.004^{A}	0.257 ± 0.001^{A}	.003
2	0.268 ± 0.001^{A}	0.250 ± 0.002^{B}	.000
3	$0.283 \pm 0.003^{B,C}$	0.251 ± 0.002^{B}	.000
4	$0.284 \pm 0.003^{B,C}$	0.251 ± 0.002^{B}	.000
5	0.270 ± 0.005^{A}	0.256 ± 0.001^{A}	.007
6	0.269 ± 0.003^{A}	0.249 ± 0.004^{B}	.002
7	0.279 ± 0.001^{B}	0.249 ± 0.004^{B}	.000
8	0.287 ± 0.005^{C}	0.248 ± 0.003^{B}	.000

Sample #	tan δ at Point 12 (Stress Amplitude = 43.6μNm) (mean ± SD)		P-value
	Method 1[£]	Method 2[£]	
1	0.275 ± 0.003^{A}	0.260 ± 0.000^{A}	.001
2	0.275 ± 0.002^{A}	$0.255 \pm 0.003^{A,B}$	.001
3	0.303 ± 0.005^{B}	$0.255 \pm 0.002^{A,B}$	.000
4	0.305 ± 0.006^{B}	$0.255 \pm 0.003^{A,B}$	.000
5	0.272 ± 0.005^{A}	0.260 ± 0.001^{A}	.012
6	0.276 ± 0.004^{A}	0.251 ± 0.004^{B}	.002
7	0.292 ± 0.004^{C}	0.251 ± 0.004^{B}	.000
8	0.310 ± 0.008^{B}	0.251 ± 0.002^{B}	.000

Sample #	tan δ at Point 25 (Stress Amplitude = 150μNm) (mean ± SD)		P-value
	Method 1[£]	Method 2[£]	
1	0.287 ± 0.010^{A}	0.273 ± 0.001^{A}	.061
2	0.299 ± 0.007^{A}	$0.267 \pm 0.006^{B,C}$	.003
3	3.717 ± 1.758^{B}	$0.264 \pm 0.002^{C,D}$	.032
4	1.071 ± 0.075^{A}	$0.269 \pm 0.002^{A,B,C}$	.000
5	0.285 ± 0.007^{A}	$0.271 \pm 0.002^{A,B}$	.024
6	0.307 ± 0.012^{A}	0.259 ± 0.004^{D}	.003
7	0.442 ± 0.142^{A}	0.260 ± 0.002^{D}	.041
8	1.342 ± 0.400^{A}	0.261 ± 0.003^{D}	.009

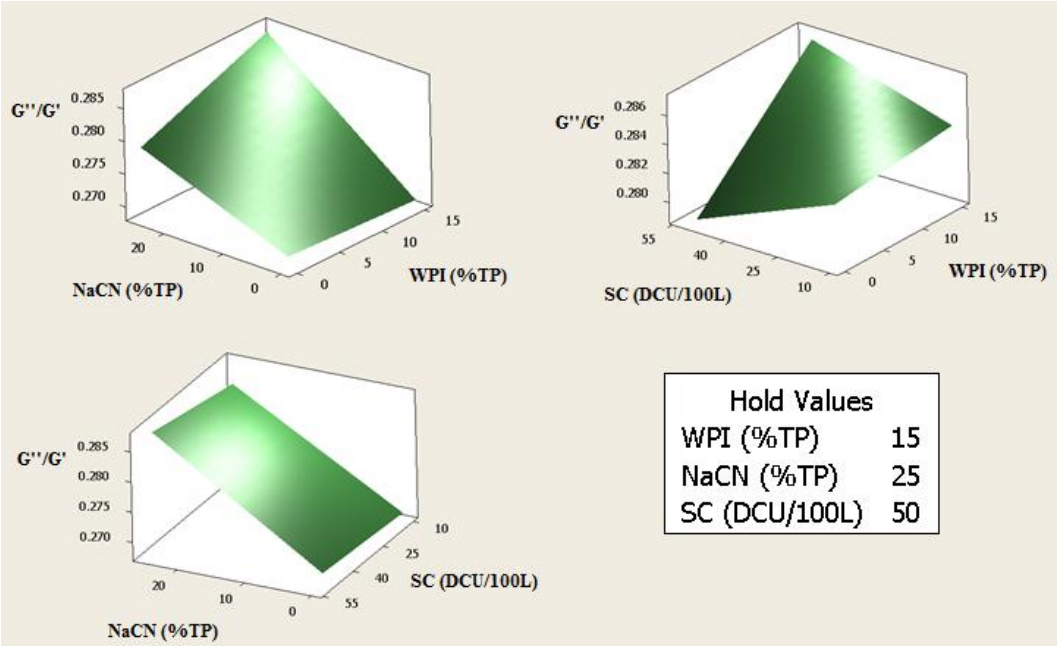
[¥] Presented values are the means of 3 replications. [†]Appendix B (Tables B-4 to B-19) provides the experimental data used to construct this table. [£] Numbers with different letters within the same column are significantly different ($P < 0.05$).

Almost all the same samples produced by different manufacturing methods presented significantly different tan δ values at the three stress amplitude points selected ($P < 0.05$). Samples produced by method 2 had significantly lower tan δ values than samples produced by method 1. This dissimilarity can be attributed to the difference in the level of denatured whey proteins that were present in the same type of samples produced by the different methods.

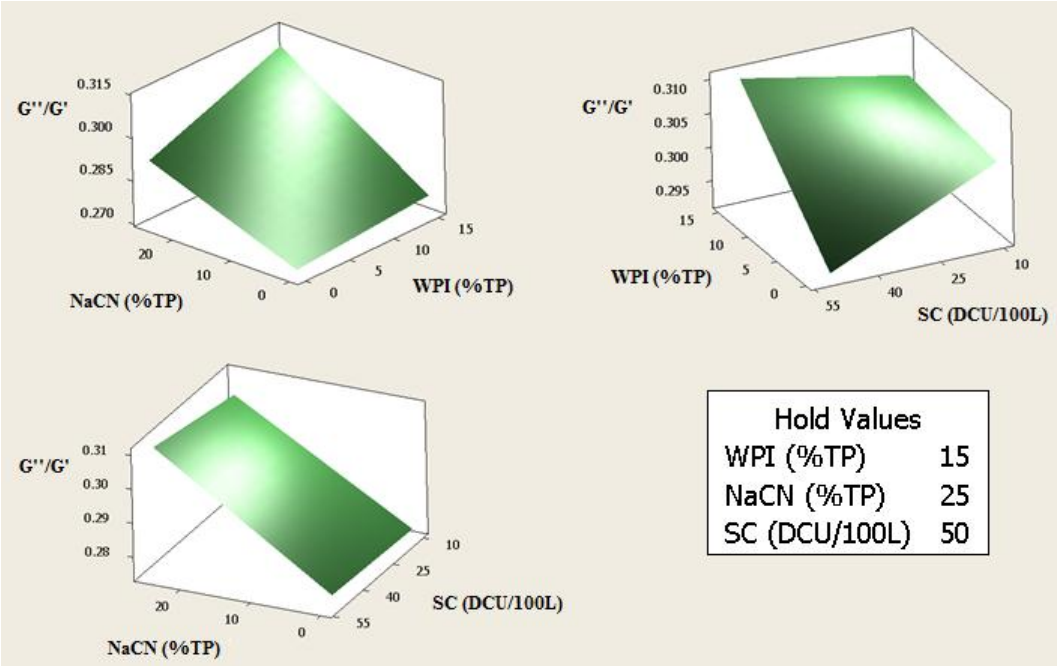
High tan δ values are related to the high number of relaxation bonds present in the gel matrix (van Vliet *et al*., 1991; Lucey & Singh, 1998). Hence, from the results obtained, it can be affirmed that the heat treatment applied in production method 2 had a significant positive effect on the formation of non-relaxing protein bonds within the gel network. In this way, samples produced by method 2 had significantly lower tan δ values than those produced by method 1. Despite the method of production used, SC contents did not have a relevant influence on the tan δ at the different stress amplitude points tested.

Figure 3-13 shows the effects of the different protein sources on the tan δ of samples produced by the production method 1. In regards to this production method, for the first two stress amplitude points considered, samples containing high amounts of MPC-85 (samples 1,2,5, and 6) had significantly lower tan δ values ($P < 0.05$) than the rest of the samples. Hence, samples containing high levels of MPC-85 presented a higher elastic behavior ($G' > G''$).

A. Point 1 (Stress Amplitude = 14.6μNm)



B. Point 12 (Stress Amplitude = 43.6μNm)



C. Point 25 (Stress Amplitude = 150μNm)

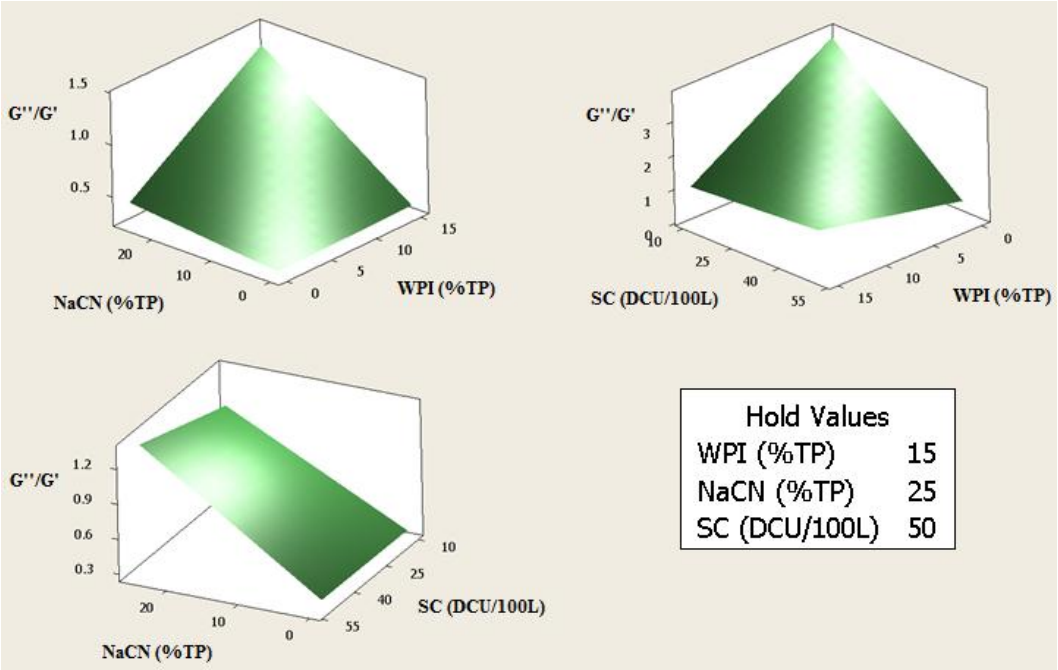
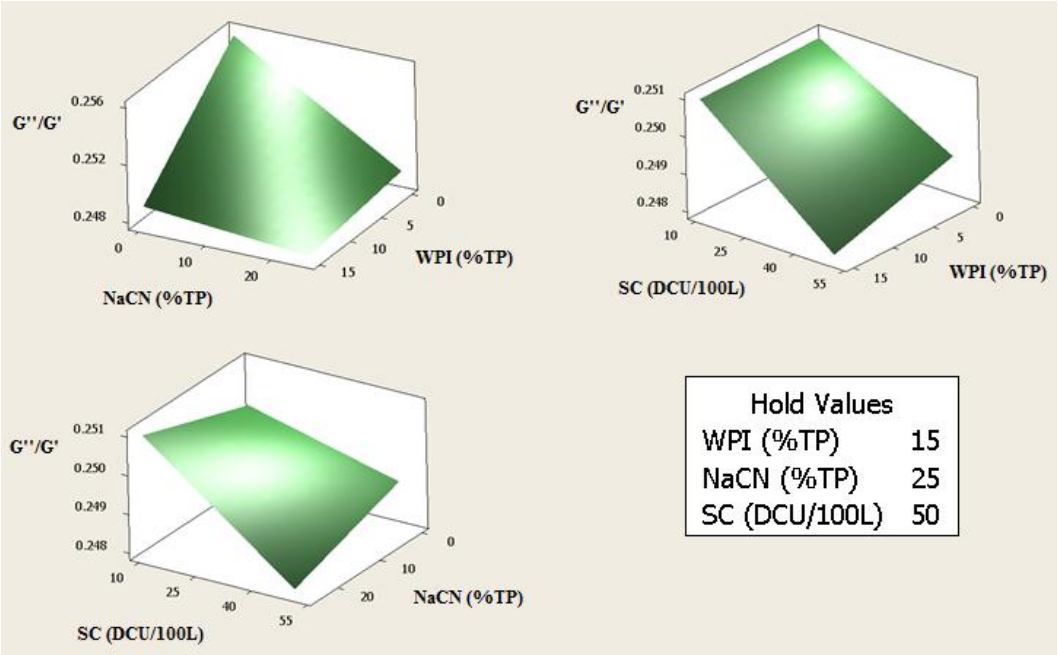


Figure 3-13: Effects of MPC-85, NaCN, WPI-90, and SC concentrations on the loss tangent (tan δ = G''/G') of samples produced by production method 1 at three selected points of the stress amplitude range applied

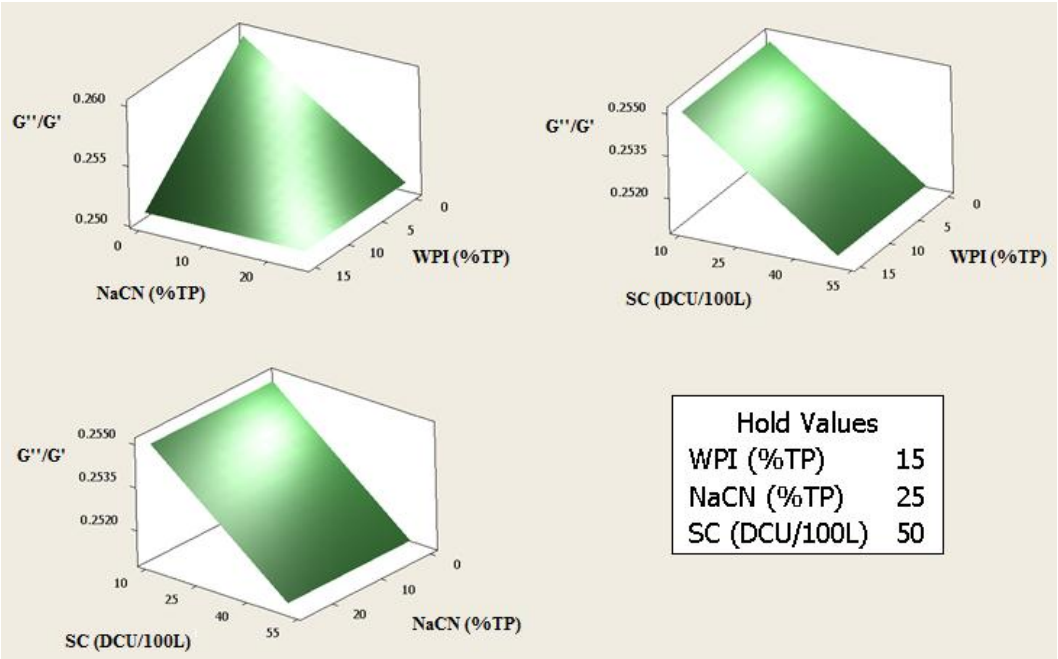
Surface plots were constructed using the means of triplicate measurements. Appendix B (Tables B-4 to B-11) provides the experimental data used to construct these graphs.

Figure 3-14 illustrates the effects of the different protein sources on the tan δ of samples produced by production method 2. Regarding production method 2, at point 1, samples with higher MPC-85 contents (samples 1 and 5) had significantly higher tan δ ($P < 0.05$) than the other samples. However, for the other tested points there was not a clear relationship between the different samples' compositions and their tan δ values.

A. Point 1 (Stress Amplitude = 14.6μNm)



B. Point 12 (Stress Amplitude = 43.6μNm)



C. Point 25 (Stress Amplitude = 150μNm)

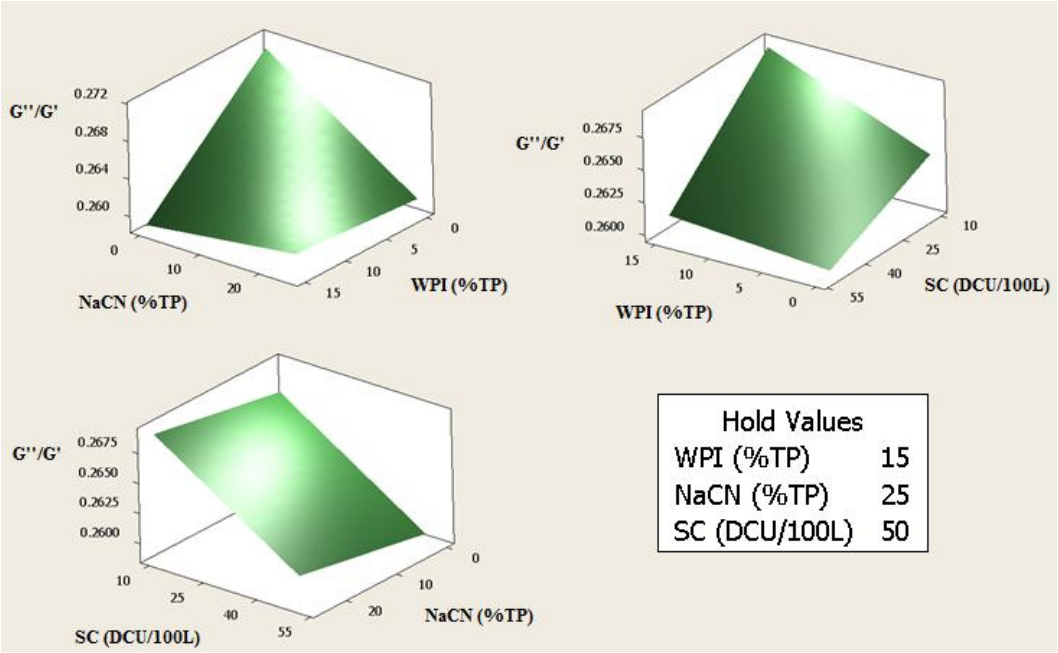


Figure 3-14: Effects of MPC-85, NaCN, WPI-90, and SC concentrations on the loss tangent (tan δ = G''/G') of samples produced by production method 2 at three selected points of the stress amplitude range applied

Surface plots were constructed using the means of triplicate measurements. Appendix B (Tables B-12 to B-19) provides the experimental data used to construct these graphs.

These results are different from those reported by several authors. Peng *et al.* (2009) reported that yogurts fortified with NaCN had higher maximum tan δ values than samples fortified with other milk powders. They stated that the partial removal of CCP by NaCN before fermentation may have increased rearrangements in yogurt gel resulting in high maximum tan δ values. Opposed to this, Roefs & van Vliet (1990) indicated that tan δ is not affected by the presence of NaCN. Furthermore, Özer *et al.* (1999a) stated that tan δ values were independent of casein concentrations of samples.

The tan δ value is related to the nature of the bonds forming the protein network and the relative importance of the different types of bond rather than to the spatial distribution of protein junction points (Özer *et al.*, 1997). Hence, statistical differences between the tan δ values of the different samples indicated that gel structures were composed by a different nature of interaction forces, suggesting the formation of different network structures. Samples produced by method 1 presented a higher number of

relaxation bonds; therefore, the resultant gels had a higher relaxation behavior (higher tan δ) than samples made by method 2.

3.3.2.2 Physicochemical & incubation time analyses

The different manufacture methods and ingredients applied to produce experimental samples had a significant impact on their physicochemical characteristics.

3.3.2.2.1 Surface whey-off (SWO)

Table 3-9 shows the level of free whey present on top of the gels before applying the homogenization step required to produce the samples (SWO_{BH}). None of the samples presented visible levels of SWO after their homogenization and storage for 1 day 5°C.

Table 3-9: Surface whey-off present in samples before applying homogenization (SWO_{BH}) [€, Δ]

Sample #	Level of SWO_{BH} (%m/m) (mean ± SD)		P-value
	Method 1[£]	Method 2[£]	
1	1.010 ± 0.128^{A}	0.767 ± 0.112^{A}	.068
2	0.657 ± 0.114^{B}	0.377 ± 0.075^{B}	.024
3	0.000 ± 0.000^{C}	0.000 ± 0.000^{C}	†¥
4	0.000 ± 0.000^{C}	0.000 ± 0.000^{C}	†¥
5	0.890 ± 0.098^{A}	0.630 ± 0.075^{D}	.022
6	0.480 ± 0.145^{D}	0.137 ± 0.121^{F}	.035
7	0.000 ± 0.000^{C}	0.000 ± 0.000^{C}	†¥
8	0.000 ± 0.000^{C}	0.000 ± 0.000^{C}	†¥

[€] Presented values are the means of 3 replications. [Δ] Appendix B (Table B-3) provides the experimental data used to construct this table. [£] Numbers with different letters within the same column are significantly different ($P < 0.05$). [†]The independent samples T test could not be computed because the standard deviations of both groups are zero. [¥] Neither group presented visible SWO_{BH}.

The different production methods applied did not have a significant influence ($P < 0.05$) on the SWO_{BH} level of the majority of the samples tested. Only samples 2, 5, and 6 presented significant different values of SWO_{BH} ($P < 0.05$) when made by different methods. These samples presented a higher level of SWO_{BH} when they were produced by

method 1. Regardless of the production method, samples containing higher amounts of MPC-85 presented higher SWO_{BH} levels. As WPI-90 and NaCN concentrations increased, the SWO_{BH} level diminished. The SC content had significant influence ($P < 0.05$) on samples that did not contain NaCN (except for samples 1 and 5 made by method 1). These types of samples containing higher SC content presented lower SWO_{BH}. This last observation agrees with Lee & Lucey (2004a), who reported that whey separation decreases as the inoculation rate increases. All these trends can be clearly seen in **Figures 3-15** and **3-16**.

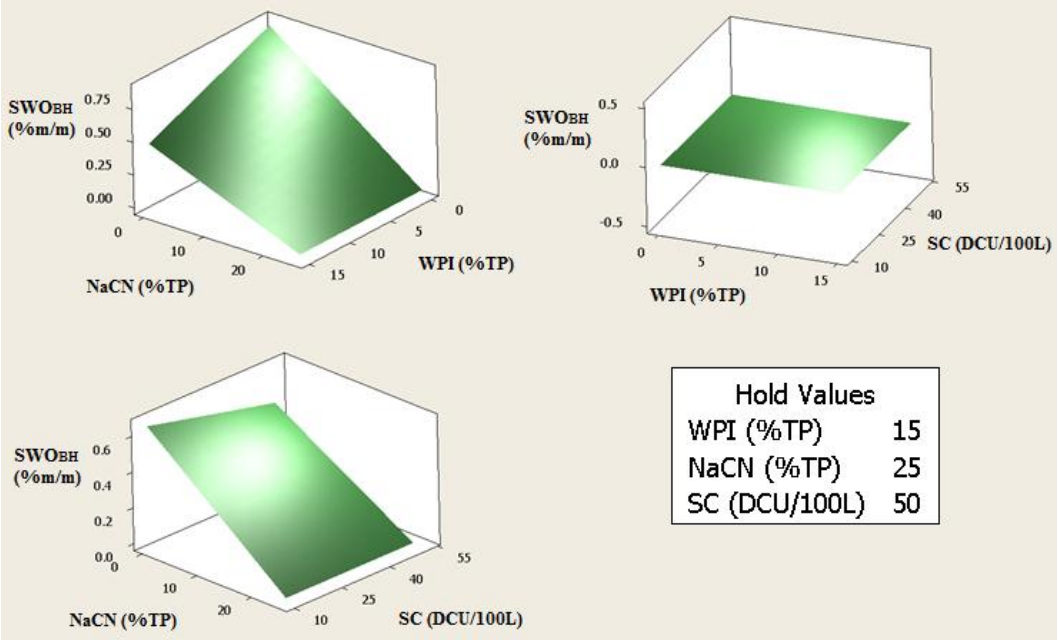


Figure 3-15: Effects of MPC-85, NaCN, WPI-90, and SC concentrations on the SWO_{BH} level of samples produced by production method 1

Surface plots were constructed using the means of triplicate measurements. Appendix B (Table B-3) provides the experimental data used to construct these graphs.

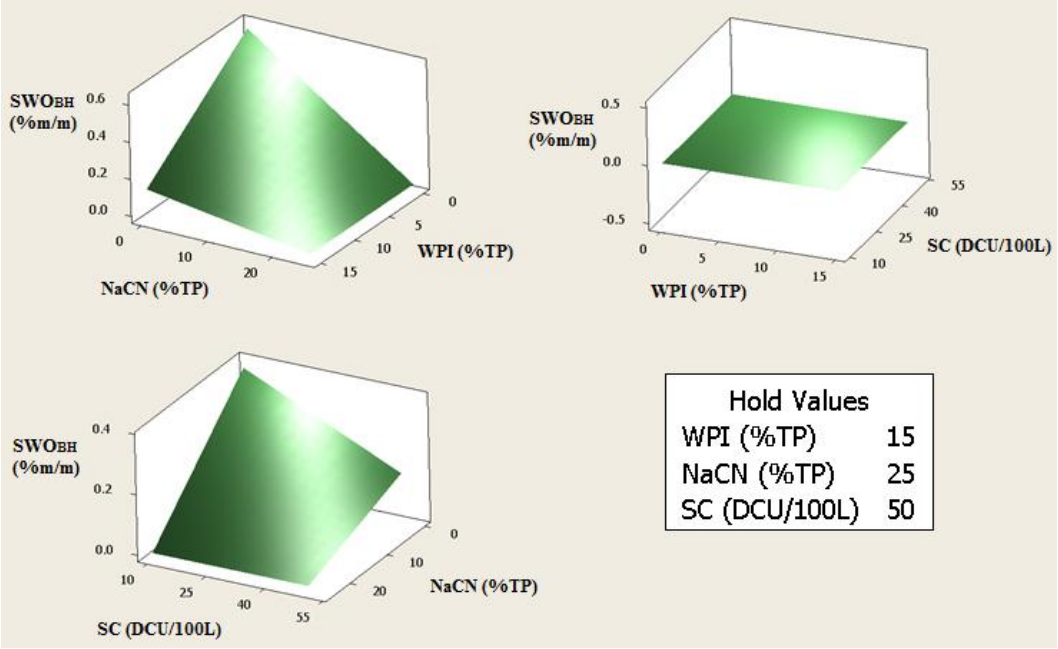


Figure 3-16: Effects of MPC-85, NaCN, WPI-90, and SC concentrations on the SWO_{BH} level of samples produced by production method 2

Surface plots were constructed using the means of triplicate measurements. Appendix B (Table B-3) provides the experimental data used to construct these graphs.

According to the previous figures, similar effects of factors on the SWO_{BH} level were observed for the different production methods considered. However, dissimilar arguments can be made to explain the results generated from different methods.

Samples made by method 1 that did not contain WPI-90 or NaCN presented the highest SWO_{BH} values. This result relies on the fact that almost all the undenatured whey proteins (present in WPI-90) or casein particles (present in NaCN) added to the milk base could not strongly interact with other proteins during the formation of the gel network. Due to this fact, added molecules probably acted as a filter in the gel matrix, increasing the permeability of the resultant gel. As a result, samples with higher WPI-90 and/or NaCN contents presented highly porous structures, hence, the free whey present inside these samples did not appear on top of the gels but drained towards to bottom through the large pores in the gel matrix. In this way, samples with high WPI-90 contents presented low levels of SWO_{BH} but high levels of WD. However, samples containing high NaCN contents had lower levels of WD than the previous ones because of casein's strong water-binding properties (Mistry & Hassan, 1992).

On the other hand, samples produced by method 2 that contained high levels of WPI-90 had lower SWO_{BH} values than samples containing low amounts of WPI-90. Increasing the whey protein to casein ratio in the milk base before heat treatment improves the water binding capacity of the yogurt coagulum (Sodini *et al*., 2004). High amounts of denatured whey proteins resulted in an increase in the degree of protein-protein interactions in the gel matrix, which increased the compactness of the yogurt's microstructure. Puvanenthiran *et al*. (2002) studied the microstructure of acid yogurt gels using scanning electron microscopy and noted that gels with lower casein to whey protein ratios had finer structures with numerous small pores and a dense network of cross-links. Thus, they suggested that an increase in the compactness of the yogurt's microstructure due to a reduction in the casein to protein ratio led to high level of immobilization of free water in the yogurt gel. As a result, a lower level of syneresis is expected for yogurts with low casein to whey protein ratios. These results are consistent with the observations made by Guzman-Gonzalez *et al.* (1999); Bhullar *et al.* (2002); Remeuf *et al.* (2003); Alkalin *et al.* (2012); Amatayakul *et al*. (2006); and Isleten & Karagul-Yuceer (2006).

None of the samples containing NaCN presented visible SWO_{BH}. These results agree with Mistry & Hassan (1992), who stated that yogurt prepared with casein-supplemented milk had low whey separation because of water binding by casein. Peng *et al*. (2009) reported that fortification with NaCN resulted in yogurt products with less syneresis than yogurts enriched with skim milk powder. Modler *et al*. (1983) stated that yogurts made with additional casein-based ingredients were firmer and showed less syneresis than yogurts fortified at the same protein level with whey protein-based ingredients. However, other authors reported different results. Akalin *et al*, 2012 and Guinee *et al.* (1995) reported that yogurts fortified with caseinate had lower WHC than those enriched with WPC. This was also confirmed by Guzman-Gonzalez *et al.* (1999, 2000), who found that the WHC of yogurt was increased by 77% when WPC partially replaced skim milk concentrate, whereas the increase was only 39% and 2%, respectively, when coprecipitate and caseinate were used instead of WPC (Sodini *et al*., 2004).

Excessive rearrangements of particles in the gel network are responsible for high levels of whey separation (Lucey, 2001). Several authors stated correlations between some possible rheological parameters of the gel network that may indicate rearrangements of milk gels and their tendency to exhibit whey separation. The following correlations were identified:

I. An increase in the value of the maximum in the loss tangent (tan δ) goes along with higher whey separation (van Vliet *et al*., 1991; Lucey, 2001; Lee & Lucey, 2004a; Lee & Lucey, 2004b)

II. Low storage modulus (G') values are related to higher whey separation (Lucey, 2001; Lee & Lucey, 2004a; Lee & Lucey, 2004b; Weidendorfer *et al*., 2008)

III. A low fracture stress ($\sigma_{fracture}$) or yield stress(σ_{yield}) is associated with higher whey separation (van Vliet *et al*., 1991; Lucey, 2001)

IV. A low fracture strain ($\gamma_{fracture}$) or shear deformation is related to higher whey separation (Lucey, 2001).

It should be emphasized that for syneresis to occur, a combination of these conditions must be met, e.g., a low value for G' and a low $\sigma_{fracture}$. If only one of these conditions is met, spontaneous whey separation may not occur, e.g., a low value for storage modulus alone is not responsible for spontaneous whey separation (Lucey, 2001).

As discussed previously, high values of tan δ are related to a high number of relaxation bonds present in the gel matrix; hence, this parameter can be used as a qualitative measure of the gel's relaxation behavior (van Vliet *et al*., 1991; Lucey & Singh, 1998). Therefore, high levels of tan δ are associated with an increased possibility for rearrangements of particles in the gel network, which favors spontaneous whey separation (Lee & Lucey, 2004b).

A low G' denotes that the number and/or strength of non-relaxing protein bonds in the gel network are low enough to be easily broken by stresses in the network caused by ongoing fusion of particles and/or strands (Özer *et al*., 1998a; Lucey, 2001). In gels with high G' there would be a high counter pressure to prevent excessive syneresis of the network. This high counter pressure is probably the reason that gels with high G' have little whey separation. In contrast, gels with low G' exhibit a lower counter pressure to prevent syneresis; thus, they present a high affinity for exhibiting considerable levels of surface whey-off (Lucey, 2001).

The $\sigma_{fracture}$ and $\gamma_{fracture}$ determine the susceptibility of the protein strands to breakage (Lucey & Singh, 1998). The $\sigma_{fracture}$ is the value of share stress at which the gel network starts to break down (this value is dependent on the rate of shearing). The $\gamma_{fracture}$ is the value of strain at the point the network starts to break down (Lucey, 2001). Both

parameters will depend on the number of intermolecular protein-protein bonds per cross section and their properties (van Vliet *et al*., 1991). A low $\sigma_{fracture}$ value indicates a weak or soft gel while low values of $\gamma_{fracture}$ denote a "brittle" or "short" texture. Both fracture properties can be used as indicators of possible rearrangements in the gel network, e.g., a reduction in the $\gamma_{fracture}$ can often be related to straightening of the strands that in turn may be caused by rearrangements due to the fusion of particles and the formation of extra bonds between particles (Lucey, 2001).

All these correlations demonstrate that rearrangements of casein particles in the gel network are an important driving force responsible for whey separation (Lee & Lucey, 2004a). Weak yogurt gels, which have high tan δ, low G', and low $\sigma_{fracture}$ and $\gamma_{fracture}$, favor rearrangements in the network; hence, they have a higher tendency to exhibit whey separation (Lee & Lucey, 2004b).

These correlations match the results obtained for production method 1. In this case, samples that presented the highest tan δ, the lowest G', and the lowest $\sigma_{fracture}$ values exhibited the highest levels of whey separation as whey drainage (due to their highly porous structures), with the exception of samples containing high levels of NaCN. Previous correlations can also be used to explain the level of SWO obtained in samples produced by method 2. Samples containing high levels of WPI-90 and/or NaCN presented a high number and/or strength of non-relaxing protein bonds in their network (high G' values), a low susceptibility of the strands to breakage (high $\sigma_{fracture}$ values), a low relaxation behavior (low tan δ), and low levels of SWO_{BH}. As the amounts of NaCN and WPI-90 diminished, the G' and $\sigma_{fracture}$ values decreased and the tan δ and SWO_{BH} values increased. Consequently, as the concentration of these protein sources decreased, the possibilities for rearrangements in the gel network increased. This is the reason that the concentrations of NaCN and WPI-90 were inversely related to the probability of obtaining high levels of SWO_{BH} in the experimental samples.

3.3.2.2.2 Whey drainage (WD)

Table 3-10 illustrates the different degrees of WD for the all the samples tested. The manufacturing method used had a significant influence ($P < 0.05$) on the level of WD in the final samples. With the exception of samples 5 and 7, all samples produced by method 1 had significantly higher WD levels than samples made by method 2. None of

the samples produced by method 2 presented visible levels of WD. Method 1 samples that contained high WPI-90 contents presented the highest degree of WD. SC contents had a significant influence ($P < 0.05$) on the WD level in method 1 samples (with exception of samples containing high amounts of MPC-85). Higher amounts of SC resulted in lower WD in the final gels.

Table 3-10: Whey drainage present in samples [€, Δ]

Sample #	Level of Whey Drainage (mean ± SD)		P-value
	Method 1[£]	Method 2[£]	
1	$0.333 \pm 0.577^{A,B}$	0.000 ± 0.000^{A}	.374
2	2.000 ± 0.000^{C}	0.000 ± 0.000^{A}	†§
3	0.667 ± 0.577^{B}	0.000 ± 0.000^{A}	.116
4	2.000 ± 0.000^{C}	0.000 ± 0.000^{A}	†§
5	0.000 ± 0.000^{A}	0.000 ± 0.000^{A}	†¥
6	2.000 ± 0.000^{C}	0.000 ± 0.000^{A}	†§
7	0.000 ± 0.000^{A}	0.000 ± 0.000^{A}	†¥
8	1.333 ± 0.577^{D}	0.000 ± 0.000^{A}	.016

[€] Presented values are the means of 3 replications. [Δ]Appendix B (Table B-3) provides the experimental data used to construct this table. [£] Numbers with different letters within the same column are significantly different ($P < 0.05$). [†]The independent samples T test could not be computed because the standard deviations of both groups are zero. [¥] Both groups presented the same level of whey drainage. [§] The difference between both groups was ≥ 1.

The following surface plots (**Figure 3-17**) represent the effect of the different factors on the level of WD of yogurt produced by method 1.

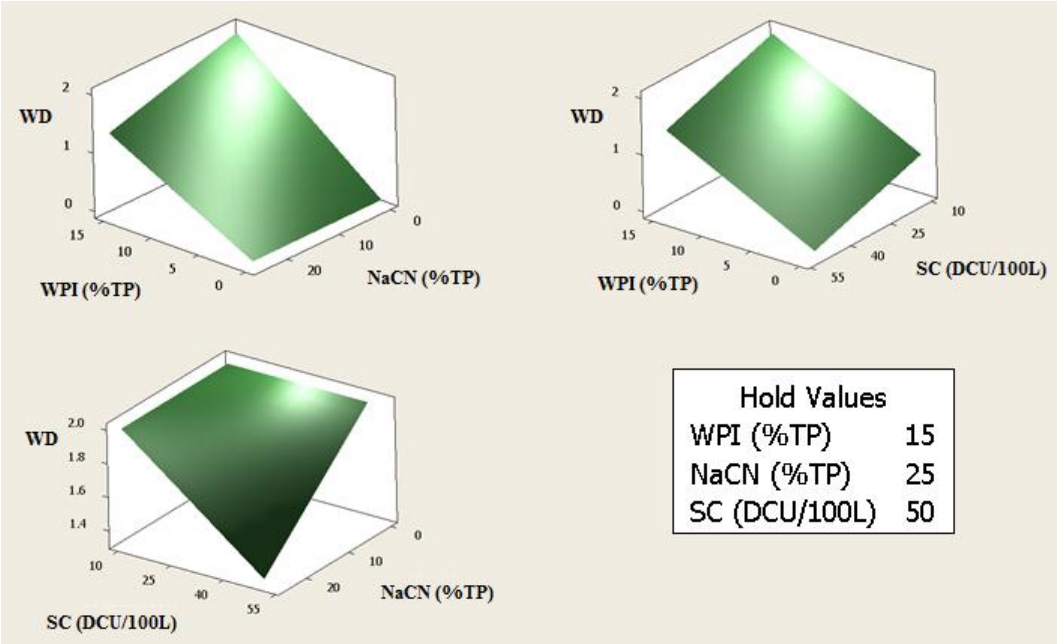


Figure 3-17: Effects of MPC-85, NaCN, WPI-90, and SC concentrations on the level of WD of samples produced by production method 1

Surface plots were constructed using the means of triplicate measurements. Appendix B (Table B-3) provides the experimental data used to construct these graphs.

The difference in WD levels in the different samples produced by method 1 is related to the different amounts of undenatured whey proteins, caseins and SC present in the final product. As explained before, samples produced by method 1, with high levels of WPI-90, presented high amounts of undenatured whey proteins in the final gels. These proteins could not interact with κ-casein; thus, a lower degree of protein interactions was obtained inside these gel networks. Due to this fact, undenatured proteins probably acted as a filter in the gel networks, increasing the permeability of the final gels (reducing their WHC) and, increasing the level of WD (Lucey *et al*., 1999). Basically the same effect occurred when adding high levels of NaCN, although lower levels of WD were observed in these cases because of the strong water-binding properties of casein (Mistry & Hassan, 1992). On the other hand, WD increased when the SC amount decreased. This last observation agrees with the findings of Lee & Lucey (2004a), who stated that permeability, pore size, and whey separation of yogurt gels increased with decreased inoculation rate.

Samples produced by method 1 had considerably lower levels of denatured whey proteins in their structure than samples produced by method 2. For this reason, gels made by method 2 presented finer structures with numerous smaller pores and a denser network of crosslinks than gels produced by method 1. As the movement of fluid out of the gel under the force of gravity is essentially related to the gel's permeability, coarser, more open structures have a higher drainage than finer networks (Puvanenthiran *et al*., 2002).

3.3.2.2.3 Presence and size of clusters

According to **Table 3-11**, all tested samples presented visible clusters. The production methods applied to the manufacture of samples had a significant effect ($P < 0.05$) on the size of clusters present in the final products. Samples 2,4 ,6, and 8 made by method 2 had significantly bigger clusters than the same samples made by method 1. Regardless of the production method, the SC content did not have significant influence ($P > 0.05$) on the magnitudes of the clusters formed.

Table 3-11: Size of visible clusters present in samples [€, Δ]

Sample #	Size of Visible Clusters (mean ± SD)		P-value
	Method 1[£]	Method 2[£]	
1	2.667 ± 0.577^{A}	2.333 ± 0.577^{A}	.519
2	2.000 ± 0.000^{B}	3.000 ± 0.000^{A}	†§
3	1.000 ± 0.000^{C}	1.000 ± 0.000^{B}	†¥
4	1.000 ± 0.000^{C}	3.000 ± 0.000^{A}	†§
5	$2.333 \pm 0.577^{A,B}$	2.667 ± 0.577^{A}	.519
6	2.000 ± 0.000^{B}	3.000 ± 0.000^{A}	†§
7	1.000 ± 0.000^{C}	1.000 ± 0.000^{B}	†¥
8	1.000 ± 0.000^{C}	2.667 ± 0.577^{A}	.007

[€] Presented values are the means of 3 replications. [Δ] Appendix B (Table B-3) provides the experimental data used to construct this table. [£] Numbers with different letters within the same column are significantly different ($P < 0.05$). [†]The independent samples T test could not be computed because the standard deviations of both groups are zero. [¥] Both groups presented the same level of clusters. [§] The difference between both groups is ≥ 1.

Concerning production method 1, there was a direct correlation between the amount of MPC-85 and the size of the clusters formed. As the level of MPC-85 was reduced, smaller clusters were obtained (regardless of the type of protein source added to substitute MPC-85). These tendencies can be clearly seen in the following figure.

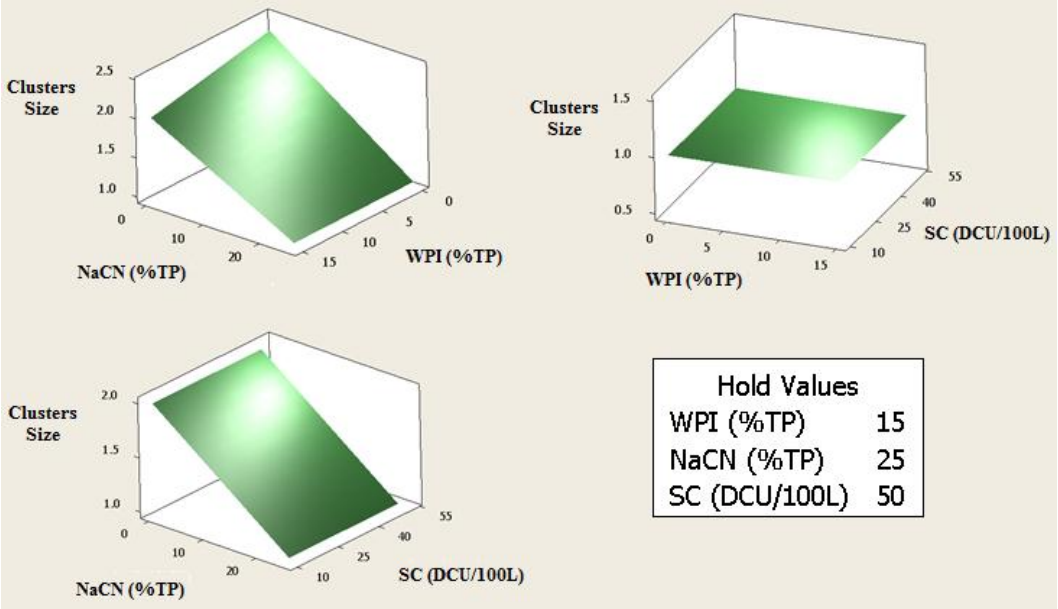


Figure 3-18: Effects of MPC-85, NaCN, WPI-90, and SC concentrations on the size of clusters present in samples produced by production method 1

Surface plots were constructed using the means of triplicate measurements. Appendix B (Table B-3) provides the experimental data used to construct these graphs.

In relation to production method 2, as the casein to whey protein ratios of samples decreased, the size of clusters increased (**Figure 3-19**). However, samples containing casein to whey protein ratios of 2.2:1, 2.8:1, and 4.6:1 did not present a significant difference ($P < 0.05$) in cluster size. These results agree with those obtained by Puvanenthiran *et al.* (2002), Kucukcetin (2008), Mistry & Hassan (1992), Remeuf *et al*. (2003), Beaulieu *et al.* (1999), and Krzeminski *et al.* (2011) who reported that the size of clusters present in yogurt samples increased as the ratio of casein to whey protein in the milk base decreased.

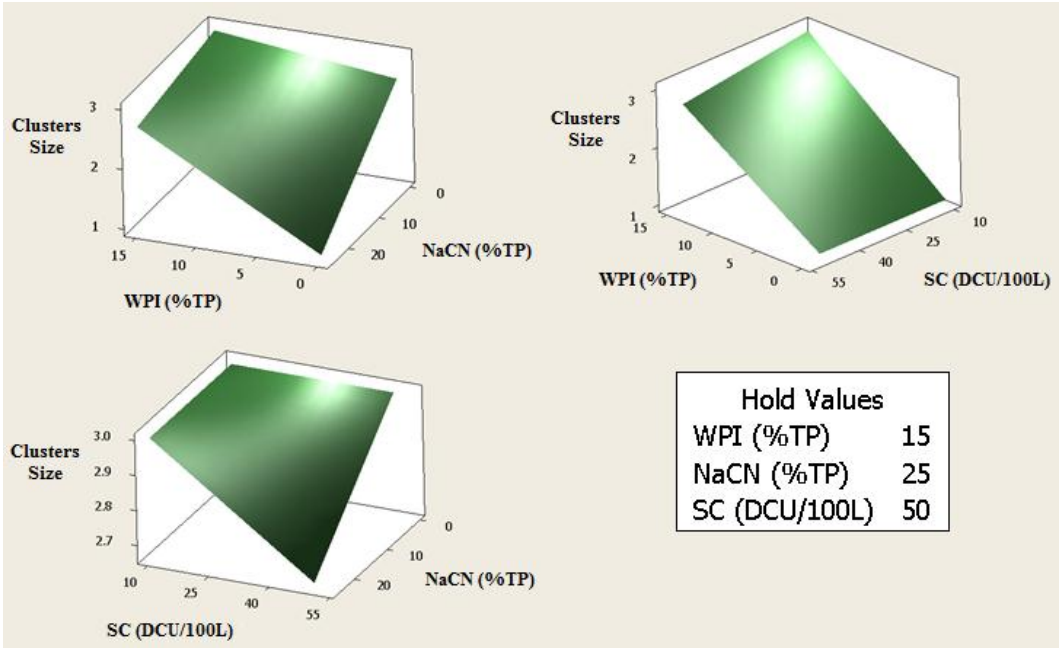


Figure 3-19: Effects of MPC-85, NaCN, WPI-90, and SC concentrations on the size of clusters present in samples produced by production method 2

Surface plots were constructed using the means of triplicate measurements. Appendix B (Table B-3) provides the experimental data used to construct these graphs.

Generally, enriching the milk base with whey protein often gives a granny texture, particularly when milk is submitted to severe heat (Sodini *et al.*, 2004). Remeuf *et al*. (2003) reported that milk enriched with WPC led to a marked increase of micelle size after heating (90^{o}C/5minutes). They argued that the formation of aggregates involving cross-links between the casein micelles and denatured whey proteins can be responsible for an increase of micelle size after heating, and this mechanism is enhanced when the whey protein to casein ratio increases. On the other hand, Beaulieue *et al*. (1999) stated that high levels of whey proteins can lead to the saturation of all of the capacity for binding of κ-casein to whey protein, and that once this occurs, aggregates of whey proteins are formed (Puvanenthiran *et al.*, 2002). The formation of large aggregates of whey proteins after heating is also possible. Heat-induced formation of aggregates could contribute to the increase of mean particle size in milk bases highly enriched with WPC (Remeuf *et al*., 2003).

These two mechanisms (the increase of micelles size and the formation of whey protein aggregates) are responsible for the presence of large clusters in yogurt samples enriched with whey proteins. As the concentration of whey protein increases, it is

expected that there will be a corresponding increase in the micelles diameter and in the occurrence of whey protein to whey protein interactions (Puvanenthiran *et al.*, 2002; Remeuf *et al*., 2003). This is the reason why samples produced by method 2 which contained the highest levels of WPI-90 presented the biggest clusters.

Moreover, samples made by method 2 presented bigger clusters than those made by method 1 because the latter method did not involve a heat treatment step. However, samples produced by method 1 that contained high amounts of MPC-85 presented bigger clusters than those containing low levels of MPC-85. These results suggest that part of the whey proteins present in the MPC-85 were denatured. For this reason, samples produced by method 1 which had high amounts of MPC-85 presented higher dynamic moduli and bigger clusters than the other samples manufactured by the same production method.

3.3.2.2.4 Incubation time (IT)

According to **Table 3-12**, the method used for the production of samples had a significant influence ($P < 0.05$) on the incubation times needed for most of the tested samples. Samples required significantly shorter incubation times when produced by method 2 (with the exception of samples 5 and 8). As stated by Oliveira *et al.* (2001), significant interaction was noted between milk supplementation and culture composition on acidifying kinetics. SC contents had a significant effect on the incubation times required for all experimental samples ($P < 0.05$). Samples containing higher SC contents needed shorter incubation times to reach pH 4.6 ± 0.3. Regardless of the production method applied, the incubation times required for all yogurts were inversely related to the amount of MPC-85 present in the samples. Samples containing the same SC content but higher amounts of MPC-85 presented significantly higher incubation times ($P < 0.05$).

Table 3-12: Incubation times required for the production of samples [€, Δ]

Sample #	Incubation Times (Hours) (mean ± SD)		P-value
	Method 1[£]	Method 2[£]	
1	14.600 ± 0.265[A]	13.967 ± 0.115[A]	.019
2	13.700 ± 0.200[B]	13.033 ± 0.115[B]	.007
3	13.233 ± 0.058[B]	12.600 ± 0.100[C]	.001
4	12.133 ± 0.058[C]	11.367 ± 0.058[D]	.000
5	11.633 ± 0.666[D]	10.967 ± 0.153[E]	.166
6	11.267 ± 0.058[D,E]	10.633 ± 0.058[F]	.000
7	11.067 ± 0.208[E]	10.500 ± 0.173[F]	.022
8	10.033 ± 0.153[F]	9.967 ± 0.058[G]	.519

[€] Presented values are the means of 3 replications. [Δ] Appendix B (Table B-3) provides the experimental data used to construct this table. [£] Numbers with different letters within the same column are significantly different ($P < 0.05$).

All these trends can be clearly seen in **Figures 3-20** and **3-21,** which illustrate the effects of the different factors on the incubation times.

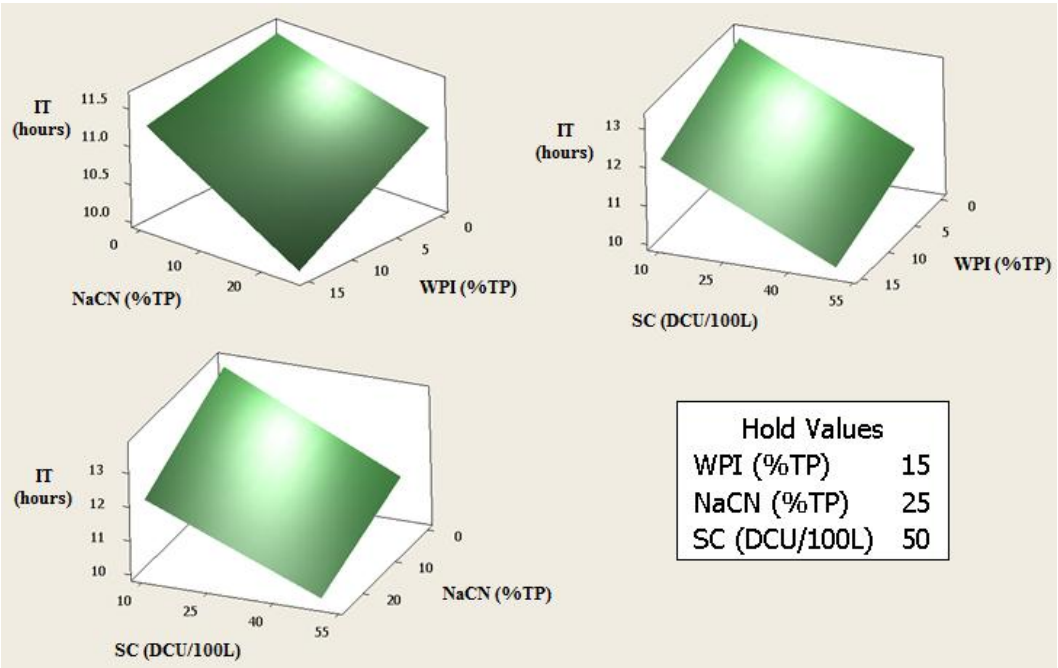


Figure 3-20: Effects of MPC-85, NaCN, WPI-90, and SC concentrations on the incubation times of samples produced by production method 1

Surface plots were constructed using the means of triplicate measurements. Appendix B (Table B-3) provides the experimental data used to construct these graphs.

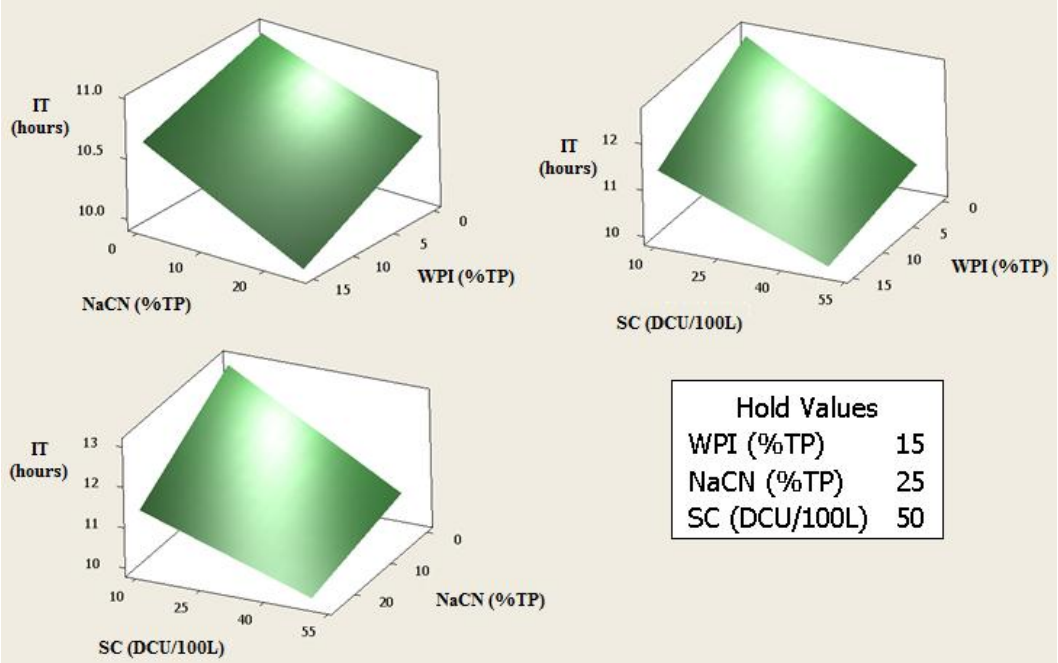


Figure 3-21: Effects of MPC-85, NaCN, WPI-90, and SC concentrations on the incubation times of samples produced by production method 2

Surface plots were constructed using the means of triplicate measurements. Appendix B (Table B-3) provides the experimental data used to construct these graphs.

The different effects of factors on the incubation times agree with results from literature. Jumah *et al.* (2001b) and Wu *et al*. (2009) reported that as the SC concentration was increased, gelation time was reduced. Lucey *et al.* (1999) stated that the addition of whey protein to milk followed by heat treatment caused a reduction in the gelation time. Due to this fact, almost all samples with the same casein to whey protein ratio produced by method 2 required significantly lower fermentation times than those produced by method 1. On the other hand, Peng *et al*. (2009) and Damin *et al*. (2009) noticed a decrease in fermentation time when enriching the milk base with NaCN. The addition of NaCN significantly reduces the buffering capacity of the yogurt mix by apparently solibilizing part of the indigenous CCP present in the milk base; consequently, a lower incubation time is expected for samples supplemented with NaCN (Peng *et al.*, 2009).

3.3.3 Proposed formulation

Based on the rheological and physicochemical results obtained from the different combinations of factors and methods applied, a potential formulation was proposed for the production of Greek-style yogurt powder.

This work proved that the level of denaturation of whey proteins greatly affects the formation and properties of acid milk gels. As the application of production method 2 gave rise to products with rheological and physicochemical aspects that were more similar to the reference samples than those made by production method 1, it was demonstrated that heat-treated milk powders (especially whey protein powders) should be recommended for the production of Greek-style yogurt powder. Furthermore, significant effects on rheological and physicochemical properties were noted when varying the total protein composition and the SC levels of samples. Therefore, contour plots were used to select the combination of protein sources and SC that best suit the rheological and physicochemical aspects of the references samples.

According to the results obtained, one of the most important physicochemical aspects that limits the range of possible formulations that can be applied for the production of Greek-style yogurt powder is the size of clusters formed in the final products. As noted, the size of clusters present in samples was significantly affected ($P < 0.05$) by the amounts of the different protein sources added. However, SC content did not have a significant effect ($P > 0.05$) on the size of clusters. The following contour plot (**Figure 3-22**) represents the effects of the different concentrations of protein sources (at high SC level) on the size of visible clusters present in final products.

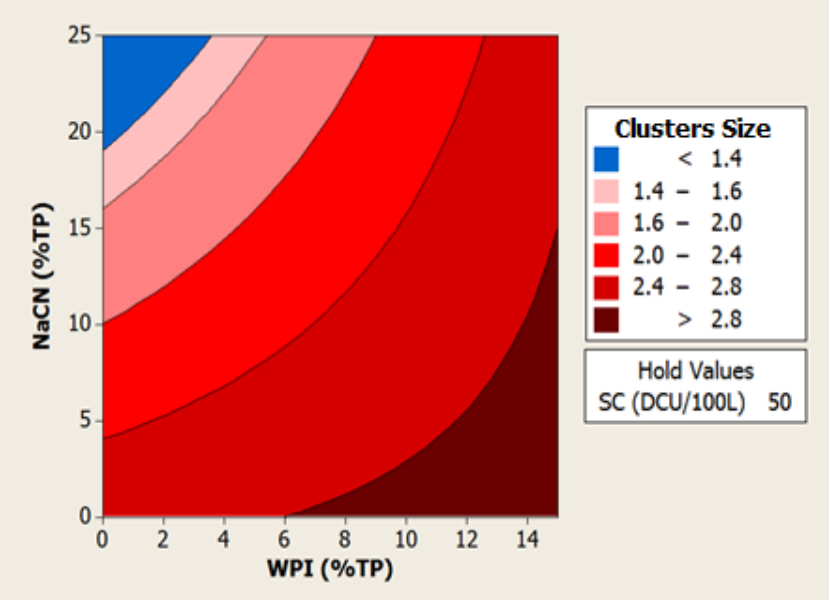


Figure 3-22: Effects of MPC-85, NaCN and WPI-90 concentrations (at high SC level) on the size of clusters present in samples produced by production method 2

This contour plot was constructed using the means of triplicate measurements. Appendix B (Table B-3) provides the experimental data used to construct this graph.

Combinations of factors inside the blue area (cluster size ≤ 1.4) were considered to produce small clusters (clusters classified at an ordinal level = 1) similar to the ones present in the reference samples. Hence, the combinations of factors available for the production of yogurt, with visible clusters similar in size to those in the reference samples, were limited to the range of formulations present in the blue area.

The following set of counter plots (**Figure 3-23**) was constructed in order to observe if the previous selected formulations (present in the blue area) could be used to obtain G' values significantly similar to those in the reference samples throughout all the sweeping amplitude range applied.

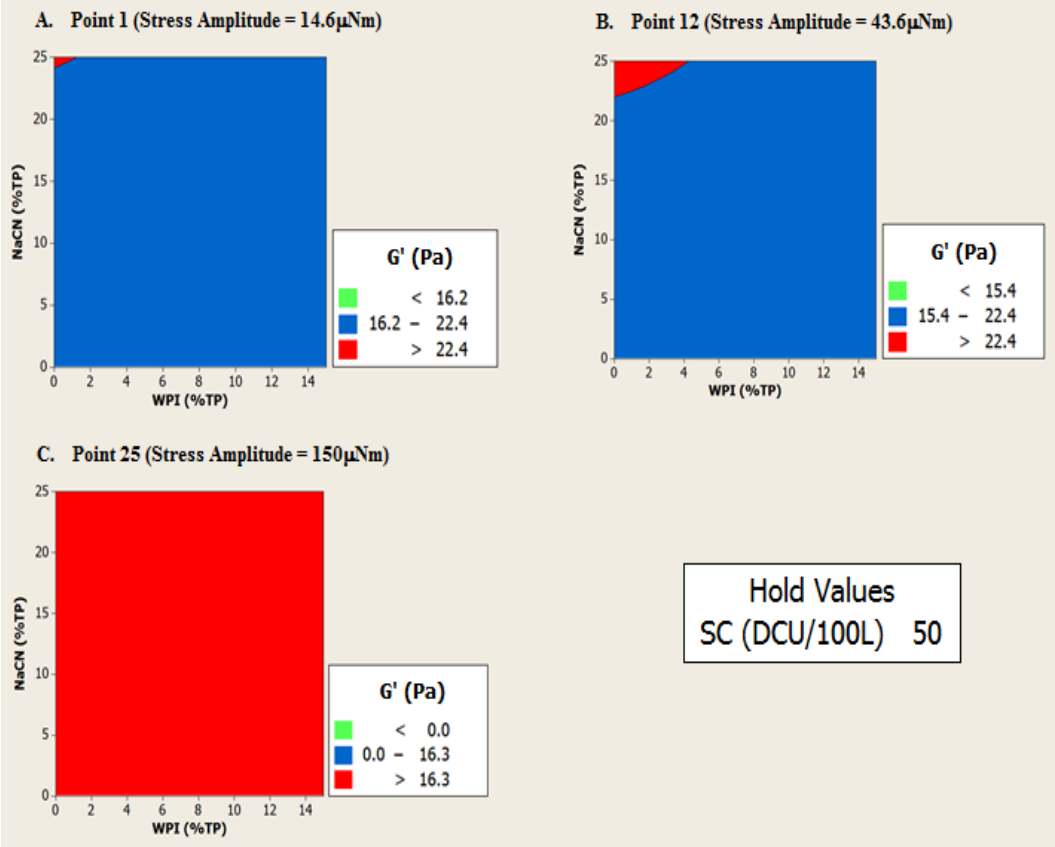


Figure 3-23: Effects of MPC-85, NaCN and WPI-90 concentrations (at high SC level) on the storage modulus (G') of samples produced by production method 2 at three selected points of the stress amplitude range applied

Contour plots were constructed using the means of triplicate measurements. Appendix B (Tables B-12 to B-19) provides the experimental data used to construct these graphs.

The two-sided confidence interval range ($\alpha = 0.05$) of the reference samples (See **Table 3-5**) was used for comparative purposes in order to establish which combinations of factors could give G' values significantly similar ($P > 0.05$) to those of the reference samples at three main points of the sweep amplitude range applied. According to the previous figure, blue areas represent the combinations of factors that could be used to obtain G' values within the reference confidence interval ranges. Red areas indicate the combination of factors which resulted in G' values significantly different ($P < 0.05$) from those of the reference samples.

Consistent with **Figure 3-23**, at point 25 of the sweep amplitude range applied (stress amplitude = 150 µNm), all possible combinations of protein sources at high SC level resulted in G' values significantly different ($P < 0.05$) from those of the reference

samples. Due to this fact, the SC contents were varied in order to see if another SC level could be used to obtain yogurts with small clusters and non-significantly different G' values at the three amplitude points tested. For the range of formulations that resulted in small clusters, no SC concentration within the range considered (10-50DCU/100L) could be used to obtain G' values inside the confidence interval limits of the reference samples at point 25 of the sweep amplitude range applied. This means that, none of the combinations of the different factors considered could result in a product significantly similar ($P > 0.05$) to the reference samples in terms of cluster size and G' values at the three amplitude points considered.

Figure 3-24 was designed in order to learn which combination of factors could be used to obtain tan δ values significantly similar to those of the reference samples ($P > 0.05$).

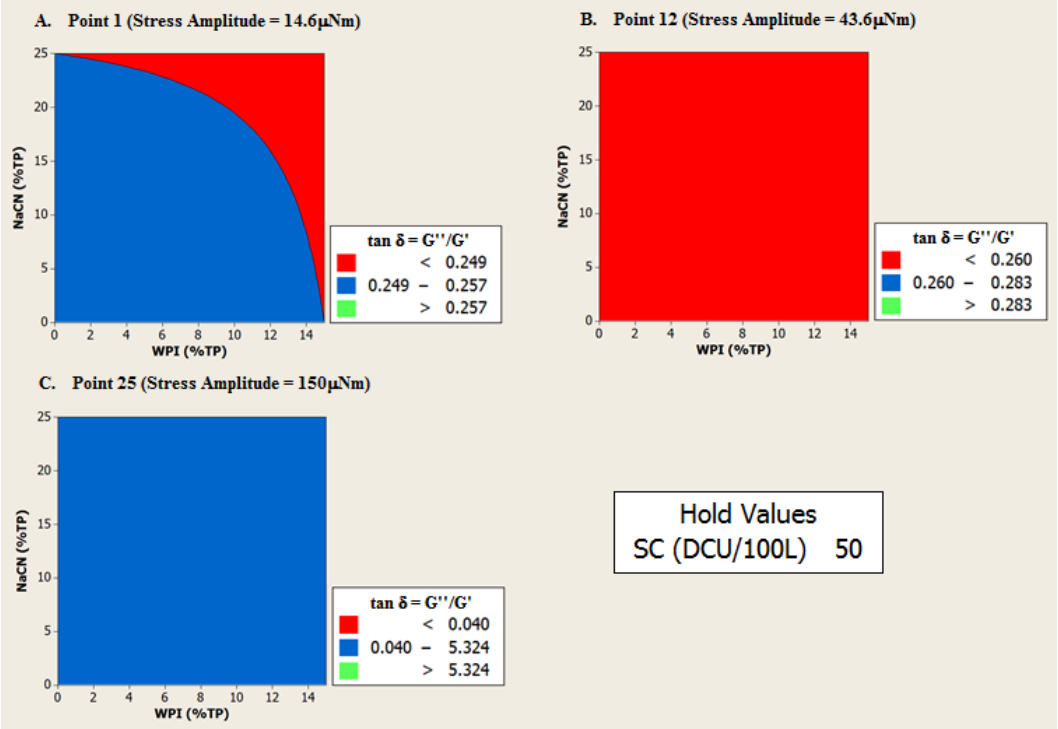


Figure 3-24: Effects of MPC-85, NaCN and WPI-90 concentrations (at high SC level) on the loss tangent (tan δ = G''/G') of samples produced by production method 2 at three selected points of the stress amplitude range applied

Contour plots were constructed using the means of triplicate measurements. Appendix B (Tables B-12 to B-19) provides the experimental data used to construct these graphs.

According to the previous figure, at point 12 of the sweep amplitude range applied (stress amplitude = 43.6 μNm), all possible combinations of protein sources at the highest SC level resulted in tan δ values significantly different ($P < 0.05$) from those of the reference samples. For the range of formulations that resulted in small clusters, no SC concentration within the range considered (10-50DCU/100L) could be used to obtain tan δ values inside the confidence interval limits of the reference samples at point 12 of the sweep amplitude range applied.

Based on the results obtained in the previous graphs, the following overlaid contour plot (**Figure 3-25**) represents the range of formulations that best suited the rheological and physicochemical characteristics of the market reference samples considered. Formulations within the blue area were considered to present the closest physicochemical and rheological characteristics to the market references considered.

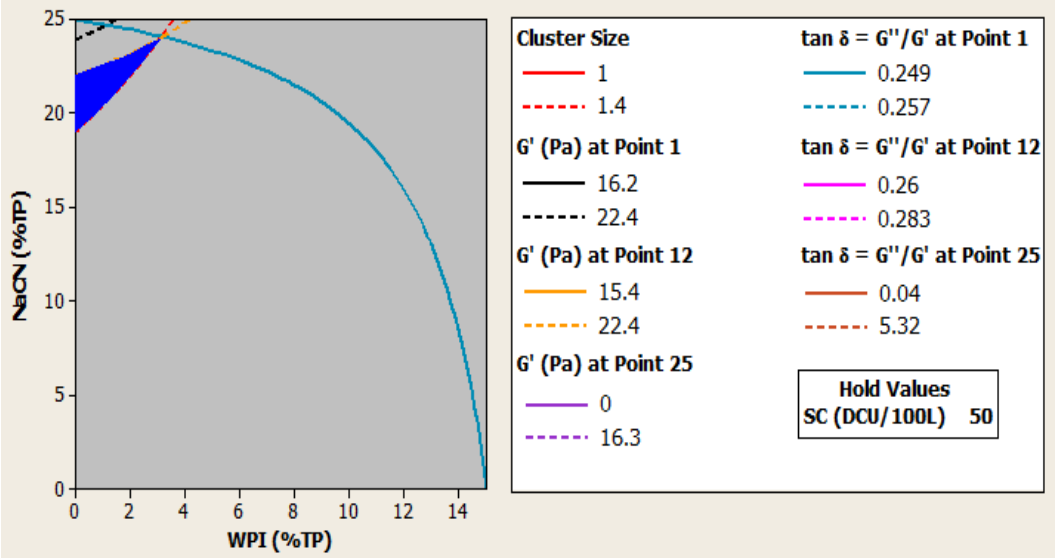


Figure 3-25: Overlaid contour plot of the effects of MPC-85, NaCN and WPI-90 concentrations (at high SC level) on the clusters' size and rheological properties of samples at three selected points of the stress amplitude range applied

This contour plot was constructed using the means of triplicate measurements. Lines represent the two sided confidence interval limits ($\alpha = 0.05$) of reference samples. Appendix B (Tables B-3 and B-12 to B-19) provides the experimental data used to construct this graph.

Presented results show that for the method of production applied (production method 2), no combination of factors resulted in a product with cluster size, G' and tan δ values (at the three amplitude points tested) significantly similar ($P > 0.05$) to those of the reference samples. However, it was possible to obtain a range of formulations that resulted in small clusters, and G' and tan δ values significantly similar to the reference samples ($P > 0.05$) at points 1 and 12, and 1 and 25 of the sweep amplitude range applied, respectively.

It is important to point out that SWO_{BH}, SWO_{AH}, WD, and IT were not considered as limiting parameters for the selection of formulations because none of the formulations that resulted in small clusters (samples containing high amounts of NaCN) presented considerable SWO (before or after homogenization) or WD levels, nor did they require long incubation times.

In order to select one formulation from the range of formulations that resulted in yogurts with small clusters and similar rheological parameters to the reference samples, the effects of the different factors on the physicochemical and rheological aspects of the final product were evaluated, as was the economic cost of each ingredient.

Due to its positive effect on the SWO_{BH}, WD, and IT of the final product, the highest SC level was recommended for use. Furthermore, the combination of protein sources that contained the lowest amount of NaCN was selected from the possible combinations to decrease the final product's production costs. **Table 3-13** illustrates the physicochemical composition of the inoculated recombined milk base that resulted from the reconstitution of the proposed formulation.

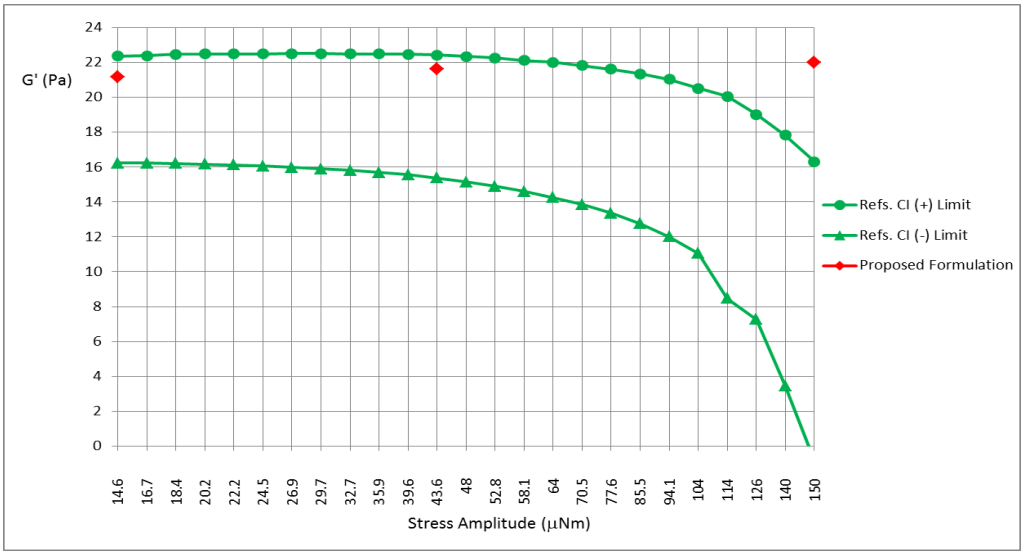
Table 3-13: Physicochemical composition of the inoculated reconstituted milk base obtained by the proposed formulation [§]

Component	Amount present in milk base
Total Solids (%)	13.7 min.
Total Protein (%) [MPC-85: 81%TP; NaCN: 19% TP]	10.3 min.
Ratio of casein to whey protein	6 : 1
Lactose (%)	2.3 max.
Fat (%)	0.5 max.
Ash (%)	1.1 max.
Starter Culture (DCU/100L)	50

[§] Data was calculated according to the composition of the ingredients used to reach the final formulation (See Appendix B, Table B-2).

Figure 3-26 shows the G' and tan δ values expected for the acid milk gel obtained by the proposed formulation at three selected points of the stress amplitude range applied and compares them with the reference samples' two-sided confidence interval limits (α = 0.05).

A.



B.

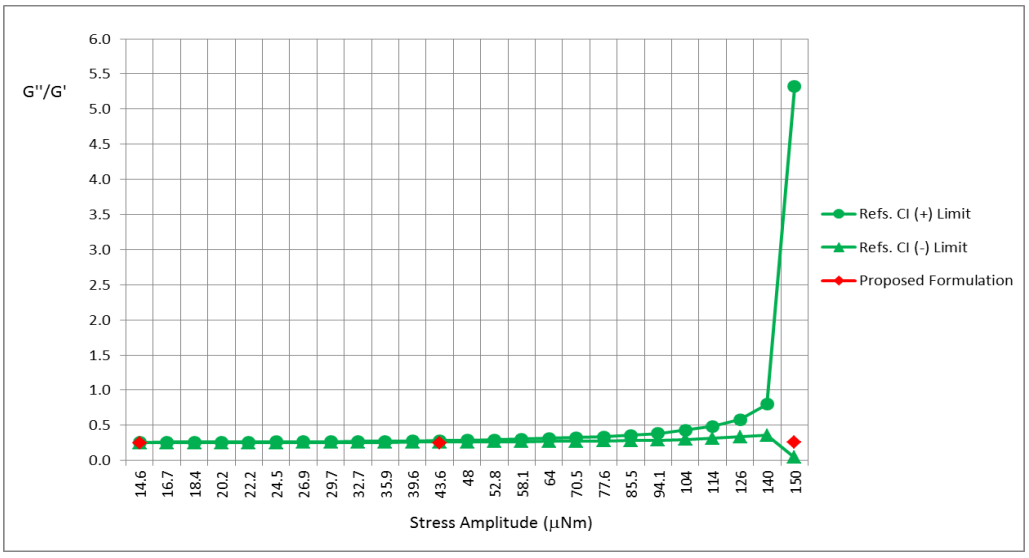


Figure 3-26: Storage modulus (G') and loss tangent (tan δ = G''/G') expected for the acid milk gel obtained by the proposed formulation at three selected points of the stress amplitude range applied

Refs. CI (+) Limit: Reference Confidence Interval Positive Limit ($\alpha = 0.05$). Refs. CI (-) Limit: Reference Confidence Interval Negative Limit ($\alpha = 0.05$). Data used to construct these graphs can be found in Appendices A (Tables A-6, A-7) and B (Tables B-12 to B-19).

According to the previous figures, the proposed formulation did not present rheological characteristics significantly similar ($P < 0.05$) to those of the reference samples for all the sweeping amplitude range applied. To obtain a product with rheological characteristics more similar to those of the reference samples (e.g., lower $\sigma_{fracture}$) it is suggested to work with a lower level of whey protein denaturation (less intense heat treatment). In this way, a higher quantity of whey proteins can be added to the final formulation (WPI-90; MPC-85), to substitute part of the NaCN, without resulting in the formation of big clusters. As discussed before, a lower amount of NaCN and a higher quantity of whey proteins at a lower denaturation level will result in gel networks with lower elasticity (G') and strength ($\sigma_{fracture}$); therefore, the rheological behavior of the final product will be much more similar to the reference samples. Although, it must be kept in mind that low levels of whey protein denaturation can lower the WHC of the resultant gel, causing syneresis defects. Thus, further investigation seems necessary to distinguish which level of whey protein denaturation can be used to obtain a final product with optimum qualities.

3.4 Conclusion

By means of the materials and methods applied in this study, it was feasible to produce a recombined, non-fat, additive-free type of acid milk gel with rheological and physicochemical aspects similar to those found in plain Greek-style yogurts (0% M.F.) commercialized in Edmonton, AB, Canada. This work demonstrated that the use of heat-treated milk powders (especially whey protein powders) should be recommended for the production of Greek-style yogurt powder. The results showed that heat-treated formulations containing high amounts of NaCN and low amounts or no presence of WPI-90 (casein-to-whey protein ratio ≈ 6:1) resulted in products with the best physicochemical characteristics. These findings are consistent with the results of Peng *et al.* (2009), Remeuf *et al.* (2003), and Isleten & Karagul-Yuceer (2006), who stated that yogurts fortified with NaCN to high casein to whey protein ratios had very good textural and sensory attributes. However, further research is needed to establish an optimal whey

protein denaturation level in order to obtain a final formulation with rheological characteristics closer to those found in the market reference samples.

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4. Physicochemical and rheological stability of the recombined yogurt obtained from the dry formulation proposed, and the application of a simplified method for the hydration of the yogurt powder

4.1 Introduction

Greek-style (concentrate) yogurt is produced by removing a proportion of the whey from cow's milk yogurt until fat and total solids contents of 9 to 11 and 23 to 25%, are attained, respectively (Al-Kadamany *et al*., 2002). The product obtained from this draining action has a better keeping quality than normal yogurt, mainly as a result of the higher concentration of lactic acid (Tamime & Robinson, 2007). Regular yogurt should have a shelf-life at 4-5^{o}C of 2-3 weeks, while for concentrated yogurt, storage for 4-6 weeks under refrigeration should be feasible. Spoilage, however, can occur through the activities of acid-tolerant yeasts, or occasionally moulds. Also, widely distributed yeasts including *Candida* or *Saccharomyces* spp., can be associated with gas formation and/or carton "doming" in fruit yogurts (Robinson, 2002).

Nevertheless, the storage ability of yogurt depends not only on its microbiological quality but also on its overall visual appearance, microstructure, and rheological properties, which contribute to the overall sensory perception and functionality of this product throughout its shelf-life (Lucey, 2002). A major concern facing the concentrated yogurt industry is the production and maintenance of a product with optimum consistency, stability and texture properties during its storage (Abu-Jdayil, *et al*., 2000). Due to this fact, when developing a new formulation for the production of a dried, non-fat, additive-free (no stabilizers, emulsifiers or preservatives) Greek-style yogurt, it is critical to control the rheological and physicochemical properties of the reconstituted product throughout its storage.

Another critical parameter concerning the development of a dried, concentrated yogurt is the reconstitution process needed to produce the final product before its consumption. As this preparation process is required to be done by the consumer, the reconstitution method proposed should be easy to perform, using common kitchen tools, and should be clearly explained to reduce the influence of the consumer's intervention on the quality of the final product.

The objectives of this study were: (1) to study, throughout the storage period, the rheological and physicochemical stability of the recombined acid milk gel obtained from the new formulation proposed in the previous chapter; (2) to study the effects of a new, simplified recombination method on the rheological and physicochemical aspects of the recombined product obtained from the previously proposed dry formulation.

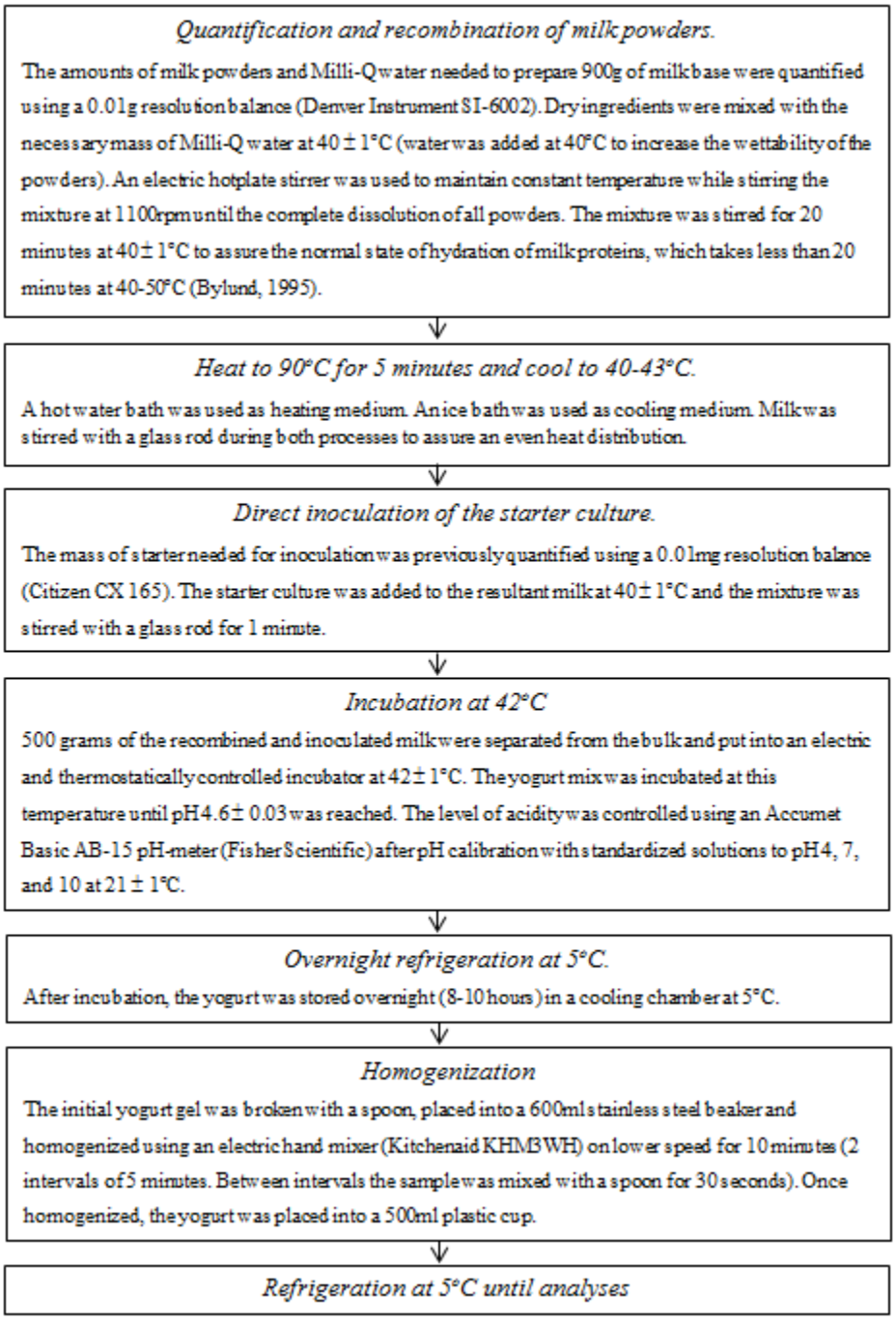
4.2 Materials & methods

Materials and determinations considered in this investigation were the same as the ones detailed in Section 3. Additionally, the total solids contents of the recombined samples were determined.

4.2.1 Yogurt manufacture

Experimental samples were produced in batch mode by direct recombination technology. All recombined yogurts were produced using the final formulation proposed in the previous chapter. Two different manufacture methods were applied in this study. Only one of these methods included a preheating step at 40^{o}C for 20 minutes (to achieve a normal state of hydration of milk proteins) before applying the heat treatment at 90^{o}C for 5 minutes. **Figure 4-1** illustrates in detail the different manufacturing procedures considered.

Method A


Quantification and recombination of milk powders.
The amounts of milk powders and Milli-Q water needed to prepare 900g of milk base were quantified using a 0.01g resolution balance (Denver Instrument SI-6002). Dry ingredients were mixed with the necessary mass of Milli-Q water at 40 ± 1°C (water was added at 40°C to increase the wettability of the powders). An electric hotplate stirrer was used to maintain constant temperature while stirring the mixture at 1100rpm until the complete dissolution of all powders. The mixture was stirred for 20 minutes at 40 ± 1°C to assure the normal state of hydration of milk proteins, which takes less than 20 minutes at 40-50°C (Bylund, 1995).
Heat to 90°C for 5 minutes and cool to 40-43°C.
A hot water bath was used as heating medium. An ice bath was used as cooling medium. Milk was stirred with a glass rod during both processes to assure an even heat distribution.
Direct inoculation of the starter culture.
The mass of starter needed for inoculation was previously quantified using a 0.01mg resolution balance (Citizen CX 165). The starter culture was added to the resultant milk at 40 ± 1°C and the mixture was stirred with a glass rod for 1 minute.
Incubation at 42°C
500 grams of the recombined and inoculated milk were separated from the bulk and put into an electric and thermostatically controlled incubator at 42 ± 1°C. The yogurt mix was incubated at this temperature until pH 4.6 ± 0.03 was reached. The level of acidity was controlled using an Accumet Basic AB-15 pH-meter (Fisher Scientific) after pH calibration with standardized solutions to pH 4, 7, and 10 at 21 ± 1°C.
Overnight refrigeration at 5°C.
After incubation, the yogurt was stored overnight (8-10 hours) in a cooling chamber at 5°C.
Homogenization
The initial yogurt gel was broken with a spoon, placed into a 600ml stainless steel beaker and homogenized using an electric hand mixer (Kitchenaid KHM3WH) on lower speed for 10 minutes (2 intervals of 5 minutes. Between intervals the sample was mixed with a spoon for 30 seconds). Once homogenized, the yogurt was placed into a 500ml plastic cup.
Refrigeration at 5°C until analyses

Method B

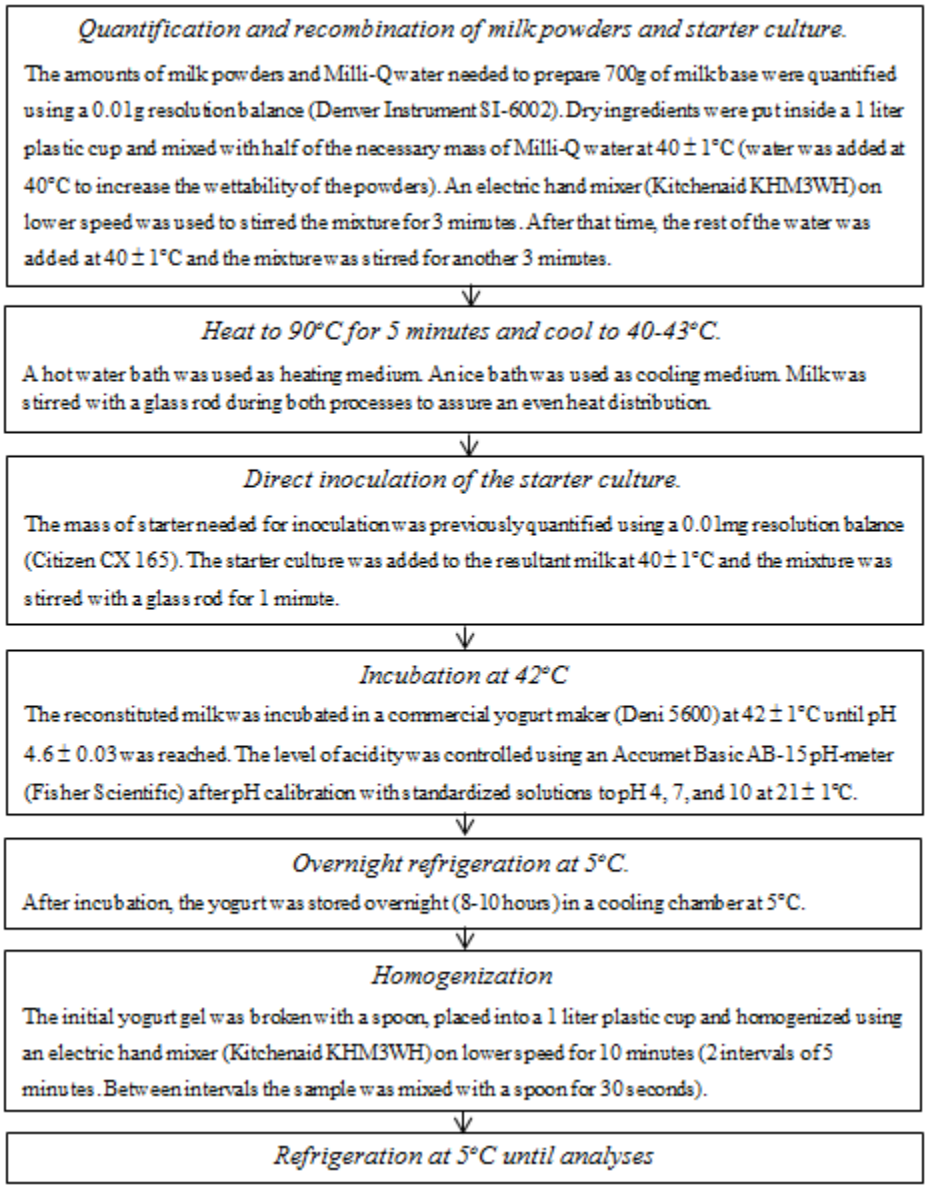


Figure 4-1: Methods of production considered for the manufacture of experimental samples

4.2.2 Total solids determination

Total solids were determined using a forced-air oven method adapted from Hooi *et al.* (2004). Each sample was gently stirred with a spoon for 30 seconds before testing. A mass of 3 grams (approximately) was put into a previously heated, desiccated and

weighed aluminum dish. A 0.01mg resolution balance (Citizen CX 165) was used for this determination. Samples were placed in a forced-air oven for drying at 110 ± 1°C for 24 hours. After drying, samples were cooled to room temperature, desiccated and weighed. Triplicate measurements and two replications were conducted for each sample. Total solids content was calculated using the following equation:

$$\%Total\ Solids = \frac{Dried\ sample\ with\ dish\ weight - Empty\ dish\ weight}{Initial\ sample\ weight}\ x\ 100 \quad \text{[Eq. 4-1]}$$

4.2.3 Experimental design and statistical analysis

The two-sided confidence interval limits ($\alpha = 0.05$) of reference samples, calculated in the previous chapter, were used for comparison with experimental data.

At first, Method A (See **Figure 4-1**) was used to produce 4 sets of samples (each set was composed of 3 independent samples) which were stored in a cooling chamber at 5°C for different time periods (1, 4, 8, and 12 days). Rheological and physicochemical measurements were carried out on these samples after their corresponding storage time in order to study the rheological and physicochemical storage stability of the reconstituted gel obtained by the proposed formulation.

Secondly, two sets of samples (each set was composed of three independent samples) were produced by Method B (See **Figure 4-1**) and stored at 5°C for different time periods (1 and 8 days). Rheological and physicochemical measurements were conducted on these samples after their corresponding storage time. Results were compared with those obtained from the same type of samples produced by Method A.

Statistical analysis of data was performed using SPSS (Inc., Chicago, IL, USA) version 19.0. Significant means of main effects between different samples produced by the same method of production were differentiated by the Duncan test at $\alpha = 0.05$. Significant differences between the mean values of the same type of samples produced by different manufacturing methods were detected by independent samples T tests at $\alpha = 0.05$.

4.3 Results & discussion

4.3.1 Rheological and physicochemical stability of the recombined yogurt throughout storage

Figures 4-2 and **4-3** illustrate the storage modulus (G') and loss tangent (tan δ) values of samples produced by method A after different storage times as a function of amplitude sweep.

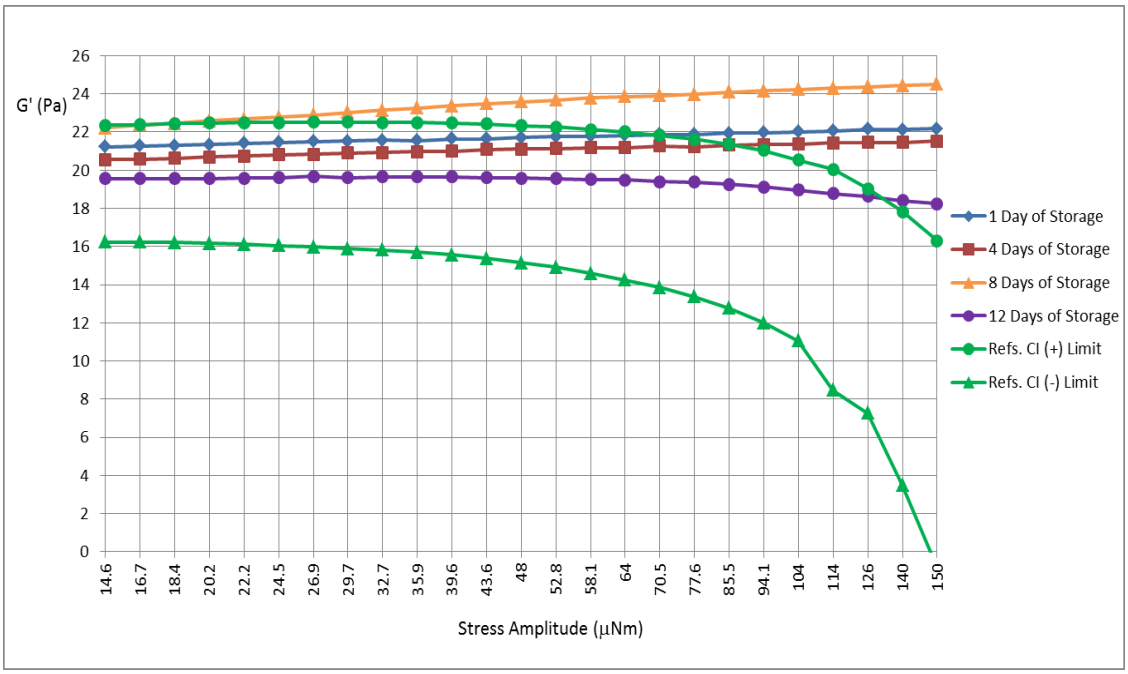


Figure 4-2: Storage modulus (G') of samples, produced by method A, after different storage times as a function of amplitude sweep

Presented values are the means of triplicate measurements. Refs. CI (+) Limit: Reference Confidence Interval Positive Limit ($\alpha = 0.05$). Refs. CI (-) Limit: Reference Confidence Interval Negative Limit ($\alpha = 0.05$). Appendices A (Table A-6) and C (Tables C-5 to C-8) provide the experimental data used to construct this graph.

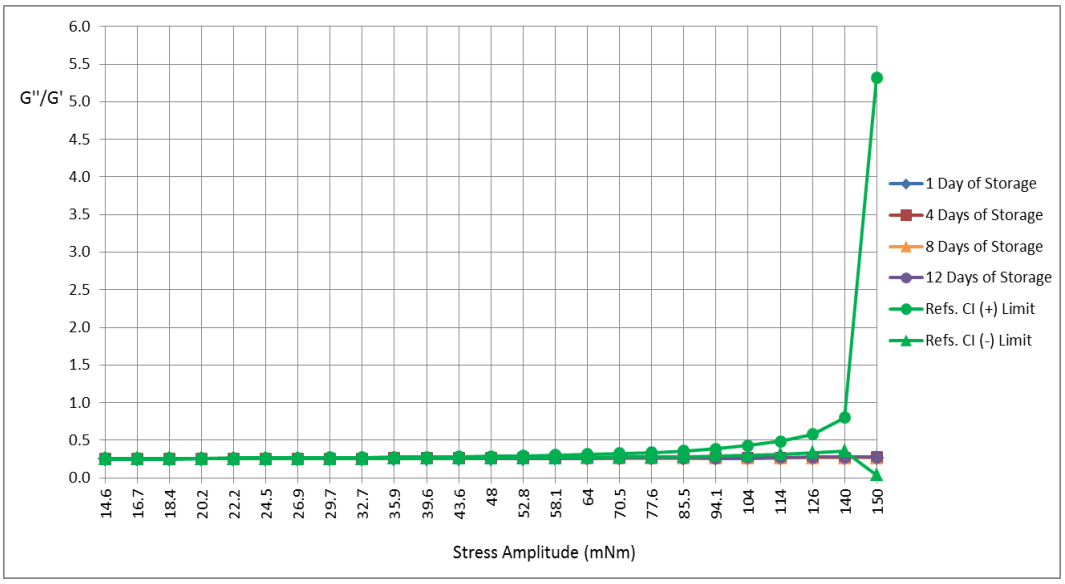


Figure 4-3: Loss tangent (tan δ = G''/G') of samples, produced by method A, after different storage times as a function of amplitude sweep

Presented values are the means of triplicate measurements. Refs. CI (+) Limit: Reference Confidence Interval Positive Limit (α = 0.05). Refs. CI (-) Limit: Reference Confidence Interval Negative Limit (α = 0.05). Appendices A (Table A-7) and C (Tables C-5 to C-8) provide the experimental data used to construct this graph.

Tables 4-1 and **4-2** present statistical differences between the G' and tan δ values of the experimental samples, produced by method A, at three points of the entire amplitude range considered.

Table 4-1: Storage modulus (G') of samples, produced by method A, after different storage times at three different points of the stress amplitude range applied [€, £, Δ]

Storage time	G' (Pa) at P.1 (Stress Amplitude = 14.6μNm)	G' (Pa) at P.12 (Stress Amplitude = 43.6μNm)	G' (Pa) at P.25 (Stress Amplitude = 150μNm)
1 Day	$21.233 \pm 0.306^{A,B}$	21.633 ± 0.419^{A}	22.183 ± 0.462^{A}
4 Days	$20.550 \pm 1.058^{A,C}$	21.083 ± 1.068^{A}	21.517 ± 1.040^{A}
8 Days	22.217 ± 0.475^{B}	23.500 ± 0.550^{B}	24.517 ± 0.586^{B}
12 Days	19.550 ± 0.737^{C}	19.600 ± 0.823^{C}	18.233 ± 1.786^{C}

[€] Presented values are the means of 3 replications (mean ± SD). [£] Numbers with different letters within the same column are significantly different (P < 0.05). [Δ] Appendix C (Tables C-5 to C-8) provides the experimental data used to construct this table.

Table 4-2: Loss tangent (tan δ = G''/G') of samples, produced by method A, after different storage times at three different points of the stress amplitude range applied [€, £, Δ]

Storage time	G''/G' at P.1 (Stress Amplitude = 14.6μNm)	G''/G' at P.12 (Stress Amplitude = 43.6μNm)	G''/G' at P.25 (Stress Amplitude = 150μNm)
1 Day	0.251 ± 0.001^{A}	0.255 ± 0.001^{A}	0.265 ± 0.001^{A}
4 Days	0.253 ± 0.000^{A}	$0.257 \pm 0.001^{A,B}$	0.267 ± 0.002^{A}
8 Days	0.252 ± 0.002^{A}	$0.257 \pm 0.001^{A,B}$	0.265 ± 0.001^{A}
12 Days	0.252 ± 0.001^{A}	0.258 ± 0.001^{B}	0.278 ± 0.007^{B}

[€] Presented values are the means of 3 replications (mean ± SD). [£] Numbers with different letters within the same column are significantly different (P < 0.05). [Δ]Appendix C (Tables C-5 to C-8) provides the experimental data used to construct this table.

According to the previous tables, recombined samples stored for 1 and 4 days presented significantly similar rheological properties (G' and tan δ) at the three different amplitude points considered (P > 0.05). Samples stored for 8 days presented a considerably higher G' than the previous samples at point 12 and 25 of the sweep amplitude range applied (P < 0.05). These results are consistent with the findings of Marafon *et al.* (2011b), Serra *et al.* (2009) and Weidendorfer *et al.* (2008), who reported an increase in G' in stirred yogurts within storage. In this way, samples stored for 8 days presented a rheological behavior that was least similar to that of the reference samples.

Even though samples stored for 8 days presented higher G' values, they had significantly similar tan δ values than samples stored for less time (P > 0.05). This is because the number and/or strength of non-relaxing protein bonds (G') and rapidly relaxing bonds (G'') increase proportionally with storage time (Özer *et al*., 1998b). Nevertheless, Marafon *et al.* (2011b) reported that stirred yogurts fortified with SMP, WPC and NaCN experienced a decrease in tan δ within storage.

Samples stored for 12 days presented significantly different rheological properties than the other samples ($P > 0.05$) for almost all the amplitude points considered. Surprisingly, these samples presented the lowest G' and the highest tan δ values. These results can be explained by considering the information illustrated in the following table (**Table 4-3**).

Table 4-3: Physicochemical properties of samples, produced by method A, after different storage times [€, £, Δ]

Storage time	Size of Visible Clusters	Level of Whey Drainage	Level of SWO_{BH} (%m/m)	Level of SWO_{AH} (%m/m)	Incubation Time (Hours)
1 Day	1.000 ± 0.000^{A}	0.000 ± 0.000^{A}	0.000 ± 0.000^{A}	0.000 ± 0.000^{A}	10.833 ± 0.153^{A}
4 Days	1.000 ± 0.000^{A}	0.000 ± 0.000^{A}	0.000 ± 0.000^{A}	0.000 ± 0.000^{A}	10.867 ± 0.058^{A}
8 Days	1.000 ± 0.000^{A}	0.000 ± 0.000^{A}	0.000 ± 0.000^{A}	0.000 ± 0.000^{A}	10.933 ± 0.153^{A}
12 Days	1.000 ± 0.000^{A}	0.667 ± 0.577^{B}	0.000 ± 0.000^{A}	0.000 ± 0.000^{A}	10.833 ± 0.153^{A}

[€] Presented values are the means of 3 replications (mean ± SD). [£] Numbers with different letters within the same column are significantly different ($P < 0.05$). [Δ] Appendix C (Table C-3) provides the experimental data used to construct this table. SWO_{BH}: Surface whey-off before homogenization. SWO_{AH}: Surface whey-off after homogenization.

According to **Table 4-3**, samples stored for 1, 4, and 8 days presented significantly similar physicochemical characteristics ($P > 0.05$). Samples stored for 12 days presented significantly higher levels of whey drainage than the other samples ($P > 0.05$). Yogurts stored for 12 days had considerable amounts of free whey below their gel networks. After mixing this free whey with the gel structure (previous to analyses), there was an increase in the viscous character and a decrease in the elastic character of the resultant gels. Due to this fact, these samples presented lower G' and higher tan δ values than samples stored for less time.

Since the proposed formulation presented a high casein to whey protein ratio (6:1), there were considerably lower amounts of denatured whey proteins than casein particles present in the gel matrix. Consequently, there were low amounts of non-relaxing protein bonds integrating the gel network and a high relaxing to non-relaxing protein bonds ratio was obtained. As the relaxation behavior of the resultant gel was high, the possibilities for rearrangements of particles inside the gel network during storage

increased. Thus, as storage time increased, the instability of the resultant gel increased and its ability to entrap the serum phase decreased (Lee & Lucey, 2004; Lucey *et al*., 1998a; van Vliet *et al*., 1991). As a result, high levels of syneresis were obtained after storing samples for 12 days. This observation agrees with Al-Kadamany *et al.* (2002) who reported that the level of free whey in concentrated yogurt produced by the traditional method increased upon storage. Additionally, Salvador & Fiszman (2004) also reported that the level of syneresis in whole and skimmed set types of yogurt increases with storage time.

On the other hand, as experimental gels presented low amounts of denatured whey proteins, a low degree of protein-protein interactions was obtained inside the gel matrix. Consequently, the compactness of the network was low. Puvanenthiran *et al*. (2002) and Remeuf *et al.* (2003) studied the microstructure of acid yogurt gels using scanning electron microscopy and noted that gels with lower casein to whey protein ratios had finer structures with numerous small pores and a dense network of cross-links. Both studies reported that an increase in the compactness of yogurt microstructure due to a reduction in the casein to protein ratio led to a high level of immobilization of free water in the yogurt gel. Remeuf *et al.* (2003) also reported that the addition of NaCN to the milk base resulted in a rather coarse and loose network structure with higher porosity than gels produced by WPC-enriched milk bases. As the movement of fluid out of the gel under the force of gravity is essentially related to the permeability of the gel, the coarser, more open structures have a higher drainage than finer networks (Puvanenthiran *et al*., 2002). Therefore, as the proposed formulation had a high casein to whey protein ratio (6:1), the reconstituted samples obtained by this formulation presented a coarser open gel network structure. Thus, high levels of syneresis (whey drainage) were expected for these samples after several days of storage.

Manufacturers try to prevent whey separation by increasing the total solids content of milk, subjecting the milk to a severe heat treatment (to increase whey protein denaturation) or by adding stabilizers such as gelatin, pectin, starches, or gums (Lucey, 2002).

There is a high level of controversy regarding the use of stabilizers in fermented products. Some countries prohibit the addition of stabilizers to plain yogurt (Peng *et al*., 2009). Moreover, there is a current growing consumer demand for more natural products that contain fewer or no additives/stabilizers. As a result, there is an emerging need to be

able to produce, without the use of stabilizers, acid milk gels that do not whey-off during storage (Lucey, 2001).

One of the most important steps for avoiding quality concerns (e.g., weak body, poor texture, whey separation, variation in consistency) in low fat yogurts production is to increase the total solids content of the yogurt mixes (Peng *et al*., 2009). This can be done by adding milk proteins, sucrose, sweeteners, lactose, etc. (Peng *et al*., 2009; Lee & Lucey, 2010). The increase of total solids increases the firmness, complex viscosity (the storage modulus and fracture stress are increased), apparent viscosity, oral viscosity, consistency index, and WHC of the resultant gel (Harwalkar & Kalab, 1986; Rohm & Schmidt, 1993; Mistry & Hassan, 1992; Lee & Lucey, 2010; Lucey, 2002; Lucey & Singh, 1998; Bhullar *et al.*, 2002; Anema, 2008; Özer, 2009; Barreto Penna *et al*., 2006; Wu *et al*., 2009; Kristo *et al*., 2003; Krzeminski *et al.*, 2011; Jumah *et al*., 2001; Amatayakul *et al*., 2006). Thus, it improves the textural attributes of the gel, giving a higher sensory acceptability to the final product (Skriver *et al*., 1999; Mahdian & Tehrani, 2007; Peng *et al.*, 2009; Marafon *et al*., 2011a). Moreover, Özer & Robinson (1999) argued that milk bases with higher total solids contents required lower incubation times. However, Puvanenthiran *et al.* (2002) stated that when the casein to whey protein and net protein content were kept constant, the whey drainage characteristics were constant, regardless of the difference in total solids used. Thus, the addition of protein should be considered to increase total solids in order to reduce whey drainage.

Recently, Le *et al*. (2011) reported that adding milk fat globule membrane (MFGM) material can also help to increase the water-holding capacity of a yogurt gel. Furthermore, they stated that supplementation with MFGM material increased the firmness of yogurt and produced denser microstructures than unfortified plain skim milk yogurts. As several health-promoting effects have been attributed to the MFGM material (Dewettinck *et al*., 2008; Veereman-Wauters *et al*., 2012), Le *et al.* (2011) stated that this ingredient has a high potential to be used as a novel component for developing new functional products, utilizing both the technological functionalities as well as the nutritional properties of the material.

As the product proposed by this investigation is a plain, fat-free, additive-free type of acid milk gel, no lipids or non-dairy additives can be added to control its physical properties. Therefore, only skim milk components can be added to the proposed formulation to increase its total solids content.

Considering the observations made by several researchers, it is evident that increasing the total protein content (increasing total solids content) of the proposed formulation by adding whey proteins to decrease the casein to whey protein ratio of the proposed formulation would decrease the level of whey drainage in the final product and increase its storage stability. However, an increase in the total protein level would result in an increase in the G' of the experimental samples, because at higher protein levels a higher amount of non-relaxing protein bonds can be formed; thus, a much denser and stronger gel structure can be expected (Anema, 2008; Özer *et al*., 1998a). Hence, if the total protein content of the proposed formulation is increased, the rheological properties of the final reconstituted product will become even more different than those of the reference samples considered.

Moreover, increasing the total protein content of the proposed formulation by increasing the whey protein concentration can result in the formation of undesired big protein aggregates (clusters) in the final product (Kucukcetin, 2008; Amatayakul *et al*., 2006). This observation is based on the fact that the cross-links between the casein micelles and denatured whey proteins can be responsible for an increase in micelle size, which can contribute to an increase of the particle size present in the gel network. This mechanism is enhanced when increasing whey protein concentrations (Remeuf *et al*., 2003). Additionally, high levels of whey proteins can lead to the saturation of all of the capacity for binding κ-casein to whey protein. Once this occurs, aggregates of whey proteins are formed and result in the formation of big clusters in the final product (Puvanenthiran *et al*., 2002).

Lowering the denaturation level of whey proteins in the final formulation can be used to reduce cross-linking and bridging within the final gel network. Hence, a decrease in the degree of denaturation of whey proteins will be accompanied by a decrease in the viscosity and strength of the final gel (Zbikowski *et al.*, 1998). Therefore, it is recommended to reduce the whey protein denaturation level in order to increase the amounts of whey protein in the final formulation without the presence of big clusters or considerable increases in the G' of the resultant gel. In this way, whey protein concentrations can be increased, reducing the percentage amount of NaCN present in the final formulation and reducing the G' and $\sigma_{fracture}$ of the resultant gel (Damin *et al*., 2009; Bremer *et al*.,1990). Consequently, the modified formulation will have a rheological behavior more similar to that of the reference samples than the previous proposed formulation.

However, decreasing the denaturation level of whey proteins will increase the probability of obtaining whey separation in the final gel, because a lower number of non-relaxing protein bonds will be present in the gel matrix (Kucukcetin, 2008; Zbikowski *et al.*, 1998). Reducing these types of bonds will result in the formation of a less denser network structure with higher porosity, and will increase the level of internal rearrangements inside the gel, which will increase the instability of the gel network and lower its ability to entrap the serum phase (Puvanenthiran *et al*., 2002; Lucey, 2002; Lucey *et al*., 1998a; Lucey & Singh, 1998).

Harwalkar & Kalab (1986) found that the WHC of yogurt made from reconstituted nonfat dry milk was proportional to the total solids content, and at 20% of total solids, the spontaneous whey drainage was stopped (Sodini *et al*., 2004). Due to this fact, high levels of whey proteins should be added in order to increase the total solids content so that whey separation is reduced in the final product. In this way, by lowering the denaturation level of whey proteins and adding a higher amount of them to the final product, it is possible to obtain a final gel that has rheological and physicochemical behaviors that are more similar to those of the reference samples, and lower whey separation than the previous proposed formulation.

To resume, a decrease in the denaturation level of whey proteins and an increase in the total protein content caused by an increase in the whey protein content (reducing the casein to whey protein ratio) should be considered in order to increase the rheological and physicochemical storage stability, improve the rheological aspects, and maintain the required physicochemical properties of the recombined gel obtained by the proposed formulation. Consequently, to obtain a recombined, non-fat, additive-free type of acid milk gel with rheological characteristics closer to those of the reference samples and with higher storage stability than the previous proposed formulation, the total solids and total protein contents of this gel should be higher than those of the reference samples. Higher levels of total solids will also help to improve probiotic growth during the fermentation period and favor bacterial viability in the recombined product (Marafon *et al*., 2011a).

4.3.2 Effects of a new simplified reconstitution method on the rheological and physicochemical aspects of the final product

Figures 4-4 and **4-5** show the G' and tan δ values of the same types of samples produced by methods A and B.

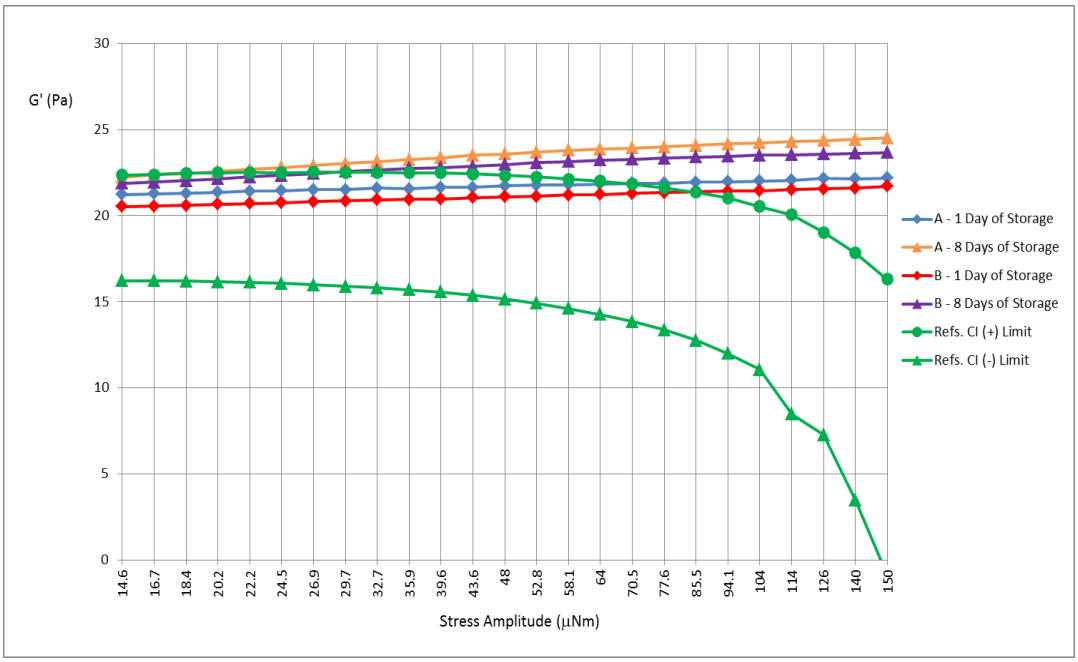


Figure 4-4: Storage modulus (G') of samples, produced by methods A and B, after 1 and 8 days of storage as a function of amplitude sweep

Presented values are the means of triplicate measurements. Refs. CI (+) Limit: Reference Confidence Interval Positive Limit ($\alpha = 0.05$). Refs. CI (-) Limit: Reference Confidence Interval Negative Limit ($\alpha = 0.05$). Appendices A (Table A-6) and C (Tables C-5, C-7, C-9, C-10) provide the experimental data used to construct this graph.

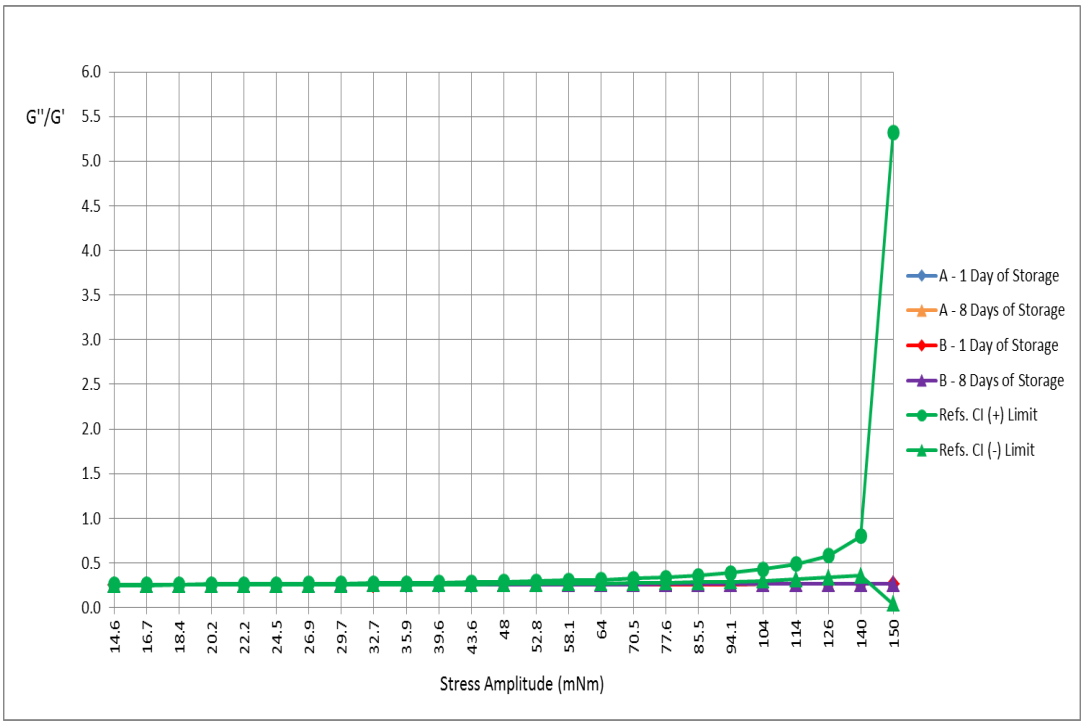


Figure 4-5: Loss tangent (tan δ = G''/G') of samples, produced by methods A and B, after 1 and 8 days of storage as a function of amplitude sweep

Presented values are the means of triplicate measurements. Refs. CI (+) Limit: Reference Confidence Interval Positive Limit ($\alpha = 0.05$). Refs. CI (-) Limit: Reference Confidence Interval Negative Limit ($\alpha = 0.05$). Appendices A (Table A-7) and C (Tables C-5, C-7, C-9, C-10) provide the experimental data used to construct this graph.

Tables 4-4 and **4-5** present a statistical comparison between the G' and the tan δ values, at three different points of the amplitude range applied, for the same types of samples obtained by different recombination methods.

Table 4-4: Storage modulus (G') of samples, produced by methods A and B, after 1 and 8 days of storage at three different points of the stress amplitude range applied [€, Δ]

Rheological Parameters	Storage time (Days)	Method used for the production of Greek-style yogurt		P-value
		A	B	
G' (Pa) at Point 1 (Stress Amplitude = 14.6μNm)	1	21.233 ± 0.306	20.517 ± 0.945	.280
G' (Pa) at Point 12 (Stress Amplitude = 43.6μNm)	1	21.633 ± 0.419	21.050 ± 1.117	.445
G' (Pa) at Point 25 (Stress Amplitude = 150μNm)	1	22.183 ± 0.462	21.700 ± 1.249	.564
G' (Pa) at Point 1 (Stress Amplitude = 14.6μNm)	8	22.217 ± 0.475	21.867 ± 0.530	.442
G' (Pa) at Point 12 (Stress Amplitude = 43.6μNm)	8	23.500 ± 0.550	22.867 ± 0.629	.260
G' (Pa) at Point 25 (Stress Amplitude = 150μNm)	8	24.517 ± 0.586	23.667 ± 0.828	.220

[€] Presented values are the means of 3 replications (mean ± SD). [Δ] Appendix C (Tables C-5, C-7, C-9, C-10) provides the experimental data used to construct this table.

Table 4-5: Loss tangent (tan δ = G''/G') of samples, produced by methods A and B, after 1 and 8 days of storage at three different points of the stress amplitude range applied [€, Δ]

Rheological parameters	Storage time (Days)	Method used for the production of Greek-style yogurt		P-value
		A	B	
G''/G' at Point 1 (Stress Amplitude = 14.6μNm)	1	0.251 ± 0.001	0.252 ± 0.001	.742
G''/G' at Point 12 (Stress Amplitude = 43.6μNm)	1	0.255 ± 0.001	0.256 ± 0.002	.588
G''/G' at Point 25 (Stress Amplitude = 150μNm)	1	0.265 ± 0.001	0.264 ± 0.004	.667
G''/G' at Point 1 (Stress Amplitude = 14.6μNm)	8	0.252 ± 0.002	0.252 ± 0.001	1.000
G''/G' at Point 12 (Stress Amplitude = 43.6μNm)	8	0.257 ± 0.001	0.258 ± 0.002	1.000
G''/G' at Point 25 (Stress Amplitude = 150μNm)	8	0.265 ± 0.001	0.266 ± 0.002	.422

[€] Presented values are the means of 3 replications (mean ± SD). [Δ] Appendix C (Tables C-5, C-7, C-9, C-10) provides the experimental data used to construct this table.

According to the presented data, samples produced by different manufacturing methods that were stored for the same period of time did not significantly differ in their G' and tan δ values ($P > 0.05$). Both manufacturing methods offered products with similar rheological properties. Therefore, it can be stated that the preheating step (40°C for 20 minutes) performed during method A, to achieve a normal state of hydration of milk proteins, did not have a significant influence on the rheological properties of the final products.

Several researchers stated the importance of the hydration level of milk proteins on the final quality of reconstituted products. Bylund (1995) stated that the hydration level of milk proteins has a very important influence on the textural properties of final recombined dairy products. He affirmed that an insufficient protein hydration level may lead to a "chalky" defect in the final product. Tamime & Kirkegaard (1991) remarked that a complete hydration level of milk proteins is very important to increase the WHC of these proteins in the final recombined product. Avisar (2010) found a positive influence of hydration time on the mechanical properties of white brined cheese. He also stated that during hydration time, the swelling properties of milk proteins improved. However, Gilles & Lawrence (1982) did not consider hydration time to be of much importance and cited several works that stated that the level of hydration of milk powders is not a significant factor in determining the final quality of recombined dairy products.

According to Kjærgaard Jensen & Nielsen (1982) and Bylund (1995), the complete hydration of milk proteins is achieved by hydrating powders for less than 20 minutes at 40-50°C. As both experimental manufacturing methods used in this study included the incubation of samples at 42 ± 1°C for several hours, it is evident that the complete hydration of milk proteins was achieved during the first stages of incubation. Due to this fact, it is believed that the total amount of proteins present in all samples obtained their normal state of hydration during the incubation time. Therefore, all samples presented the same level of protein hydration and no rheological differences were observed between samples made by the different production methods proposed.

The following tables present the physicochemical properties of samples made by the different production methods considered. **Table 4-6** shows the physicochemical aspects of recombined samples produced by methods A and B after 1 day of storage. **Table 4-7** illustrates the physicochemical characteristics of samples produced by methods A and B after 8 days of storage.

Table 4-6: Physicochemical properties of samples, produced by methods A and B, after 1 day of storage [€, Δ]

Physicochemical parameters	Method used for the production of Greek-style yogurt		P-value
	A	B	
Size of Visible Clusters	1.000 ± 0.000	1.000 ± 0.000	†¥
Level of Whey Drainage	0.000 ± 0.000	0.000 ± 0.000	†¥
Level of SWO_{BH} (%m/m)	0.000 ± 0.000	0.000 ± 0.000	†¥
Level of SWO_{AH} (%m/m)	0.000 ± 0.000	0.000 ± 0.000	†¥
Incubation Time (Hours)	10.833 ± 0.153	12.367 ± 0.153	.000

[€] Presented values are the means of 3 replications (mean ± SD). [Δ] Appendix C (Tables C-3, C-4) provides the experimental data used to construct this table. [†]The independent samples T test could not be computed because the standard deviations of both groups are zero. [¥] Both groups presented the same physicochemical properties.

Table 4-7: Physicochemical properties of samples, produced by methods A and B, after 8 days of storage [€, Δ]

Physicochemical parameters	Method used for the production of Greek-style yogurt		P-value
	A	B	
Size of Visible Clusters	1.000 ± 0.000	1.000 ± 0.000	†¥
Level of Whey Drainage	0.000 ± 0.000	0.000 ± 0.000	†¥
Level of SWO_{BH} (%m/m)	0.000 ± 0.000	0.000 ± 0.000	†¥
Level of SWO_{AH} (%m/m)	0.000 ± 0.000	0.000 ± 0.000	†¥
Incubation Time (Hours)	10.933 ± 0.153	12.400 ± 0.173	.000

[€] Presented values are the means of 3 replications (mean ± SD). [Δ] Appendix C (Tables C-3, C-4) provides the experimental data used to construct this table. [†]The independent samples T test could not be computed because the standard deviations of both groups are zero. [¥] Both groups presented the same physicochemical properties.

Consistent with the data presented in **Tables 4-6** and **4-7**, samples produced by different manufacturing methods and stored for the same period of time presented significantly similar ($P > 0.05$) physicochemical characteristics. However, samples produced by method B required considerably longer incubation times than samples

produced by method A. This may be attributed to the different instruments that were used to incubate samples in the different production methods. The yogurt maker used in method B needed a longer time to reach a stable final temperature of incubation. Due to this fact, a longer incubation time was required for samples made using method B.

As method A required an extra pre-heating step (40^{o}C for 20 minutes), final samples produced by this method were expected to have higher total solids contents. Samples produced by method A, stored for 1 day after production, presented a mean total solids content (13.871 ± 0.023 %) significantly higher ($P < 0.05$) than that of the same type of samples produced by method B (13.783 ± 0.011%). However, this difference did not have a significant influence on the rheological and physicochemical aspects of the final products.

Further investigation is needed in order to evaluate the whey protein denaturation level obtained during the intense heat treatment (90^{o}C for 5 minutes) applied. This data is required to simplify both production methods considered. Incorporating the precise amount of denatured whey proteins into the final dried formulation can eliminate the intense heat treatment (90^{o}C for 5 minutes) and the inoculation step required in the current manufacturing methods. In this way, production method B can be further simplified to offer a quicker and easier manufacturing method to reduce the influence of consumer intervention on the quality of the final recombined product.

4.4 Conclusion

The recombined acid milk gel obtained by the proposed formulation conserved its initial physicochemical and rheological properties for at least 4 days after recombination. The rheological characteristics of the product were maintained significantly unchanged for at least 4 days, while the physicochemical properties were conserved for at least 8 days. Further investigation seems necessary to optimize the physicochemical and rheological stability of the formulation proposed. Relevant importance should be given to the whey protein denaturation level and to the total protein content and composition of the final formulation.

Acid milk gels produced by the new, simplified recombination method proposed presented rheological and physicochemical aspects significantly similar ($P > 0.05$) to yogurts manufactured by the other recombination method considered. Therefore,

according to the results obtained, it can be stated that the normal state of hydration of milk proteins is achieved during the first stages of incubation; hence, there is no need to apply a preheating step (40^{o}C for 20 minutes) before incubation.

4.5 References

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5. Effects of storing the dry formulation on the rheological and physicochemical characteristics of the recombined yogurt

5.1 Introduction

Manufacturing recombined and reconstituted milk and milk products is a technology that emerged toward the end of the 20th century (Kneifel, 1993). Recombined and reconstituted milk products provide a nutritious and high-quality source of dairy products in areas where fresh raw milk is not readily available or is in short supply (Tong, 2002). This technology was initially applied to obtain fluid milk, but it was followed by production of recombined evaporated milk and sweetened condensed milk. Today recombination also includes yogurt, butter and cheese (Bylund, 1995).

The basic types of milk powders used in the recombination industry are skim-milk powder, whole milk powder and buttermilk powder (Kjærgaard Jensen, 1990). These products are the major milk powders in the marketplace (Augustin & Margetts, 2003). However, introducing membrane techniques to the dairy industry has enabled the production of other types of milk powders containing diverse protein to lactose ratios and altered whey protein to casein ratios (e.g., milk retentate, milk permeate, whey retentate, and whey permeate powders) (Avisar, 2010; Caric, 2002). The use of these latter powders has enabled the production of recombined dairy products (such as concentrated yogurt), which have high protein and low lactose contents.

Numerous investigations were done in order to evaluate the properties and applications of these various dairy powders on the production of dairy products (Jimenez-Florez & Kosikowski, 1986; El-Samragy *et al*., 1993a; El-Samragy *et al*., 1993b; Mistry & Pulgar, 1996; Patocka *et al*., 2006; Isleten & Karagul-Yuceer, 2008; Oliveira *et al*., 2001; Marafon *et al*., 2011a; Marafon *et al*., 2011b). Several researchers recommended using these types of powders to fortify the milk base during yogurt production (Mistry & Hassan, 1992; Gonzalez-Martinez *et al*., 2002; Guzman-Gonzales *et al.*, 1999; Guzman-Gonzales *et al.*, 2000). Chapter III suggested using some of these powders to produce a dried, concentrated yogurt formulation. The current study will emphasize how storing the dried formulation proposed in the preceding chapter affects the quality of the final recombined product.

Dairy powders have a very long shelf-life; they can be stored at ambient temperatures, and can be easily transported (Tamime & Robinson, 2007). However, as the quality of recombined dairy products is directly related to the composition and the physical, chemical, microbiological and sensoric standard of the ingredients used, it is of great importance to conserve the physicochemical and microbiological properties of diary powders during their storage until their final reconstitution (Kjærgaard Jensen, 1990).

Freeze-dried (direct-to-vat) thermophilic starter cultures are also required to produce yogurt powders. Freeze-dried cultures can contain higher levels of viability than dried cultures obtained by other drying techniques (10^9 to 10^{12} cells per gram). They can also be stored in a conventional refrigerator and transported at room temperature (Durso & Hutkins 2003; Wigley, 1999). These advantages are possible because lyophilized cells are somewhat stable at room temperature, although they are best maintained at -20^{o}C (Durso & Hutkins, 2003). The major disadvantages of using freeze-dried concentrate cultures is that, compared to frozen concentrate cultures, they require a longer lag phase during incubation, some commercial strains do not survive the process well and, compared to other drying techniques, freeze-drying requires higher costs and energy consumption (Surono & Hosono, 2002; Powell *et al*., 2002; Peighambardoust *et al*., 2011; Silva *et al*., 2011).

A number of factors, such as growth medium, freezing rate, drying temperature and composition of freezing medium, influence the viability of lyophilized starter cultures, together with subsequent storage conditions including temperature, atmosphere, exposure to light and relative humidity (Andersen *et al*., 1999). As the starter cultures' activities significantly affect the rheological and physicochemical aspects of acid milk gels (Lee & Lucey, 2004; Jumah *et al*., 2001; Sodini *et al*., 2004; Wu *et al*., 2009), it is essential to preserve the viability of the starter cultures during the dry storage of the powder mix in order to maintain, throughout storage, the expected rheological and physicochemical characteristics of the final recombined product.

Knowing how the yogurt powder behaves during storage is important because its shelf life is based on whether the recombined product obtained from the dried mix displays any of the physical, chemical, or sensory characteristics that are unacceptable for consumption (Salvador & Fiszman, 2004). The objective of this investigation was to study how storing the yogurt powder formulation previously proposed affected the rheological and physicochemical characteristics of the final recombined product.

5.2 Materials & methods

Materials and determinations considered in this investigation were the same as the ones detailed in Section 3. Experimental samples were produced in batch mode by means of Method B detailed in Section 4. All recombined yogurts were produced using the final formulation proposed in Section 3.

5.2.1 Experimental design and statistical analysis

To study how storing the dried yogurt formulation affects the rheological and physicochemical properties of the final recombined yogurt, the corresponding amounts of milk powders needed to produce 700g of the recombined product were placed into 1L plastic cups. The amounts of starter culture required to produce 700g of yogurt were packed into small polyethylene bags and each bag was put inside a plastic cup containing the milk powders. Plastic cups were covered with plastic lids and stored at 5^{o}C (inside a cooling chamber) and 20^{o}C (inside an electric and thermostatically controlled incubator) for different amounts of time. After storage, powders were recombined and the resultant gels were stored at 5^{o}C for 8 days before conducting the rheological and physicochemical determinations.

The two-sided confidence interval limits ($\alpha = 0.05$) of reference samples, calculated in Section 3, were used for comparison with experimental data. A one-block full factorial design 2*2 was used to investigate how storing the dried yogurt powder formulation affected the rheological and physicochemical characteristics of the final recombined product. **Table 5-1** illustrates the different factors and levels considered in the experiment. **Table 5-2** shows the composition of the different samples used in this experiment according to the combination of factors and levels detailed in **Table 5-1**.

Table 5-1: Factors and levels considered to produce experimental samples

Factors	Low Level	High Level
Storage Temperature (^{o}C)	5	20
Storage Time (Weeks)	2	8

Table 5-2: Composition of experimental samples according to the different factors and levels considered in the experimental design

Sample No.	Storage Temperature (^{o}C)	Storage Time (Weeks)
1	5	2
2	20	2
3	5	8
4	20	8

Yogurt samples were made in triplicate, producing a total of 12 batches. All measurements were carried out in triplicate at day 8 after production.

Response surface methodology was applied, using Minitab 16 software (Inc., State College, PA, USA) version 16.1.1, in order to evaluate the effects of factors on the rheological and physicochemical parameters tested. Statistical analysis of data was performed using SPSS (Inc., Chicago, IL, USA) version 19.0. Significant means of main effects between different samples were differentiated by the Duncan test at $\alpha = 0.05$.

5.3 Results & discussion

The rheological properties of recombined yogurts were significantly affected by storing the dry formulations ($P < 0.05$). Yogurts produced by means of powders stored for 2 weeks at 5^{o}C and 20^{o}C, and for 8 weeks at 5^{o}C, presented storage modulus (G') and loss tangent (tan δ = G''/G') significantly similar ($P > 0.05$) to those found in recombined acid milk gels manufactured from fresh yogurt powders (at three selected points of the amplitude sweep range considered: 14.6; 43.6; 150 μNm). Recombined samples obtained from powders stored for 8 weeks at 20^{o}C presented considerably lower G' and higher tan δ than the rest of the samples ($P < 0.05$) (at the three amplitude sweep points considered). **Figures 5-1** and **5-2** illustrate the G' and tan δ values of reconstituted samples produced from powders stored at dissimilar conditions as a function of amplitude sweep.

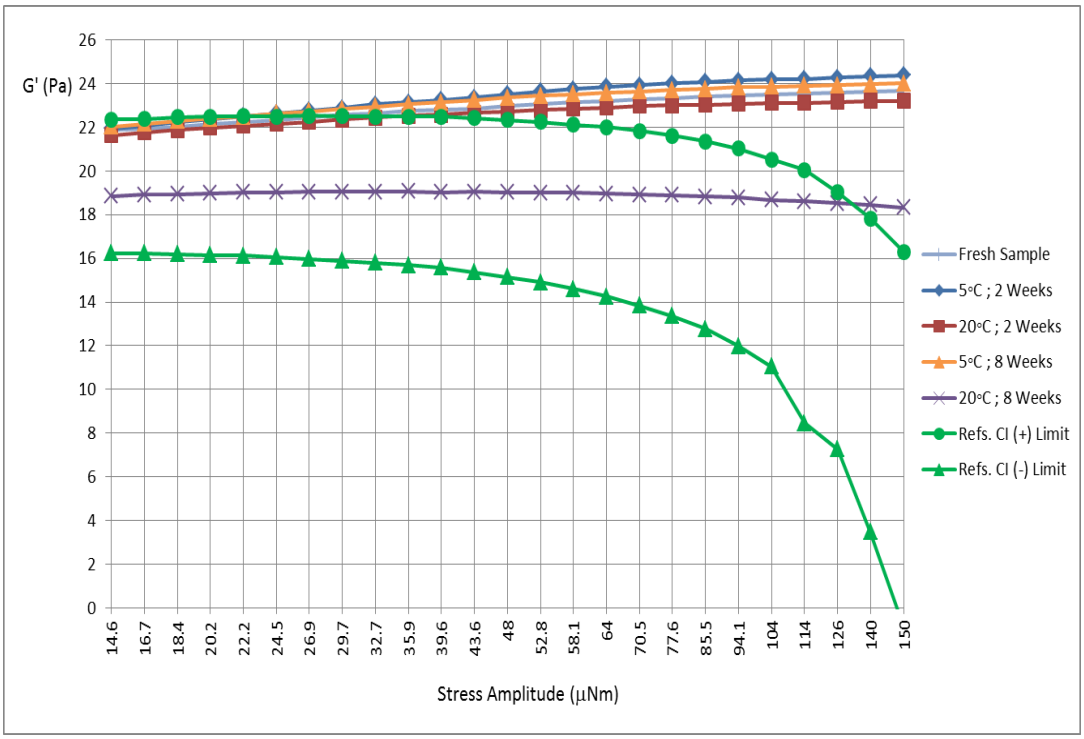

Figure 5-1: Storage modulus (G') of reconstituted samples obtained from powders stored at different conditions as a function of amplitude sweep

Presented values are the means of triplicate measurements. Refs. CI (+) Limit: Reference Confidence Interval Positive Limit ($\alpha = 0.05$). Refs. CI (-) Limit: Reference Confidence Interval Negative Limit ($\alpha = 0.05$). Appendices A (Table A-6) and D (Tables D-3 to D-6) provide the experimental data used to construct this graph.

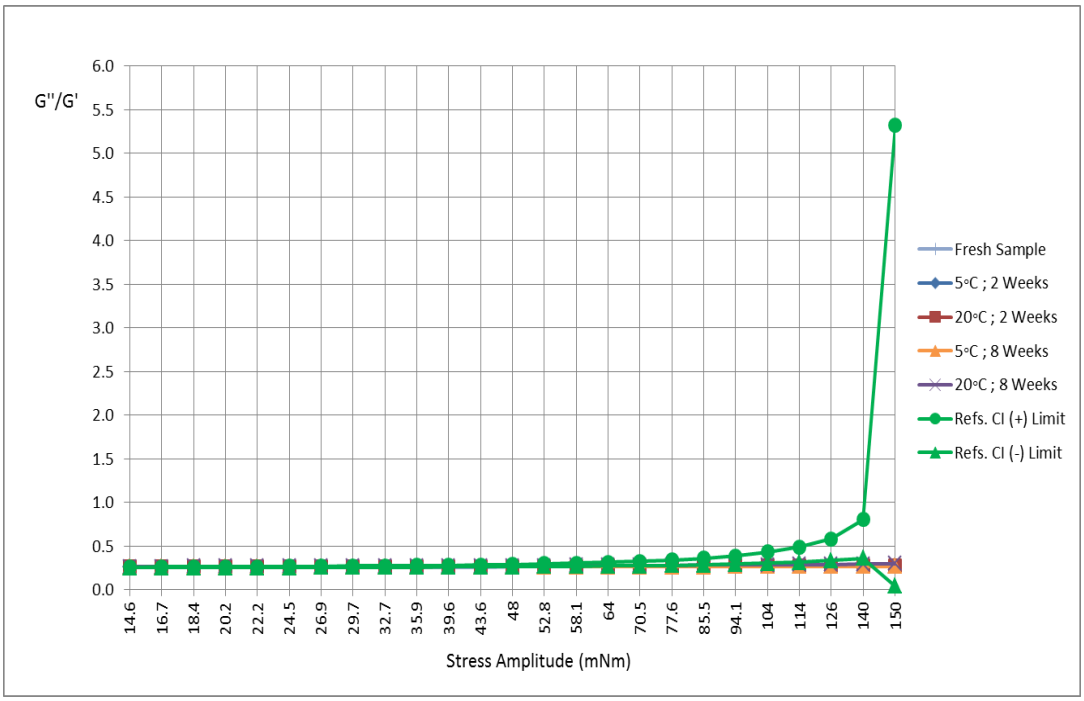

Figure 5-2: Loss tangent (tan δ = G''/G') of reconstituted samples obtained from powders stored at different conditions as a function of amplitude sweep

Presented values are the means of triplicate measurements. Refs. CI (+) Limit: Reference Confidence Interval Positive Limit ($\alpha = 0.05$). Refs. CI (-) Limit: Reference Confidence Interval Negative Limit ($\alpha = 0.05$). Appendices A (Table A-7) and D (Tables D-3 to D-6) provide the experimental data used to construct this graph.

As storage times and temperatures increased, the G' and the $\sigma_{fracture}$ of the recombined samples decreased and the tan δ increased. As a result, samples stored for 8 weeks at 20°C presented the closest rheological behavior to that of the reference market samples.

Figures 5-3 and **5-4** illustrate the effects of the factors on the rheological properties of the recombined acid milk gels.

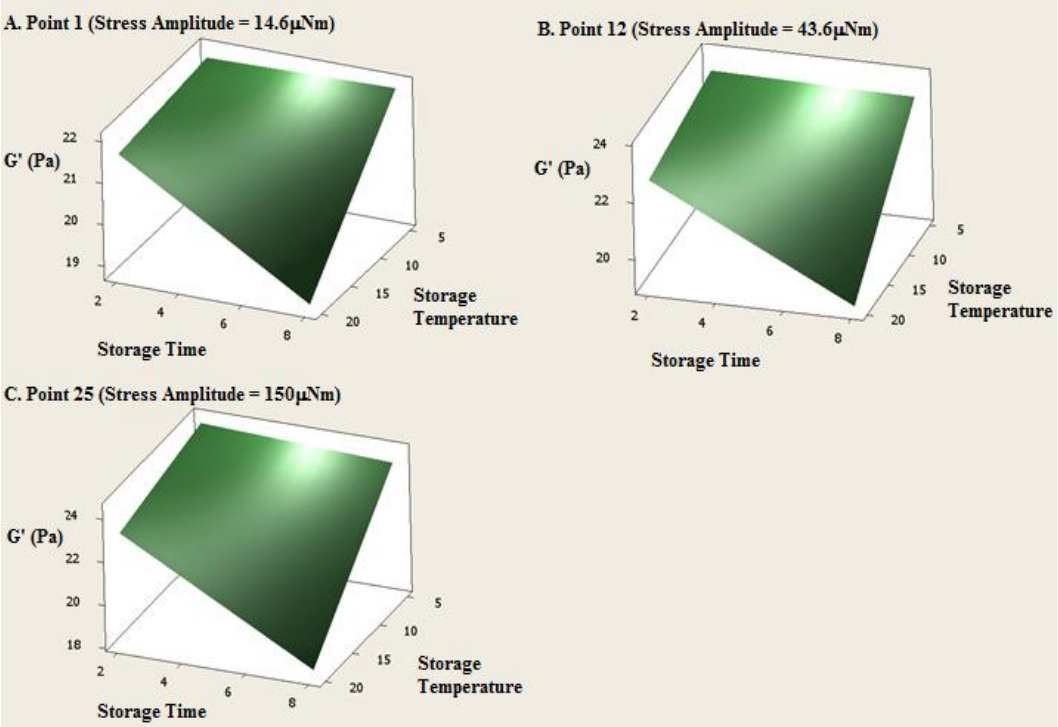

Figure 5-3: Effects of storage temperature and time, applied to yogurt powders, on the storage modulus (G') of the recombined samples at three selected points of the stress amplitude range applied

Surface plots were constructed using the means of triplicate measurements. Appendix D (Tables D-3 to D-6) provides the experimental data used to construct these graphs.

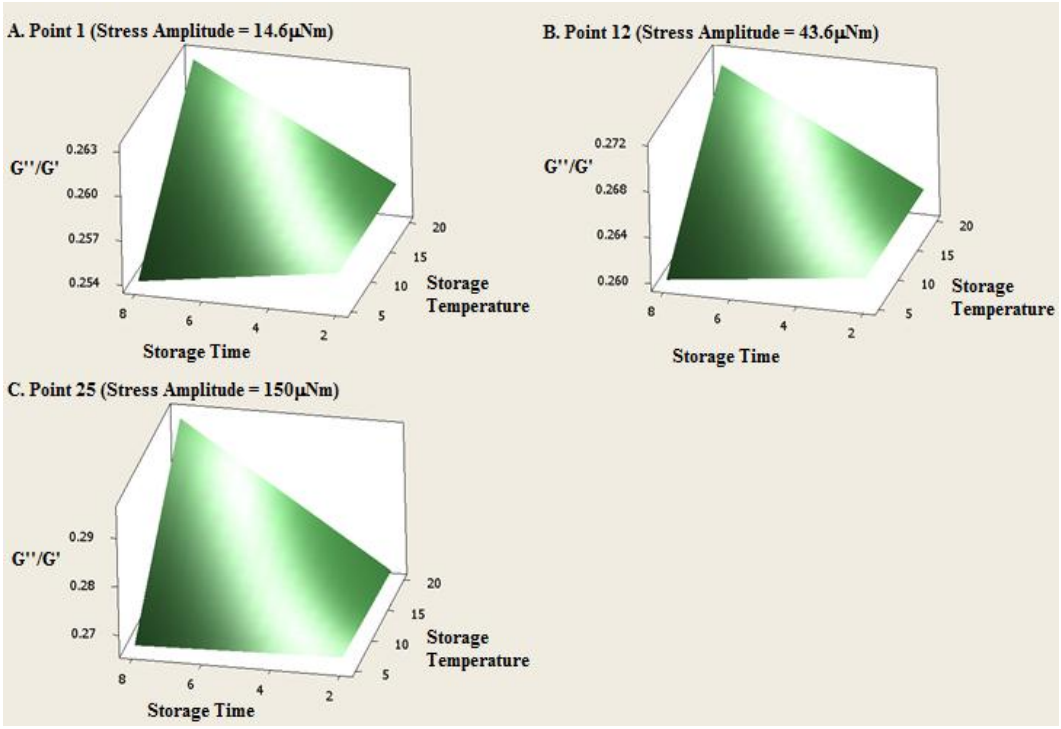

Figure 5-4: Effects of storage temperature and time, applied to yogurt powders, on the loss tangent (tan δ = G''/G') of recombined samples at three selected points of the stress amplitude range applied

Surface plots were constructed using the means of triplicate measurements. Appendix D (Tables D-3 to D-6) provides the experimental data used to construct these graphs.

Consistent with **Figures 5-3** and **5-4**, in order to preserve the initial rheological properties of the recombined product for a longer time, yogurt powders must be stored at low temperatures.

Table 5-3 illustrates the physicochemical characteristics of the different experimental samples considered. None of the reconstituted products presented physicochemical aspects significantly different from the reference samples ($P > 0.05$). All samples presented physicochemical aspects similar to those of yogurts reconstituted from fresh dried formulations ($P > 0.05$). However, samples produced from powders stored for 8 weeks at 20°C required considerably longer incubation times ($P < 0.05$) to reach pH 4.6 ± 0.03.

Table 5-3: Physicochemical properties of samples produced from powders stored at different conditions [€, £, Δ]

Sample #	Size of Visible Clusters	Level of Whey Drainage	Level of SWO_{BH} (%m/m)	Level of SWO_{AH} (%m/m)	Incubation Time (Hours)
1	1.000 ± 0.000^{A}	0.000 ± 0.000^{A}	0.000 ± 0.000^{A}	0.000 ± 0.000^{A}	12.367 ± 0.115^{A}
2	1.000 ± 0.000^{A}	0.000 ± 0.000^{A}	0.000 ± 0.000^{A}	0.000 ± 0.000^{A}	12.333 ± 0.058^{A}
3	1.000 ± 0.000^{A}	0.000 ± 0.000^{A}	0.000 ± 0.000^{A}	0.000 ± 0.000^{A}	12.300 ± 0.100^{A}
4	1.000 ± 0.000^{A}	0.333 ± 0.577^{A}	0.000 ± 0.000^{A}	0.000 ± 0.000^{A}	15.867 ± 1.686^{B}
Fresh Sample	1.000 ± 0.000^{A}	0.000 ± 0.000^{A}	0.000 ± 0.000^{A}	0.000 ± 0.000^{A}	12.400 ± 0.173^{A}

[€] Presented values are the means of 3 replications (mean ± SD). [£] Numbers with different letters within the same column are significantly different ($P < 0.05$). [Δ] Appendix D (Table D-2) provides the experimental data used to construct this table. SWO_{BH}: Surface whey-off before homogenization. SWO_{AH}: Surface whey-off after homogenization.

The following contour plot (**Figure 5-5**) shows the effects of the storage temperatures and times applied to the yogurt powders on the incubation times required to acidify the resultant recombined milk bases to pH 4.6 ± 0.03.

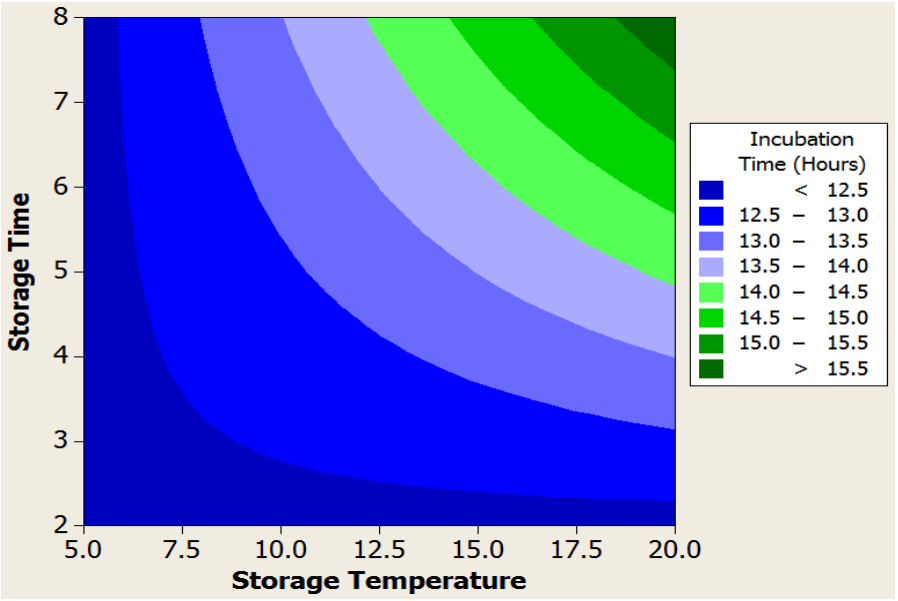

Figure 5-5: Effects of storage temperature and time, applied to yogurt powders, on the incubation times of samples

This contour plot was constructed using the means of triplicate measurements. Appendix D (Table D-2) provides the experimental data used to construct this graph.

Recombined milks obtained from powders stored at lower temperatures needed lower incubation times. As storage temperature and time increased, the incubation times increased. However, exposing yogurt powders to room temperature (20^{o}C) for less than 2.5 weeks did not cause a considerable increase in the incubation times needed to produce the final recombined product. These results suggest that after 2 weeks of storage at room temperature, the yogurt powder must be stored at lower temperatures in order to delay the increase of the incubation time required to produce the recombined product. This observation agrees with the recommendations of the starter culture producer (Danlac Canada Inc.). The manufacturer indicates that dispatching the product at ambient temperatures for a maximum of 2 weeks does not influence the culture's best before date. The manufacturer also states that after reception, the product must be stored at $\leq$ -18^{o}C. Nevertheless, this investigation proved that storing the starter culture at 5^{o}C for at least 8 weeks did not have significant effects ($P > 0.05$) on the rheological and physicochemical properties of the recombined product and did not have a considerable influence on the incubation time required to produce the final yogurt. Thus, it is believed that the storage of the starter culture at 5^{o}C for 8 weeks did not significantly affect its viability. These results are consistent with the findings of Kumar & Gandhi (2009), who affirmed that freeze-dried concentrated starter cultures are stable at 5^{o}C without loss of activity. Furthermore, Saxelin *et al.* (1999) studied the survival of eight different freeze-dried species of lactic acid bacteria during storage and stated that most cultures could be stored for one year at 5^{o}C without any significant loss in viability. However, it is important to point out that the storage stability of freeze-dried starter cultures is intimately related to the types of cryoprotectants added to the initial culture before freeze-drying (Saarela *et al.*, 2005).

According to the results of this investigation, it is believed that prolonged storage at high temperatures caused a detrimental effect on the activity of the freeze-dried starter cultures present in the yogurt powder. This observation coincides with the results obtained by several investigators. Achour *et al.* (2001) studied the survival rates of freeze-dried *Lactococcus* starter cultures and demonstrated that temperature had a destructive effect on survival rates. They reported that an average half-life of a strain maintained at 25^{o}C was equal to about 7 days as compared with about 43 days at 4^{o}C.

Bruno & Shah (2003) investigated the viability of two freeze-dried strains of *Bifidobacterium* at various temperatures during prolonged storage and concluded that strains stored at 20^{o}C showed the greatest decline in the viability of bacteria, whereas those stored at -18^{o}C showed the least.

For long-term storage, inactivation of freeze-dried starter cultures significantly depends on the storage conditions. The inactivation is correspondingly related to the storage temperature and the moisture content. This is mainly due to the state of dried starter cultures. In general, components of biological materials in dehydrated states form amorphous structures, with the typical characteristics referred to as the rubbery (amorphous liquid) and glassy (amorphous solid) states. The most important parameter describing the glassy state is the glass transition temperature (T_g), below which materials exhibit extremely high viscosity that gives them solid-like properties (Santivarangka *et al*., 2008). During storage, T_g of the dried sample is an important factor affecting the viability of cultures, and therefore moisture content becomes a key variable (Santivarangka *et al*., 2007). When the dried starter cultures are filled in moisture-permissible packages, the relative humidity of the storage environments will have additional influence besides the end moisture after drying (Santivarangka *et al*., 2008). It is thought that high viscosity of sugar glasses below or around their T_g retards molecular mobility and reaction rate, hence stabilizing the biological system. Storing the dried cultures at a temperature lower than their T_g would increase the stability (**Figure 5-6**) (Santivarangka *et al*., 2007). This fact was proved by Passot *et al*. (2012) who investigated the influence of water activity and amorphous state on the stability of co-lyophilized *Lactobacillus bulgaricus* with sucrose and reported that the optimal stability of the lyophilized bacteria was observed below T_g, in the intermediate water activity range 0.1 - 0.214. Andersen *et al*., (1999) showed that freeze-dried *Streptococcus thermophilus* in a matrix of ascorbic acid, casein and sucrose or mannitol presented significantly low inactivation rates below T_g, and that when the cultures were stored at or above T_g their loss of activity increase dramatically. Higl *et al*. (2007) reported that the inactivation of freeze-dried *Lactobacillus paracasei* ssp. *paracasei* in a lactose matrix was low below T_g; however, when the cells were stored in the non-glassy state ($T > T_g$) the inactivation was not as rapid as suggested by the temperature dependence of the viscosity above the glass transition temperature. Furthermore, Higl *et. al* (2007) stated that the first-order inactivation rate constant, *k*, was dependent on the storage temperature

per se rather than on the temperature difference between the glass transition temperature and the storage temperature (T – T_g).

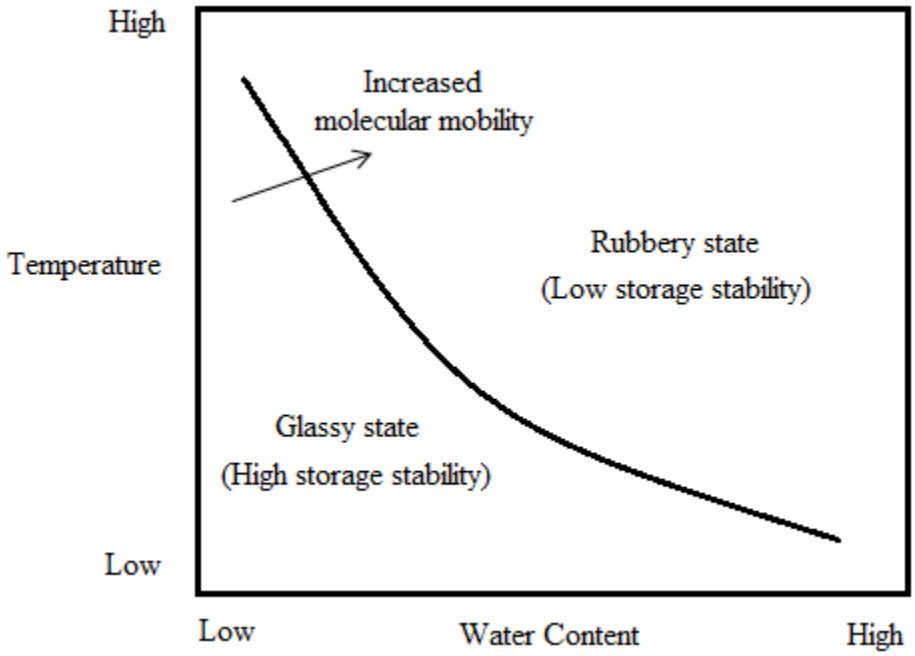

Figure 5-6: Glass transition curve scheme which relates the glass transition temperature and moisture content

Molecular mobility and deleterious reaction rates in the glassy state are extremely low, while they increase at the storage conditions above the curve (rubbery state). *Source*: Adapted from Santivarangka *et al*., 2008.

Although T_g cannot be regarded as an absolute threshold of bacterial stability during storage because not every inactivation process is diffusion limited, inactivation below T_g is low (Santivarangka *et al*., 2008; Higl *et al*. 2007). For example, the free radical reactions are not diffusion controlled, and will therefore not be reduced in the glassy state but, the diffusion of oxygen into the dry matrix will be slow, and this will decrease the production rate of free radicals (Santivarangka *et al*., 2008).

All starter cultures present in experimental samples were conserved in non-moisture-permissible packages (small polyethylene sealed bags) at an equal relative humidity but at different temperatures. Therefore, it is believed that dried cultures stored at different temperatures presented different amorphous states which resulted in different storage stabilities (starter cultures stored at 20^{o}C for eight weeks presented the lowest level of viability).

The presence of O_2 and light are other critical factors influencing the stability of cultures. The main cause of deterioration of freeze-dried starter cultures is related to membrane lipid oxidation (Andersen *et al*., 1999; Santivarangka *et al*., 2008). A study on the lipid composition of the cell membrane by gas chromatography showed that the unsaturated/saturated fatty acid index of the cell membrane changes within storage (Castro *et al*., 1995). These changes strongly affect the passive permeability of the membrane and contribute to cellular death (Santivarangka *et al*., 2007). In addition, rehydration of the cultures is another critical step that can influence the level of viability of freeze-dried microorganisms. The medium itself, its molarity and rehydration conditions, can drastically affect the rate of recovery (Kumar & Gandhi, 2009). However, as all the starter cultures present in the experimental samples were packed and rehydrated the same way, the influence of O_2, light, and rehydration cannot be used to explain the rheological differences obtained for the different recombined samples.

As the level of viability of starter cultures decreased, the incubation times required for the production of acid milk gels increased and the final gel obtained had a higher liquid-like behaviour (lower G' and $\sigma_{fracture}$, and higher tan δ). These results are consistent with the findings of Jumah *et al*. (2001), Sodini *et al*. (2004); Lee & Lucey (2004); Wu *et al*. (2009).

Low levels of viability of starter cultures resulted in low lactic acid production rates during the incubation of the recombined milk bases; hence, longer times were needed to acidify the medium up to pH 4.6. In milk, the integrity of casein micelles is controlled by a localized balance between hydrophobic interactions and electrostatic repulsions. As the pH of milk decreases during fermentation, the colloidal calcium phosphate (CCP) within casein micelles is solubilized, especially at pH < 6.0, and it is completed by pH ≈ 5.0, which leads to the partial rearrangement of the internal structure of the casein micelle. When CCP is dissolved within casein particles, there is an increase in electrostatic repulsion between the exposed phosphoserine residues. In this way, the solubilization of CCP weakens casein-casein interactions and probably contributes to a slight decrease in G' values. As the pH of milk approaches the isoelectric point (i.e., pH < 5.0), electrostatic repulsion decreases, which facilitates enhanced casein-casein attractions due to increased hydrophobic interactions. These factors increase bond formation/strength and thus increase gel stiffness, contributing to an increase in G' values (Lee & Lucey, 2004).

The solubilization of CCP in milk during acidification is a slow process, and may require a slightly lower pH to completely dissolve CCP under condition of fast acidification (i.e., high inoculation rates may be less efficient in solubilizing CCP, as there would be less time at any particular pH value during milk acidification). When CCP dissolves at a lower pH, caseins at this lower pH value may be less sensitive to excessive rearrangements (due to the fact that at lower pH values there will be lower electrostatic repulsion and higher hydrophobic interactions between casein particles), and this may make stiffer gel networks (i.e., high G' and $\sigma_{fracture}$ values) (Lee & Lucey, 2004). More rapid drops in pH may lock the protein into a more dispersed structure with greater density of possibly stronger strands (Horne, 2008). For this reason, higher levels of viability of the starter culture resulted in acid milk gels with higher G' and $\sigma_{fracture}$ and lower tan δ values.

The overall result of the lower viability level of starter cultures was the formation of weaker gels that were more prone to rearrangements. Large scale rearrangements, related to dynamics and relaxation of the protein-protein bonds, increased the instability of the gel network and reduced its ability to entrap all the serum phase (Lucey *et al*., 1998; Lucey, 2002; Lucey & Singh, 1998; Lee & Lucey, 2010). Therefore, samples containing lower amounts of viable starter culture (sample 4) presented higher levels of SWO_{BH}. However, all samples were considered to have significantly similar levels of SWO_{BH} for a significance level of 0.05.

The differences in the physicochemical and rheological properties of experimental samples were mainly attributed to the different activities of the starter cultures. Although milk powders had different moisture, lactose, protein and fat contents, and were not vacuum packed, the levels of moisture transfer (within the different powders and between powders and the surroundings), lipid oxidation, lactose crystallization and other biochemical reactions that occurred during the storage of the powder mix were not considered critical parameters responsible to affect in a significant way the rheological and physicochemical properties of the final reconstituted product (milk powders had very low lipid and moisture contents and were stored at 40-60% relative humidity). However, it is possible that the latter factors could accentuate the unfavourable marked effects caused by the loss of viability of the starter cultures. Recently, Karam *et al*. (2012) reported no differences in the G' of yogurts fortified with a micellar casein powder stored for 1 year at 20^{o}C and those fortified with a fresh micellar casein powder.

In order to reduce the incubation times needed to produce the recombined yogurt and extend the initial rheological properties of the recombined product throughout its dry storage, the freeze-dried starter culture inside the product container must be packed in a non-moisture, non-oxygen and non-light permissible package and stored at low temperatures (after 2 weeks of exposure to ambient temperature, the yogurt powder must be stored under refrigeration) (Kumar & Gandhi, 2009; Santivarangka *et al*., 2007; Santivarangka *et al*., 2008; Kurtmann *et al*., 2009).

Additionally, the storage stability of the yogurt powder can also be improved by considering the use of freeze-dried cultures containing different types of protective agents. Many compounds have been tested to improve the survival of lactic acid bacteria during the freeze-drying process and the subsequent storage period: polyols, polysaccharides, disaccharides, amino acids, proteins, vitamins and various salts (Champagne *et al*., 1996; Santivarangka *et al*., 2007). Some compounds can be added to the drying medium to raise the T_g of cultures and therefore better stabilize the storage viability at a given condition. Antioxidants can be also included to scavenge free radicals and diminish lipid oxidation (Santivarangka *et al*., 2007). The influence of these protectants proved to be species specific and therefore needs to be determined on a case-by-case basis (Strasser *et al*., 2009). Several authors reported the effectiveness of protective agents on different lactic acid bacteria (Andersen *et al*., 1999; Kurtmann *et al*., 2009; Champagne *et al.* 1996; Achour *et al*., 2001; Saarela *et al.*, 2005; Venir *et al*., 2007; Zayed & Ross, 2004; Sunny-Roberts & Knorr, 2009).

Considering the previous recommendations should make it feasible to increase the storage stability of the dried formulation proposed. In this way, the yogurt powder can be stored in its dry form for longer periods of time without considerable changes to the rheological aspects of the hydrated product obtained after its recombination. Heeding the recommendations should also prevent the need for long incubation times during the production of the final gel.

5.4 Conclusion

Exposing the yogurt powder to ambient temperature (20^{o}C) for long periods of time caused considerable changes to the rheological properties of the recombined product and the incubation times required to produce the final gel. Alterations of these two factors within storage were associated with the loss of activity of the starter cultures present in

samples exposed to high temperatures. Two main recommendations were given in order to decrease the inactivation rates of starter cultures throughout storage: conserve the dried product at low temperatures, and consider the use of different packages and cryoprotectants to shelter starter cultures from inactivation. Further work is required in order to select the appropriate cryoprotectants and packages required to extend the storage stability of the starter culture blend employed. This additional work is indispensable to the efforts to offer a yogurt powder that can be recombined using standard parameters and can result in good rheological properties throughout its shelf-life.

5.5 References

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6. Summary of findings, conclusions and recommendations for future research

6.1 Summary of findings

The growing awareness of the relationship between diet and health has led to an increasing demand for food products that support health (Cirak, 2009). Dairy products are foods of high nutrient density and that means that they have a high concentration of major nutrients in relation to their energy value (Chryssanthopoulos & Maridaki, 2009). Moreover, fermented milk products may be termed as "functional foods" that have health benefits beyond conventional nutrition (Chandan & Nauth, 2012). Greek-style yogurt (concentrated yogurt) has nutritional benefits superior to those found in regular yogurt (Mahdian & Tehrani 2007). This healthy image has led to a remarkable sale growth of this product in the United States over the past years (Palmer & Sakan, 2011).

The development of an efficient Greek-style yogurt powder formulation is a promising strategy to extend the economic impact of this food commodity to other regions and to address nutritional defects in regions that present temporary or permanent deficiencies in milk supply.

6.1.1 Development of the novel Greek-style yogurt powder formulation free of additives and fat

This experiment was designed to find an effective formulation to produce a recombined, non-fat, additive-free type of acid milk gel with similar rheological and physicochemical aspects to plain Greek-style yogurts (0% M.F.) commercialized in Edmonton, AB, Canada.

To address this objective, two commercial Greek-style yogurts (0% M.F.) were used as reference samples. Recombined acid milk gels were manufactured by two different production methods (method 1 and 2). All recombined gels had the same amount of TS and TP, 13.7% and 10.3%, respectively. Samples differed in their total protein composition, the casein to whey protein ratios and the starter culture contents. Method 1 did not include a heat treatment of the milk base over 60^{o}C. Method 2 included a severe heat treatment (90^{o}C for 5 minutes). Small amplitude oscillatory rheology tests

were performed on samples, and G' and G'' were recorded. Important physicochemical parameters such as whey separation (surface whey-off and whey drainage) and presence and size of visible clusters were evaluated. A one-block full factorial design 3*2 was used to investigate the effect of MPC-85, NaCN, WPI-90 and SC concentrations on the rheological and physicochemical properties of recombined yogurt gels obtained by each manufacturing method. According to the results obtained, a final formulation with desired rheological and physicochemical properties was proposed and compared to the reference samples to detect significant differences.

The results showed that heat treated formulations containing high amounts of NaCN and low amounts or no presence of WPI-90 (casein to whey protein ratio ≈ 6:1) resulted in products with the best physicochemical characteristics. These findings are consistent with the results of Peng *et al.* (2009), Remeuf *et al.* (2003), and Isleten & Karagul-Yuceer (2006), who stated that yogurts fortified with NaCN to high casein to whey protein ratios had very good textural and sensory attributes. However, none of the combinations between the different factors considered that resulted in significantly similar physicochemical characteristics to the reference standard ($P > 0.05$) could result in rheological properties considerably close to the market samples.

6.1.2 Physicochemical and rheological stability of the recombined yogurt obtained from the dry formulation proposed, and the application of a simplified method for the hydration of the yogurt powder

The objectives of this study were: (1) to study, throughout the storage period, the rheological and physicochemical stability of the recombined acid milk gel obtained from the new formulation proposed; (2) to study the effects of a new simplified recombination method on the rheological and physicochemical aspects of the recombined product obtained from the previously proposed dry formulation.

To address the first objective, production method 2 used in the previous study was applied to produce 4 sets of samples (each set was composed of three independent samples) which were stored in a cooling chamber at 5^{o}C for different time periods (1, 4, 8, and 12 days). Rheological and physicochemical measurements were carried out on these samples after their corresponding storage time in order to study the rheological and physicochemical storage stability of the reconstituted gel obtained by the proposed formulation. To accomplish the second objective, 2 sets of samples (each set was

composed of 3 independent samples) were produced by a simplified production method, which did not require a pre-heating step at 40^{o}C for 20 minutes to attain the normal state of hydration of milk proteins, and stored at 5^{o}C for different time periods (1 and 8 days). Rheological and physicochemical measurements were conducted on these samples after their corresponding storage times. Results were compared with those obtained from the same type of samples produced by the manufacturing method 2 used in Chapter 3.

The recombined acid milk gel manufactured by the first production method conserved its initial physicochemical and rheological properties for at least 4 days after recombination. The rheological characteristics of the product were maintained significantly unchanged for at least 4 days, while the physicochemical properties were conserved for at least 8 days. Acid milk gels produced by the new simplified recombination method proposed presented rheological and physicochemical aspects significantly similar ($P > 0.05$) to those of gels manufactured by the previous recombination method considered. Therefore, according to the results obtained, it can be stated that the normal state of hydration of milk proteins is achieved during the first stages of incubation, hence, there is no need to apply a preheating step (40^{o}C for 20 minutes) before incubation.

6.1.3 Effects of storing the dry formulation on the rheological and physicochemical characteristics of the recombined yogurt

This experiment was designed to determine how storing the previously proposed yogurt powder formulation affected the rheological and physicochemical characteristics of the final recombined product.

To address this objective, the corresponding amounts of milk powders needed to produce 700g of the recombined product were placed into 1L plastic cups. The amounts of starter culture required to produce 700g of yogurt were packed into small polyethylene bags and each bag was put inside a plastic cup containing the milk powders. Plastic cups were covered with plastic lids and stored at 5^{o}C (inside a cooling chamber) and 20^{o}C (inside an electric and thermostatically controlled incubator) for different amounts times. After storage, powders were recombined using the simplified method proposed in the previous study. A one-block full factorial design 2*2 was used to investigate how the storage time and temperature applied to the dried yogurt powder formulation affected the rheological and physicochemical characteristics of the final recombined product.

Exposing the yogurt powder to ambient temperature (20^{o}C) for long periods of time caused considerable changes in the rheological properties of the recombined product and in the incubation times required to produce the final gel. Alterations of these two factors within storage were associated with the loss of activity of the starter cultures present in samples exposed to high temperatures. Two main recommendations were made in order to decrease the inactivation rates of starter cultures throughout storage: conserve the dried product at low temperatures, and consider the use of different packages and cryoprotectants to shelter starter cultures from inactivation.

6.2 Conclusions and recommendations for future research

This study demonstrated that total protein composition, denaturation level of whey proteins, and starter culture contents are significant parameters that affect the acidification kinetics and the physical and rheological properties of recombined acid milk gels obtained from dried formulations. These statements are supported by several investigations (Wu *et al*., 2009; Damin *et al*., 2009; Isleten & Karagul-Yuceer, 2008; Peng *et al*., 2009; Amatayakul *et al*., 2006; Cho *et al*., 1999; Lucey *et al*., 1997; Lucey *et al*., 1998; Lucey *et al*., 1999; Lee & Lucey, 2004; Marafon *et al*., 2011; Krzeminski *et al*., 2011)

Although the casein to whey protein ratio of milk bases is an important parameter currently associated with the physicochemical and rheological characteristics of acid milk gels (Puvanenthiran *et al*., 2002; Amatayakul *et al*., 2006; Kucukcetin, 2008), this investigation found no direct correlation between this ratio and the physicochemical and rheological aspects of the final gels. This finding matches the observations reported by Remeuf *et al.* (2003) and Peng *et al.* (2009). Therefore, to obtain a yogurt with desired physicochemical and rheological characteristics, is very important to consider the formulation composition beyond its casein to whey protein ratio.

Even though previous investigations demonstrated that the total solids and total protein contents of milk bases have a direct influence on the physical and rheological aspects of yogurt gels (Kristo *et al*., 2003; Anema, 2008; Jumah *et al*., 2001; Mahdian & Tehrani, 2007; Barreto Penna *et al*., 2006), the present investigation attempted to propose a formulation with a fixed total protein (10.3%) (equal to the one present in both reference samples used) and total solids (13.7%) (equal to the one present in the reference sample with the lowest total solids content) contents in order to offer a highly nutritive

product with low caloric content. According to the production methods used to manufacture the experimental samples, and the factors and levels considered in the experimental design conducted in Section 3, it can be concluded that using a heat-treated, inoculated, recombined milk containing NaCN (19.0 % TP), MPC-85 (81.0 %TP), MPP (13.1 %TS), SC (50 DCU $100L^{-1}$) may produce a yogurt with the closest physicochemical and rheological characteristics considered (at day 1 after production) to the established reference standard.

However, the recombined product obtained by this formulation did not present a rheological behaviour (for the entire amplitude range considered) significantly similar ($P > 0.05$) to the reference standard at day 1 after production, and it lacked good rheological and physicochemical stability during storage at 5^oC. Consistent with the findings of previous studies (Zbikowski *et al.*, 1998; Damin *et al*., 2009; Harwalkar & Kalab, 1986; Remeuf *et al*., 2003), it should be feasible to optimize the rheological behaviour of the recombined gel, maintain its required physicochemical properties, and improve its rheological and physicochemical storage stability by considering a lower whey protein denaturation level and increasing the total protein (total solids) content by adding WPI-90 to the final formulation (reducing the NaCN to WPI-90 ratio in the final formulation).

On the other hand, recombined acid milk gels obtained by the proposed dried formulation stored at 20^oC for 8 weeks required significantly longer incubation times ($P < 0.05$) and presented rheological characteristics that were considerably different ($P < 0.05$) from gels produced by the fresh, dried formulation. To offer a yogurt powder which can be recombined using standard parameters and result in good rheological properties throughout all its shelf-life, it is recommended to conserve the dried product at low temperatures and consider using different packaging and cryoprotectants to shelter starter cultures from inactivation.

According to the observations and results obtained in the present study, further work seems necessary to optimize the rheological characteristics and improve the physicochemical and rheological storage stability of the recombined gel obtained by the proposed formulation. Relevant importance should be given to the whey protein denaturation level and to the total protein content and composition of the final formulation. Additional research is also needed in order to select the appropriate cryoprotectants and packaging required to extend the storage stability of the starter culture blend employed in the formulation. This further work will help to improve the storage stability of the suggested formulation in its powder form.

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7. Appendices

Appendix A – Market reference samples

Table A-1: Physicochemical analyses of market reference samples

Ref. sample [¥]	No. of repetition	Surface whey-off (%m/m)	Whey drainage (Ordinal scale: 0;1;2)	Size of visible clusters (Ordinal scale: 0;1;2;3)
A – Day 18	1	0.00	0	1
	2	0.00	0	1
	3	0.15	0	1
	Mean ± SD	**0.050 ± 0.087**	**0.000 ± 0.000**	**1.000 ± 0.000**
A – Day 35	1	0.22	0	1
	2	0.18	0	1
	3	0.21	0	1
	Mean ± SD	**0.203 ± 0.021**	**0.000 ± 0.000**	**1.000 ± 0.000**
B – Day 18	1	0.09	0	1
	2	0.00	0	1
	3	0.00	0	1
	Mean ± SD	**0.030 ± 0.052**	**0.000 ± 0.000**	**1.000 ± 0.000**
B – Day 35	1	0.30	0	1
	2	0.00	0	1
	3	0.16	0	1
	Mean ± SD	**0.153 ± 0.150**	**0.000 ± 0.000**	**1.000 ± 0.000**

[¥] A-Day 18: Reference sample A stored for 18 days after production; A-Day 35: Reference sample A stored for 18 days after production; B-Day 18: Reference sample B stored for 18 days after production; B-Day 35: Reference sample B stored for 35 days after production.

Table A-2: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of reference sample A at day 18 after its production

Meas. Pts.	Stress Amp. (μNm)	A 1-1		A 1-2		A 2-1		A 2-2		A 3-1		A 3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	16.5	4.19	16.2	4.09	16.2	4.12	15.7	3.99	14.6	3.79	15.3	4	**15.750**	**4.030**	**0.256**
2	16.7	16.6	4.23	16.2	4.12	16.2	4.14	15.7	4.02	14.6	3.82	15.3	4.02	**15.767**	**4.058**	**0.257**
3	18.4	16.5	4.21	16.2	4.14	16.2	4.15	15.7	4.05	14.6	3.85	15.2	4.03	**15.733**	**4.072**	**0.259**
4	20.2	16.4	4.2	16.2	4.15	16.2	4.17	15.6	4.07	14.5	3.87	15.3	4.09	**15.700**	**4.092**	**0.261**
5	22.2	16.4	4.22	16.2	4.17	16.2	4.21	15.6	4.09	14.5	3.89	15.1	4.07	**15.667**	**4.108**	**0.262**
6	24.5	16.4	4.25	16.1	4.19	16	4.19	15.6	4.12	14.5	3.92	15	4.08	**15.600**	**4.125**	**0.264**
7	26.9	16.1	4.2	16.1	4.22	15.9	4.19	15.5	4.15	14.4	3.95	15	4.1	**15.500**	**4.135**	**0.267**
8	29.7	16.1	4.24	16	4.24	15.8	4.18	15.5	4.16	14.3	3.98	15	4.16	**15.450**	**4.160**	**0.269**
9	32.7	16.1	4.26	16	4.28	15.7	4.2	15.4	4.19	14.2	4.01	14.8	4.15	**15.367**	**4.182**	**0.272**
10	35.9	16.1	4.28	15.9	4.3	15.6	4.21	15.2	4.23	14.1	4.03	14.7	4.17	**15.267**	**4.203**	**0.275**
11	39.6	15.9	4.3	15.8	4.35	15.5	4.22	15.1	4.25	14	4.05	14.6	4.22	**15.150**	**4.232**	**0.279**
12	43.6	15.7	4.31	15.6	4.37	15.3	4.23	14.9	4.27	13.8	4.08	14.3	4.25	**14.933**	**4.252**	**0.285**
13	48	15.5	4.34	15.4	4.4	15.1	4.25	14.7	4.3	13.6	4.12	14	4.24	**14.717**	**4.275**	**0.290**
14	52.8	15.3	4.35	15.2	4.43	14.8	4.26	14.5	4.31	13.3	4.15	13.8	4.26	**14.483**	**4.293**	**0.296**
15	58.1	15	4.38	15	4.44	14.5	4.29	14.2	4.34	13	4.19	13.4	4.28	**14.183**	**4.320**	**0.305**
16	64	14.7	4.38	14.7	4.47	14.2	4.29	13.9	4.36	12.6	4.22	13	4.33	**13.850**	**4.342**	**0.313**
17	70.5	14.3	4.39	14.4	4.49	13.9	4.32	13.6	4.41	12.2	4.26	12.5	4.38	**13.483**	**4.375**	**0.324**
18	77.6	13.9	4.43	14	4.5	13.5	4.32	13.1	4.44	11.6	4.33	11.9	4.46	**13.000**	**4.413**	**0.339**
19	85.5	13.5	4.46	13.6	4.52	13	4.36	12.6	4.49	11	4.43	11.1	4.56	**12.467**	**4.470**	**0.359**
20	94.1	12.9	4.48	13	4.54	12.5	4.36	11.9	4.57	10	4.62	10.1	4.71	**11.733**	**4.547**	**0.388**
21	104	12	4.63	12.4	4.58	11.8	4.42	11.1	4.67	8.83	5.03	8.9	5	**10.838**	**4.722**	**0.436**
22	114	11.1	4.71	11.6	4.65	10.5	4.79	10	4.87	0.679	1.42	0.352	1.18	**7.372**	**3.603**	**0.489**
23	126	9.98	4.89	10.7	4.79	9.14	5.08	8.62	5.31	0.108	0.77	0.0778	0.709	**6.438**	**3.591**	**0.558**
24	140	8.24	5.47	9.31	5.08	0.427	1.29	0.455	1.23	0.036	0.45	0.0249	0.396	**3.082**	**2.319**	**0.753**
25	150	0.187	0.886	0.551	1.37	0.0914	0.751	0.131	0.765	0.0417	0.24	0.042	0.205	**0.174**	**0.703**	**4.037**

Table A-3: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of reference sample A at day 35 after its production

Meas. Pts.	Stress Amp. (μNm)	A 1-1		A 1-2		A 2-1		A 2-2		A 3-1		A 3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	18.6	4.55	18.3	4.51	17.3	4.29	17.9	4.47	17.5	4.42	17.6	4.51	**17.867**	**4.458**	**0.250**
2	16.7	18.5	4.56	18.3	4.53	17.3	4.30	17.8	4.46	17.5	4.42	17.6	4.53	**17.833**	**4.467**	**0.250**
3	18.4	18.5	4.56	18.3	4.58	17.3	4.33	17.8	4.49	17.5	4.45	17.5	4.54	**17.817**	**4.492**	**0.252**
4	20.2	18.4	4.57	18.1	4.57	17.2	4.36	17.8	4.49	17.5	4.47	17.5	4.55	**17.750**	**4.502**	**0.254**
5	22.2	18.3	4.58	18.1	4.61	17.2	4.37	17.7	4.5	17.5	4.48	17.5	4.58	**17.717**	**4.520**	**0.255**
6	24.5	18.3	4.61	18.1	4.63	17.1	4.41	17.6	4.53	17.4	4.50	17.5	4.59	**17.667**	**4.545**	**0.257**
7	26.9	18.2	4.62	18	4.65	17.1	4.42	17.5	4.54	17.4	4.52	17.4	4.62	**17.600**	**4.562**	**0.259**
8	29.7	18.1	4.66	17.8	4.68	17	4.46	17.3	4.56	17.2	4.53	17.4	4.63	**17.467**	**4.587**	**0.263**
9	32.7	18	4.65	17.8	4.7	16.9	4.46	17.2	4.57	17.1	4.55	17.3	4.67	**17.383**	**4.600**	**0.265**
10	35.9	17.8	4.69	17.6	4.72	16.8	4.50	17.1	4.58	17	4.56	17.2	4.68	**17.250**	**4.622**	**0.268**
11	39.6	17.7	4.7	17.5	4.75	16.6	4.54	16.9	4.62	16.9	4.64	17.1	4.71	**17.117**	**4.660**	**0.272**
12	43.6	17.5	4.75	17.3	4.78	16.5	4.55	16.7	4.64	16.8	4.66	16.9	4.73	**16.950**	**4.685**	**0.276**
13	48	17.3	4.77	17.1	4.82	16.2	4.59	16.4	4.66	16.6	4.68	16.7	4.74	**16.717**	**4.710**	**0.282**
14	52.8	17.1	4.8	16.9	4.86	16	4.60	16.1	4.7	16.4	4.70	16.4	4.78	**16.483**	**4.740**	**0.288**
15	58.1	16.8	4.81	16.5	4.94	15.7	4.64	15.8	4.74	16.1	4.75	16.1	4.82	**16.167**	**4.783**	**0.296**
16	64	16.4	4.86	16.2	4.98	15.3	4.69	15.4	4.77	15.8	4.77	15.8	4.84	**15.817**	**4.818**	**0.305**
17	70.5	16	4.92	15.8	5	14.8	4.73	14.9	4.82	15.4	4.80	15.4	4.89	**15.383**	**4.860**	**0.316**
18	77.6	15.6	4.93	15.3	5.05	14.3	4.79	14.4	4.88	15	4.82	14.9	4.92	**14.917**	**4.898**	**0.328**
19	85.5	14.8	5.09	14.8	5.09	13.6	4.85	13.6	4.97	14.5	4.86	14.3	4.98	**14.267**	**4.973**	**0.349**
20	94.1	14.3	5.05	14.1	5.16	12.7	4.95	12.6	5.09	13.8	4.91	13.5	5.02	**13.500**	**5.030**	**0.373**
21	104	13.4	5.13	13.2	5.23	11.7	5.06	11.4	5.23	13	4.99	12.6	5.11	**12.550**	**5.125**	**0.408**
22	114	12.3	5.25	12.2	5.35	10.4	5.23	10.1	5.42	11.9	5.15	11.5	5.22	**11.400**	**5.270**	**0.462**
23	126	10.9	5.4	10.7	5.64	8.75	5.62	8.14	6.14	10.2	5.71	10.2	5.41	**9.815**	**5.653**	**0.576**
24	140	9.17	5.81	8.43	6.56	0.273	1.17	0.186	1.17	8.4	6.18	7.93	6.47	**5.732**	**4.560**	**0.796**
25	150	0.299	1.32	0.342	1.52	0.0553	0.59	0.0244	0.627	0.228	1.25	0.154	1.11	**0.184**	**1.070**	**5.822**

Table A-4: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of reference sample B at day 18 after its production

Meas. Pts.	Stress Amp. (μNm)	B 1-1		B 1-2		B 2-1		B 2-2		B 3-1		B 3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	20.6	5.3	20.4	5.29	21.5	5.46	19.9	5.2	20.8	5.28	20.8	5.39	**20.667**	**5.320**	**0.257**
2	16.7	20.6	5.3	20.4	5.32	21.6	5.54	19.9	5.24	20.9	5.32	20.9	5.43	**20.717**	**5.358**	**0.259**
3	18.4	20.7	5.33	20.5	5.35	21.7	5.56	20	5.27	21	5.36	20.9	5.45	**20.800**	**5.387**	**0.259**
4	20.2	20.8	5.36	20.6	5.38	21.7	5.59	20	5.3	21.1	5.4	21	5.49	**20.867**	**5.420**	**0.260**
5	22.2	20.9	5.4	20.5	5.39	21.7	5.61	20.1	5.33	21.2	5.45	21	5.5	**20.900**	**5.447**	**0.261**
6	24.5	20.7	5.37	20.4	5.38	21.6	5.6	20.1	5.36	21.2	5.47	21	5.51	**20.833**	**5.448**	**0.262**
7	26.9	20.8	5.42	20.4	5.41	21.7	5.63	20.1	5.38	21.3	5.5	21	5.53	**20.883**	**5.478**	**0.262**
8	29.7	20.9	5.44	20.4	5.42	21.7	5.64	20.2	5.44	21.3	5.54	21.1	5.57	**20.933**	**5.508**	**0.263**
9	32.7	20.9	5.46	20.4	5.44	21.8	5.68	20.2	5.48	21.3	5.56	21	5.57	**20.933**	**5.532**	**0.264**
10	35.9	20.9	5.49	20.4	5.46	21.8	5.7	20.2	5.49	21.3	5.55	20.9	5.59	**20.917**	**5.547**	**0.265**
11	39.6	20.9	5.51	20.4	5.49	21.8	5.73	20.3	5.53	21.3	5.58	21	5.62	**20.950**	**5.577**	**0.266**
12	43.6	20.9	5.54	20.4	5.51	21.7	5.76	20.2	5.53	21.3	5.62	20.9	5.63	**20.900**	**5.598**	**0.268**
13	48	20.6	5.55	20.3	5.53	21.7	5.81	20.1	5.56	21.3	5.66	20.9	5.66	**20.817**	**5.628**	**0.270**
14	52.8	20.7	5.6	20.2	5.54	21.6	5.8	20.1	5.61	21.2	5.69	20.7	5.65	**20.750**	**5.648**	**0.272**
15	58.1	20.4	5.63	20	5.56	21.5	5.8	20.1	5.62	21	5.7	20.8	5.74	**20.633**	**5.675**	**0.275**
16	64	20.4	5.66	20	5.6	21.4	5.86	20	5.62	20.9	5.74	20.6	5.75	**20.550**	**5.705**	**0.278**
17	70.5	20.1	5.7	19.9	5.64	21.3	5.92	19.9	5.68	20.7	5.74	20.3	5.79	**20.367**	**5.745**	**0.282**
18	77.6	19.8	5.79	19.7	5.72	21.1	5.96	19.7	5.75	20.5	5.82	20.2	5.84	**20.167**	**5.813**	**0.288**
19	85.5	19.3	5.81	19.5	5.76	20.8	6.02	19.5	5.86	20.3	5.87	19.9	5.9	**19.883**	**5.870**	**0.295**
20	94.1	19.2	5.88	19.1	5.88	20.5	6.1	19.2	5.94	19.9	5.94	19.6	5.96	**19.583**	**5.950**	**0.304**
21	104	18.7	5.94	18.5	5.95	20.1	6.2	18.7	6	19.3	5.96	18.9	6.03	**19.033**	**6.013**	**0.316**
22	114	17.9	6.11	17.6	6.05	19.3	6.29	18	6.06	18.4	6.03	18	6.1	**18.200**	**6.107**	**0.336**
23	126	17	6.19	16.7	6.25	18.5	6.41	17	6.16	17.3	6.13	16.8	6.21	**17.217**	**6.225**	**0.362**
24	140	15.8	6.33	15.4	6.5	17.1	6.53	15.8	6.3	16	6.19	15.5	6.3	**15.933**	**6.358**	**0.399**
25	150	13.9	6.49	14	6.7	15.3	6.69	14	6.52	14.6	6.33	14	6.44	**14.300**	**6.528**	**0.457**

Table A-5: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of reference sample B at day 35 after its production

Meas. Pts.	Stress Amp. (μNm)	B 1-1		B 1-2		B 2-1		B 2-2		B 3-1		B 3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	22.6	5.68	22.2	5.56	23.4	5.82	22.5	5.61	23.2	5.8	23.4	5.82	**22.883**	**5.715**	**0.250**
2	16.7	22.6	5.69	22.2	5.56	23.4	5.84	22.5	5.64	23.3	5.81	23.4	5.84	**22.900**	**5.730**	**0.250**
3	18.4	22.7	5.73	22.2	5.56	23.5	5.86	22.6	5.64	23.3	5.81	23.5	5.86	**22.967**	**5.743**	**0.250**
4	20.2	22.6	5.71	22.2	5.55	23.5	5.85	22.6	5.67	23.4	5.82	23.5	5.86	**22.967**	**5.743**	**0.250**
5	22.2	22.6	5.73	22.2	5.58	23.5	5.86	22.6	5.68	23.4	5.85	23.5	5.87	**22.967**	**5.762**	**0.251**
6	24.5	22.6	5.74	22.2	5.59	23.6	5.9	22.6	5.68	23.4	5.85	23.5	5.88	**22.983**	**5.773**	**0.251**
7	26.9	22.7	5.79	22.2	5.6	23.6	5.93	22.6	5.69	23.4	5.89	23.5	5.88	**23.000**	**5.797**	**0.252**
8	29.7	22.6	5.8	22.2	5.61	23.6	5.91	22.6	5.69	23.3	5.88	23.5	5.89	**22.967**	**5.797**	**0.252**
9	32.7	22.5	5.82	22.2	5.63	23.6	5.93	22.5	5.7	23.3	5.88	23.5	5.89	**22.933**	**5.808**	**0.253**
10	35.9	22.5	5.86	22.2	5.63	23.6	5.95	22.5	5.73	23.3	5.91	23.5	5.93	**22.933**	**5.835**	**0.254**
11	39.6	22.4	5.87	22.1	5.65	23.6	5.99	22.5	5.75	23.3	5.92	23.4	5.94	**22.883**	**5.853**	**0.256**
12	43.6	22.3	5.88	22	5.67	23.5	6.01	22.4	5.75	23.2	5.94	23.4	5.96	**22.800**	**5.868**	**0.257**
13	48	22.2	5.87	21.9	5.69	23.5	6.03	22.3	5.76	23.1	5.96	23.3	5.97	**22.717**	**5.880**	**0.259**
14	52.8	22.1	5.91	21.8	5.74	23.4	6.05	22.2	5.8	23	5.97	23.2	6	**22.617**	**5.912**	**0.261**
15	58.1	22	5.98	21.7	5.74	23.2	6.06	22	5.81	22.8	6.05	23	6.03	**22.450**	**5.945**	**0.265**
16	64	21.8	5.96	21.5	5.77	23.1	6.1	21.9	5.83	22.7	6.04	22.9	6.05	**22.317**	**5.958**	**0.267**
17	70.5	21.6	5.99	21.3	5.83	22.9	6.13	21.7	5.87	22.5	6.08	22.7	6.08	**22.117**	**5.997**	**0.271**
18	77.6	21.4	6.03	21.1	5.87	22.7	6.17	21.4	5.91	22.3	6.12	22.4	6.11	**21.883**	**6.035**	**0.276**
19	85.5	21.2	6.06	20.8	5.94	22.5	6.22	21.1	5.96	22	6.17	22.1	6.14	**21.617**	**6.082**	**0.281**
20	94.1	20.9	6.11	20.4	6.01	22.2	6.26	20.6	6.05	21.7	6.24	21.7	6.2	**21.250**	**6.145**	**0.289**
21	104	20.4	6.14	19.8	6.1	21.7	6.38	20.1	6.16	21.2	6.32	21.2	6.28	**20.733**	**6.230**	**0.300**
22	114	19.8	6.24	19.1	6.22	21.2	6.41	19.2	6.34	20.5	6.43	20.6	6.38	**20.067**	**6.337**	**0.316**
23	126	19.1	6.35	17.9	6.37	20.4	6.54	18.1	6.49	19.5	6.58	19.6	6.48	**19.100**	**6.468**	**0.339**
24	140	18.2	6.44	16.4	6.52	19.2	6.71	16.6	6.6	18.1	6.74	18.5	6.56	**17.833**	**6.595**	**0.370**
25	150	16.9	6.58	14.7	6.63	17.6	6.86	14.8	6.63	16.4	6.85	17.1	6.67	**16.250**	**6.703**	**0.413**

Two-sided confidence intervals (α= 0.05) were calculated using the following equation:

$$X \pm \frac{z_{1-\alpha/2}\,\sigma}{\sqrt{N}} \qquad \text{[Eq. A-1]}$$

where: X is the sample mean, $z_{1-\alpha/2}$ is the 1-α/2 critical value of the standard normal distribution, σ is the known population standard deviation, and N is the sample size.

Table A-6: Two-sided confidence interval limits (α = 0.05) for the mean storage modulus (G') of all reference samples tested

Meas. Pts.	Stress Amp. (μNm)	Mean G' values of samples (Pa) †				**Total Mean G' Value (Pa) ± SD**	**CI (+) Limit ¥**	**CI (-) Limit ¥**
		A - Day 18	A - Day 35	B – Day 18	B – Day 35			
1	14.6	15.750	17.867	20.667	22.883	**19.292 ± 3.129**	**22.358**	**16.226**
2	16.7	15.767	17.833	20.717	22.900	**19.304 ± 3.141**	**22.383**	**16.226**
3	18.4	15.733	17.817	20.800	22.967	**19.329 ± 3.194**	**22.460**	**16.199**
4	20.2	15.700	17.750	20.867	22.967	**19.321 ± 3.228**	**22.484**	**16.157**
5	22.2	15.667	17.717	20.900	22.967	**19.313 ± 3.251**	**22.499**	**16.126**
6	24.5	15.600	17.667	20.833	22.983	**19.271 ± 3.280**	**22.485**	**16.057**
7	26.9	15.500	17.600	20.883	23.000	**19.246 ± 3.342**	**22.521**	**15.970**
8	29.7	15.450	17.467	20.933	22.967	**19.204 ± 3.379**	**22.516**	**15.892**
9	32.7	15.367	17.383	20.933	22.933	**19.154 ± 3.412**	**22.498**	**15.810**
10	35.9	15.267	17.250	20.917	22.933	**19.092 ± 3.469**	**22.492**	**15.692**
11	39.6	15.150	17.117	20.950	22.883	**19.025 ± 3.524**	**22.478**	**15.572**
12	43.6	14.933	16.950	20.900	22.800	**18.896 ± 3.594**	**22.418**	**15.374**
13	48	14.717	16.717	20.817	22.717	**18.742 ± 3.670**	**22.338**	**15.145**
14	52.8	14.483	16.483	20.750	22.617	**18.583 ± 3.750**	**22.258**	**14.909**
15	58.1	14.183	16.167	20.633	22.450	**18.358 ± 3.836**	**22.118**	**14.599**
16	64	13.850	15.817	20.550	22.317	**18.133 ± 3.960**	**22.015**	**14.252**
17	70.5	13.483	15.383	20.367	22.117	**17.838 ± 4.070**	**21.826**	**13.849**
18	77.6	13.000	14.917	20.167	21.883	**17.492 ± 4.213**	**21.620**	**13.363**
19	85.5	12.467	14.267	19.883	21.617	**17.058 ± 4.383**	**21.354**	**12.763**
20	94.1	11.733	13.500	19.583	21.250	**16.517 ± 4.611**	**21.036**	**11.998**
21	104	10.838	12.550	19.033	20.733	**15.789 ± 4.830**	**20.522**	**11.056**
22	114	7.372	11.400	18.200	20.067	**14.260 ± 5.912**	**20.054**	**8.466**
23	126	6.438	9.815	17.217	19.100	**13.142 ± 6.003**	**19.026**	**7.259**
24	140	3.082	5.732	15.933	17.833	**10.645 ± 7.325**	**17.824**	**3.466**
25	150	0.174	0.184	14.300	16.250	**7.727 ± 8.752**	**16.304**	**-0.850**

¥ CI (+) Limit: Confidence interval positive limit. CI (-) Limit: confidence interval negative limit. † A-Day 18: Reference sample A stored for 18 days after production; A-Day 35: Reference sample A stored for 18 days after production; B-Day 18: Reference sample B stored for 18 days after production; B-Day 35: Reference sample B stored for 35 days after production.

Table A-7: Two-sided confidence interval limits ($\alpha = 0.05$) for the mean loss tangent (G''/G') of all reference samples tested

Meas. Pts.	Stress Amp. (μNm)	Mean G''/G' values of samples				**Total Mean G''/G' Value ± SD**	**CI (+) Limit** [¥]	**CI (-) Limit** [¥]
		A - Day 18	A - Day 35	B – Day 18	B – Day 35			
1	14.6	0.256	0.250	0.257	0.250	**0.253 ± 0.004**	**0.257**	**0.249**
2	16.7	0.257	0.250	0.259	0.250	**0.254 ± 0.004**	**0.259**	**0.250**
3	18.4	0.259	0.252	0.259	0.250	**0.255 ± 0.005**	**0.259**	**0.251**
4	20.2	0.261	0.254	0.260	0.250	**0.256 ± 0.005**	**0.261**	**0.251**
5	22.2	0.262	0.255	0.261	0.251	**0.257 ± 0.005**	**0.262**	**0.252**
6	24.5	0.264	0.257	0.262	0.251	**0.259 ± 0.006**	**0.264**	**0.253**
7	26.9	0.267	0.259	0.262	0.252	**0.260 ± 0.006**	**0.266**	**0.254**
8	29.7	0.269	0.263	0.263	0.252	**0.262 ± 0.007**	**0.269**	**0.255**
9	32.7	0.272	0.265	0.264	0.253	**0.264 ± 0.008**	**0.271**	**0.256**
10	35.9	0.275	0.268	0.265	0.254	**0.266 ± 0.009**	**0.274**	**0.257**
11	39.6	0.279	0.272	0.266	0.256	**0.268 ± 0.010**	**0.278**	**0.259**
12	43.6	0.285	0.276	0.268	0.257	**0.272 ± 0.012**	**0.283**	**0.260**
13	48	0.290	0.282	0.270	0.259	**0.275 ± 0.014**	**0.289**	**0.262**
14	52.8	0.296	0.288	0.272	0.261	**0.279 ± 0.016**	**0.295**	**0.264**
15	58.1	0.305	0.296	0.275	0.265	**0.285 ± 0.018**	**0.303**	**0.267**
16	64	0.313	0.305	0.278	0.267	**0.291 ± 0.022**	**0.312**	**0.269**
17	70.5	0.324	0.316	0.282	0.271	**0.298 ± 0.026**	**0.324**	**0.273**
18	77.6	0.339	0.328	0.288	0.276	**0.308 ± 0.031**	**0.338**	**0.278**
19	85.5	0.359	0.349	0.295	0.281	**0.321 ± 0.038**	**0.359**	**0.283**
20	94.1	0.388	0.373	0.304	0.289	**0.338 ± 0.049**	**0.386**	**0.290**
21	104	0.436	0.408	0.316	0.300	**0.365 ± 0.067**	**0.431**	**0.300**
22	114	0.489	0.462	0.336	0.316	**0.401 ± 0.088**	**0.486**	**0.315**
23	126	0.558	0.576	0.362	0.339	**0.458 ± 0.126**	**0.582**	**0.335**
24	140	0.753	0.796	0.399	0.370	**0.579 ± 0.226**	**0.801**	**0.358**
25	150	4.037	5.822	0.457	0.413	**2.682 ± 2.696**	**5.324**	**0.040**

[¥] CI (+) Limit: Confidence interval positive limit. CI (-) Limit: confidence interval negative limit. [†] A-Day 18: Reference sample A stored for 18 days after production; A-Day 35: Reference sample A stored for 18 days after production; B-Day 18: Reference sample B stored for 18 days after production; B-Day 35: Reference sample B stored for 35 days after production.

Appendix B – Development of the novel Greek-style yogurt powder formulation free of additives and fat

Table B-1: Density measurements carried out on recombined milks [¥]

Sample No.	Mass of 100ml (g)		Mean mass of 100ml (g)	Density (g/ml)	Mean density (g/ml)
	I	II			
1-1	104.07880	104.08041	104.07961	1.04079	1.04080
1-2	104.08011	104.08062	104.08037	1.04080	
1-3	104.08006	104.07992	104.07999	1.04080	

[¥] A set of samples containing 13.7% of total solids; 10.3% of total protein (100% MPC-85) was used for density calculations. No significant difference was assumed between the densities of samples containing different total protein composition.

Table B-2: Mass of ingredients used to produce experimental samples [¥]

Production method 1

Sample No.	Ingredients					
	MPC-85 (g)	WPI-90 (g)	NaCN (g)	MPP (g)	H_2O (g)	SC (mg)
1-1	115.44	-	-	14.66	769.89	13.18
1-2	115.44	-	-	14.66	769.90	13.08
1-3	115.44	-	-	14.66	769.90	13.16
2-1	98.13	15.91	-	15.96	769.99	13.15
2-2	98.13	15.91	-	15.96	770.00	13.06
2-3	98.13	15.91	-	15.96	769.99	13.18
3-1	86.58	-	26.33	17.25	769.83	13.09
3-2	86.58	-	26.33	17.25	769.83	13.02
3-3	86.58	-	26.33	17.25	769.82	13.15
4-1	69.26	15.91	26.33	18.55	769.94	13.13
4-2	69.26	15.91	26.33	18.55	769.93	13.10
4-3	69.26	15.91	26.33	18.55	769.93	13.00
5-1	115.44	-	-	14.66	769.89	65.08
5-2	115.44	-	-	14.66	769.90	65.09
5-3	115.44	-	-	14.66	769.90	65.19
6-1	98.13	15.91	-	15.96	770.01	65.07
6-2	98.13	15.91	-	15.96	770.01	65.08
6-3	98.13	15.91	-	15.96	770.00	65.04
7-1	86.58	-	26.33	17.25	769.82	65.03
7-2	86.58	-	26.33	17.25	769.83	65.01
7-3	86.58	-	26.33	17.25	769.83	65.04
8-1	69.26	15.91	26.33	18.55	769.93	65.07
8-2	69.26	15.91	26.33	18.55	769.95	65.12
8-3	69.26	15.91	26.33	18.55	769.93	65.09

Production method 2

Sample No.	Ingredients					
	MPC-85 (g)	**WPI-90 (g)**	**NaCN (g)**	**MPP (g)**	**H_2O (g)**	**SC (mg)**
1-1	115.44	-	-	14.66	769.92	13.04
1-2	115.44	-	-	14.66	769.88	13.11
1-3	115.44	-	-	14.66	769.90	13.07
2-1	98.13	15.91	-	15.96	770.01	13.12
2-2	98.13	15.91	-	15.96	769.99	13.16
2-3	98.13	15.91	-	15.96	769.99	13.11
3-1	86.58	-	26.33	17.25	769.83	12.98
3-2	86.58	-	26.33	17.25	769.83	13.07
3-3	86.58	-	26.33	17.25	769.83	13.10
4-1	69.26	15.91	26.33	18.55	769.92	13.04
4-2	69.26	15.91	26.33	18.55	769.93	13.13
4-3	69.26	15.91	26.33	18.55	769.92	13.03
5-1	115.44	-	-	14.66	769.91	65.02
5-2	115.44	-	-	14.66	769.89	65.14
5-3	115.44	-	-	14.66	769.89	65.05
6-1	98.13	15.91	-	15.96	770.00	65.09
6-2	98.13	15.91	-	15.96	769.99	65.10
6-3	98.13	15.91	-	15.96	769.99	65.07
7-1	86.58	-	26.33	17.25	769.83	65.03
7-2	86.58	-	26.33	17.25	769.83	65.00
7-3	86.58	-	26.33	17.25	769.85	65.07
8-1	69.26	15.91	26.33	18.55	769.92	64.98
8-2	69.26	15.91	26.33	18.55	769.92	65.04
8-3	69.26	15.91	26.33	18.55	769.93	65.13

[¥]Presented amounts were used to prepare 900g of recombined and inoculated milks.

Table B -3: Physicochemical analyses of experimental samples [¥]

Production method 1

Sample No.	Incubation time (hours)	Surface whey-off before homogenization (%m/m)	Surface whey-off after homogenization (%m/m)	Whey drainage (Ordinal scale: 0;1;2)	Size of visible clusters (Ordinal scale: 0;1;2;3)
1-1	14.9	1.12	0.00	0	3
1-2	14.5	0.87	0.00	0	3
1-3	14.4	1.04	0.00	1	2
Mean ± SD	**14.600 ± 0.265**	**1.010 ± 0.128**	**0.000 ± 0.000**	**0.333 ± 0.577**	**2.667 ± 0.577**
2-1	13.7	0.69	0.00	2	2
2-2	13.5	0.53	0.00	2	2
2-3	13.9	0.75	0.00	2	2
Mean ± SD	**13.700 ± 0.200**	**0.657 ± 0.114**	**0.000 ± 0.000**	**2.000 ± 0.000**	**2.000 ± 0.000**
3-1	13.3	0.00	0.00	1	1
3-2	13.2	0.00	0.00	0	1
3-3	13.2	0.00	0.00	1	1
Mean ± SD	**13.233 ± 0.058**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.667 ± 0.577**	**1.000 ± 0.000**
4-1	12.2	0.00	0.00	2	1
4-2	12.1	0.00	0.00	2	1
4-3	12.1	0.00	0.00	2	1
Mean ± SD	**12.133 ± 0.058**	**0.000 ± 0.000**	**0.000 ± 0.000**	**2.000 ± 0.000**	**1.000 ± 0.000**
5-1	12.4	0.78	0.00	0	2
5-2	11.3	0.92	0.00	0	3
5-3	11.2	0.97	0.00	0	2
Mean ± SD	**11.633 ± 0.666**	**0.890 ± 0.098**	**0.000 ± 0.000**	**0.000 ± 0.000**	**2.333 ± 0.577**
6-1	11.3	0.49	0.00	2	2
6-2	11.2	0.62	0.00	2	2
6-3	11.3	0.33	0.00	2	2
Mean ± SD	**11.267 ± 0.058**	**0.480 ± 0.145**	**0.000 ± 0.000**	**2.000 ± 0.000**	**2.000 ± 0.000**
7-1	11.3	0.00	0.00	0	1
7-2	10.9	0.00	0.00	0	1
7-3	11.0	0.00	0.00	0	1
Mean ± SD	**11.067 ± 0.208**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**1.000 ± 0.000**
8-1	10.2	0.00	0.00	1	1
8-2	10.0	0.00	0.00	1	1
8-3	9.9	0.00	0.00	2	1
Mean ± SD	**10.033 ± 0.153**	**0.000 ± 0.000**	**0.000 ± 0.000**	**1.333 ± 0.577**	**1.000 ± 0.000**

Production method 2

Sample No.	Incubation time (hours)	Surface whey-off before homogenization (%m/m)	Surface whey-off after homogenization (%m/m)	Whey drainage (Ordinal scale: 0;1;2)	Size of visible clusters (Ordinal scale: 0;1;2;3)
1-1	14.1	0.74	0.00	0	2
1-2	13.9	0.89	0.00	0	3
1-3	13.9	0.67	0.00	0	2
Mean ± SD	**13.967 ± 0.115**	**0.767 ± 0.112**	**0.000 ± 0.000**	**0.000 ± 0.000**	**2.333 ± 0.577**
2-1	12.9	0.45	0.00	0	3
2-2	13.1	0.38	0.00	0	3
2-3	13.1	0.3	0.00	0	3
Mean ± SD	**13.033 ± 0.115**	**0.377 ± 0.075**	**0.000 ± 0.000**	**0.000 ± 0.000**	**3.000 ± 0.000**
3-1	12.6	0.00	0.00	0	1
3-2	12.5	0.00	0.00	0	1
3-3	12.7	0.00	0.00	0	1
Mean ± SD	**12.600 ± 0.100**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**1.000 ± 0.000**
4-1	11.4	0.00	0.00	0	3
4-2	11.4	0.00	0.00	0	3
4-3	11.3	0.00	0.00	0	3
Mean ± SD	**11.367 ± 0.058**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**3.000 ± 0.000**
5-1	11.0	0.56	0.00	0	2
5-2	11.1	0.62	0.00	0	3
5-3	10.8	0.71	0.00	0	3
Mean ± SD	**10.967 ± 0.153**	**0.630 ± 0.075**	**0.000 ± 0.000**	**0.000 ± 0.000**	**2.667 ± 0.577**
6-1	10.6	0.00	0.00	0	3
6-2	10.6	0.18	0.00	0	3
6-3	10.7	0.23	0.00	0	3
Mean ± SD	**10.633 ± 0.058**	**0.137 ± 0.121**	**0.000 ± 0.000**	**0.000 ± 0.000**	**3.000 ± 0.000**
7-1	10.7	0.00	0.00	0	1
7-2	10.4	0.00	0.00	0	1
7-3	10.4	0.00	0.00	0	1
Mean ± SD	**10.500 ± 0.173**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**1.000 ± 0.000**
8-1	9.9	0.00	0.00	0	3
8-2	10.0	0.00	0.00	0	3
8-3	10.0	0.00	0.00	0	2
Mean ± SD	**9.967 ± 0.058**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**2.667 ± 0.577**

[¥] Physicochemical analyses were conducted at day 1 after samples production.

Table B-4: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of experimental sample 1 manufactured by production method 1 [¥]

Meas. Pts.	Stress Amp. (μNm)	1-1-1		1-1-2		1-2-1		1-2-2		1-3-1		1-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	13.9	3.7	13.5	3.62	13.9	3.81	14.5	3.97	13.3	3.61	13.4	3.67	**13.750**	**3.730**	**0.271**
2	16.7	13.9	3.7	13.5	3.61	13.9	3.8	14.5	3.97	13.4	3.64	13.4	3.67	**13.767**	**3.732**	**0.271**
3	18.4	14.2	3.79	13.6	3.65	14	3.84	14.5	3.97	13.4	3.65	13.4	3.68	**13.850**	**3.763**	**0.272**
4	20.2	14	3.74	13.6	3.66	14	3.84	14.6	3.99	13.5	3.67	13.5	3.7	**13.867**	**3.767**	**0.272**
5	22.2	14	3.74	13.7	3.69	14	3.85	14.5	3.97	13.5	3.68	13.5	3.71	**13.867**	**3.773**	**0.272**
6	24.5	14	3.75	13.8	3.72	14	3.84	14.6	4	13.6	3.7	13.6	3.74	**13.933**	**3.792**	**0.272**
7	26.9	14.2	3.81	13.9	3.75	14	3.85	14.6	4	13.6	3.71	13.5	3.71	**13.967**	**3.805**	**0.272**
8	29.7	14.3	3.84	13.9	3.76	14	3.86	14.6	4.01	13.6	3.72	13.6	3.74	**14.000**	**3.822**	**0.273**
9	32.7	14.3	3.85	14	3.79	14	3.86	14.6	4.02	13.7	3.75	13.6	3.74	**14.033**	**3.835**	**0.273**
10	35.9	14.3	3.86	14.1	3.82	13.9	3.84	14.5	4.01	13.7	3.76	13.6	3.74	**14.017**	**3.838**	**0.274**
11	39.6	14.2	3.85	14.2	3.85	13.9	3.84	14.5	4.01	13.7	3.78	13.6	3.75	**14.017**	**3.847**	**0.274**
12	43.6	14.2	3.85	14.2	3.85	13.9	3.84	14.5	4.01	13.7	3.78	13.5	3.73	**14.000**	**3.843**	**0.275**
13	48	14.2	3.85	14.3	3.88	13.8	3.83	14.4	4	13.7	3.8	13.5	3.75	**13.983**	**3.852**	**0.275**
14	52.8	14.1	3.82	14.3	3.89	13.8	3.83	14.4	4	13.7	3.8	13.5	3.76	**13.967**	**3.850**	**0.276**
15	58.1	14.1	3.82	14.4	3.92	13.7	3.82	14.4	4.01	13.7	3.82	13.4	3.75	**13.950**	**3.857**	**0.276**
16	64	14.1	3.82	14.5	3.95	13.7	3.83	14.3	4	13.6	3.8	13.3	3.73	**13.917**	**3.855**	**0.277**
17	70.5	14	3.8	14.6	3.98	13.6	3.82	14.3	4	13.6	3.81	13.3	3.75	**13.900**	**3.860**	**0.278**
18	77.6	13.9	3.79	14.6	3.98	13.6	3.82	14.2	3.99	13.5	3.8	13.2	3.73	**13.833**	**3.852**	**0.278**
19	85.5	13.8	3.77	14.7	4.02	13.5	3.8	14.2	3.99	13.4	3.8	13.1	3.72	**13.783**	**3.850**	**0.279**
20	94.1	13.7	3.75	14.7	4.03	13.4	3.79	14.1	3.98	13.3	3.79	13	3.71	**13.700**	**3.842**	**0.280**
21	104	13.6	3.71	14.7	4.03	13.3	3.78	14	3.98	13.2	3.79	12.9	3.71	**13.617**	**3.833**	**0.282**
22	114	13.4	3.67	14.8	4.06	13.2	3.76	13.9	3.96	13.1	3.77	12.8	3.7	**13.533**	**3.820**	**0.282**
23	126	13.3	3.66	14.8	4.06	13	3.7	13.7	3.9	12.9	3.75	12.7	3.69	**13.400**	**3.793**	**0.283**
24	140	13.2	3.63	14.8	4.06	12.9	3.72	13.6	3.92	12.7	3.7	12.5	3.66	**13.283**	**3.782**	**0.285**
25	150	13	3.59	14.7	4.07	12.7	3.7	13.4	3.88	12.5	3.69	12.3	3.63	**13.100**	**3.760**	**0.287**

[¥] Rheological analyses were conducted at day 1 after samples production.

Table B-5: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of experimental sample 2 manufactured by production method 1 [¥]

Meas. Pts.	Stress Amp. (μNm)	2-1-1		2-1-2		2-2-1		2-2-2		2-3-1		2-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	10.9	2.94	11.5	3.08	11.9	3.2	11.3	3.05	11.8	3.14	10.2	2.74	**11.267**	**3.025**	**0.268**
2	16.7	10.9	2.95	11.5	3.09	11.9	3.2	11.3	3.05	11.8	3.16	10.2	2.75	**11.267**	**3.033**	**0.269**
3	18.4	10.9	2.95	11.6	3.11	11.9	3.21	11.3	3.06	11.8	3.16	10.2	2.74	**11.283**	**3.038**	**0.269**
4	20.2	11	2.98	11.6	3.11	11.9	3.22	11.3	3.07	11.8	3.16	10.2	2.75	**11.300**	**3.048**	**0.270**
5	22.2	11	2.98	11.6	3.12	11.9	3.22	11.3	3.07	11.8	3.16	10.2	2.76	**11.300**	**3.052**	**0.270**
6	24.5	11	2.99	11.6	3.12	12	3.25	11.3	3.08	11.8	3.17	10.2	2.77	**11.317**	**3.063**	**0.271**
7	26.9	11	3	11.6	3.12	12	3.26	11.3	3.08	11.8	3.17	10.2	2.77	**11.317**	**3.067**	**0.271**
8	29.7	11	3.01	11.6	3.13	12	3.27	11.3	3.09	11.8	3.18	10.2	2.78	**11.317**	**3.077**	**0.272**
9	32.7	11.1	3.03	11.5	3.11	12	3.28	11.3	3.1	11.8	3.19	10.2	2.79	**11.317**	**3.083**	**0.272**
10	35.9	11.1	3.04	11.5	3.11	12	3.29	11.3	3.1	11.7	3.17	10.2	2.8	**11.300**	**3.085**	**0.273**
11	39.6	11.1	3.05	11.5	3.12	12	3.3	11.3	3.11	11.7	3.19	10.2	2.82	**11.300**	**3.098**	**0.274**
12	43.6	11	3.02	11.5	3.12	12	3.32	11.2	3.1	11.7	3.2	10.1	2.8	**11.250**	**3.093**	**0.275**
13	48	11.1	3.06	11.4	3.1	11.9	3.3	11.2	3.11	11.7	3.2	10.1	2.81	**11.233**	**3.097**	**0.276**
14	52.8	11	3.05	11.4	3.11	11.9	3.32	11.1	3.1	11.6	3.19	10	2.8	**11.167**	**3.095**	**0.277**
15	58.1	11	3.06	11.3	3.09	11.9	3.34	11	3.09	11.6	3.2	9.98	2.8	**11.130**	**3.097**	**0.278**
16	64	11	3.06	11.3	3.1	11.8	3.33	11	3.11	11.5	3.2	9.92	2.8	**11.087**	**3.100**	**0.280**
17	70.5	10.9	3.05	11.2	3.09	11.7	3.32	10.9	3.09	11.4	3.19	9.85	2.79	**10.992**	**3.088**	**0.281**
18	77.6	10.8	3.03	11.1	3.08	11.7	3.33	10.8	3.09	11.3	3.18	9.74	2.79	**10.907**	**3.083**	**0.283**
19	85.5	10.8	3.05	11	3.07	11.6	3.32	10.7	3.08	11.2	3.17	9.63	2.78	**10.822**	**3.078**	**0.284**
20	94.1	10.7	3.03	10.9	3.05	11.5	3.32	10.6	3.07	11.1	3.16	9.5	2.76	**10.717**	**3.065**	**0.286**
21	104	10.5	3	10.8	3.04	11.4	3.31	10.5	3.06	10.9	3.14	9.36	2.75	**10.577**	**3.050**	**0.288**
22	114	10.4	2.98	10.7	3.02	11.2	3.26	10.4	3.04	10.8	3.12	9.21	2.73	**10.452**	**3.025**	**0.289**
23	126	10.2	2.95	10.5	2.99	11.1	3.25	10.2	3.02	10.6	3.09	9.02	2.7	**10.270**	**3.000**	**0.292**
24	140	10	2.92	10.4	2.97	10.9	3.22	10	3	10.4	3.07	8.82	2.67	**10.087**	**2.975**	**0.295**
25	150	9.8	2.89	10.2	2.94	10.7	3.2	9.78	2.97	10.1	3.04	8.57	2.64	**9.858**	**2.947**	**0.299**

[¥] Rheological analyses were conducted at day 1 after samples production.

Table B-6: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of experimental sample 3 manufactured by production method 1 [¥]

Meas. Pts.	Stress Amp. (μNm)	3-1-1		3-1-2		3-2-1		3-2-2		3-3-1		3-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	8.07	2.25	7.31	2.12	8.49	2.39	8.54	2.37	8.66	2.47	8.23	2.34	**8.217**	**2.323**	**0.283**
2	16.7	8.07	2.26	7.31	2.12	8.46	2.39	8.54	2.38	8.65	2.48	8.25	2.35	**8.213**	**2.330**	**0.284**
3	18.4	8.03	2.27	7.33	2.13	8.45	2.39	8.54	2.39	8.66	2.49	8.28	2.36	**8.215**	**2.338**	**0.285**
4	20.2	8.02	2.28	7.3	2.15	8.42	2.39	8.52	2.4	8.67	2.5	8.29	2.38	**8.203**	**2.350**	**0.286**
5	22.2	8	2.31	7.28	2.15	8.4	2.4	8.51	2.4	8.64	2.5	8.3	2.39	**8.188**	**2.358**	**0.288**
6	24.5	7.98	2.31	7.26	2.15	8.37	2.4	8.5	2.41	8.63	2.52	8.3	2.4	**8.173**	**2.365**	**0.289**
7	26.9	7.93	2.31	7.22	2.15	8.33	2.4	8.47	2.41	8.6	2.52	8.29	2.41	**8.140**	**2.367**	**0.291**
8	29.7	7.87	2.31	7.17	2.15	8.27	2.4	8.43	2.42	8.55	2.52	8.26	2.42	**8.092**	**2.370**	**0.293**
9	32.7	7.8	2.3	7.11	2.15	8.23	2.4	8.38	2.42	8.49	2.52	8.21	2.42	**8.037**	**2.368**	**0.295**
10	35.9	7.71	2.29	7.05	2.16	8.17	2.41	8.32	2.43	8.43	2.54	8.17	2.43	**7.975**	**2.377**	**0.298**
11	39.6	7.62	2.29	6.98	2.16	8.09	2.4	8.23	2.42	8.37	2.53	8.12	2.43	**7.902**	**2.372**	**0.300**
12	43.6	7.54	2.3	6.88	2.15	8.02	2.41	8.14	2.41	8.3	2.52	8.06	2.44	**7.823**	**2.372**	**0.303**
13	48	7.44	2.29	6.8	2.15	7.92	2.4	8.05	2.41	8.2	2.52	8	2.44	**7.735**	**2.368**	**0.306**
14	52.8	7.3	2.28	6.7	2.15	7.79	2.39	7.94	2.41	8.09	2.51	7.91	2.44	**7.622**	**2.363**	**0.310**
15	58.1	7.17	2.28	6.57	2.15	7.66	2.39	7.83	2.41	7.97	2.51	7.81	2.44	**7.502**	**2.363**	**0.315**
16	64	6.98	2.26	6.42	2.14	7.5	2.38	7.68	2.4	7.82	2.5	7.69	2.43	**7.348**	**2.352**	**0.320**
17	70.5	6.78	2.25	6.24	2.13	7.31	2.37	7.49	2.39	7.65	2.49	7.5	2.42	**7.162**	**2.342**	**0.327**
18	77.6	6.53	2.23	6	2.12	7.06	2.36	7.27	2.37	7.45	2.49	7.31	2.41	**6.937**	**2.330**	**0.336**
19	85.5	6.2	2.21	5.7	2.1	6.73	2.35	6.99	2.35	7.19	2.48	7.08	2.39	**6.648**	**2.313**	**0.348**
20	94.1	5.78	2.17	5.27	2.07	6.29	2.32	6.61	2.32	6.83	2.45	6.77	2.38	**6.258**	**2.285**	**0.365**
21	104	5.17	2.13	4.62	2	5.69	2.27	6.06	2.28	6.36	2.41	6.37	2.35	**5.712**	**2.240**	**0.392**
22	114	4.23	2	3.66	1.86	4.83	2.18	5.27	2.19	5.72	2.33	5.75	2.29	**4.910**	**2.142**	**0.436**
23	126	1.88	1.44	0.0445	0.254	3.47	1.97	4.14	2.02	4.88	2.2	4.94	2.19	**3.226**	**1.679**	**0.520**
24	140	0.0449	0.213	0.0521	0.141	0.0335	0.3	0.0447	0.251	3.72	1.98	3.81	1.98	**1.284**	**0.811**	**0.631**
25	150	0.0519	0.135	0.0534	0.0912	0.0501	0.158	0.0516	0.144	0.0396	0.2	0.0943	0.539	**0.057**	**0.211**	**3.717**

[¥] Rheological analyses were conducted at day 1 after samples production.

Table B-7: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of experimental sample 4 manufactured by production method 1 [¥]

Meas. Pts.	Stress Amp. (μNm)	4-1-1		4-1-2		4-2-1		4-2-2		4-3-1		4-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	6.29	1.78	6.41	1.83	5.88	1.65	6	1.68	6.18	1.77	6.22	1.78	**6.163**	**1.748**	**0.284**
2	16.7	6.29	1.78	6.5	1.85	5.85	1.65	5.97	1.68	6.15	1.77	6.21	1.78	**6.162**	**1.752**	**0.284**
3	18.4	6.28	1.79	6.5	1.85	5.83	1.65	5.95	1.69	6.14	1.77	6.19	1.79	**6.148**	**1.757**	**0.286**
4	20.2	6.27	1.79	6.5	1.86	5.81	1.65	5.93	1.69	6.12	1.78	6.17	1.79	**6.133**	**1.760**	**0.287**
5	22.2	6.27	1.80	6.43	1.85	5.78	1.65	5.92	1.7	6.1	1.78	6.15	1.79	**6.108**	**1.762**	**0.288**
6	24.5	6.2	1.79	6.43	1.86	5.76	1.66	5.89	1.7	6.06	1.78	6.12	1.79	**6.077**	**1.763**	**0.290**
7	26.9	6.19	1.79	6.42	1.86	5.74	1.66	5.86	1.7	6.01	1.78	6.09	1.8	**6.052**	**1.765**	**0.292**
8	29.7	6.19	1.79	6.4	1.86	5.7	1.67	5.82	1.71	5.93	1.78	6.06	1.8	**6.017**	**1.768**	**0.294**
9	32.7	6.07	1.78	6.4	1.87	5.66	1.67	5.77	1.71	5.89	1.78	6.02	1.81	**5.968**	**1.770**	**0.297**
10	35.9	6.06	1.78	6.33	1.87	5.6	1.67	5.71	1.71	5.83	1.78	5.96	1.8	**5.915**	**1.768**	**0.299**
11	39.6	5.97	1.77	6.21	1.86	5.52	1.67	5.64	1.71	5.76	1.78	5.9	1.8	**5.833**	**1.765**	**0.303**
12	43.6	5.89	1.76	6.21	1.86	5.44	1.66	5.56	1.71	5.68	1.77	5.82	1.8	**5.767**	**1.760**	**0.305**
13	48	5.77	1.73	6.1	1.85	5.35	1.65	5.46	1.7	5.58	1.77	5.74	1.8	**5.667**	**1.750**	**0.309**
14	52.8	5.61	1.7	5.99	1.83	5.24	1.65	5.34	1.7	5.46	1.76	5.63	1.79	**5.545**	**1.738**	**0.313**
15	58.1	5.5	1.68	5.86	1.81	5.1	1.64	5.19	1.69	5.31	1.75	5.51	1.79	**5.412**	**1.727**	**0.319**
16	64	5.27	1.65	5.71	1.8	4.92	1.62	4.98	1.69	5.12	1.75	5.35	1.78	**5.225**	**1.713**	**0.328**
17	70.5	5.19	1.63	5.54	1.76	4.7	1.61	4.7	1.68	4.87	1.73	5.16	1.77	**5.027**	**1.697**	**0.338**
18	77.6	4.98	1.6	5.32	1.72	4.4	1.59	4.26	1.67	4.55	1.7	4.89	1.75	**4.733**	**1.672**	**0.353**
19	85.5	4.74	1.55	5.05	1.68	3.95	1.54	3.52	1.61	4.12	1.66	4.49	1.73	**4.312**	**1.628**	**0.378**
20	94.1	4.44	1.49	4.73	1.61	3.25	1.46	1.78	1.27	3.49	1.58	3.86	1.68	**3.592**	**1.515**	**0.422**
21	104	3.99	1.41	4.32	1.53	0.0922	0.435	0.0482	0.159	0.11	0.505	2.42	1.43	**1.830**	**0.912**	**0.498**
22	114	0.0494	0.124	0.339	0.823	0.0496	0.157	0.0533	0.108	0.051	0.122	0.0492	0.154	**0.099**	**0.248**	**2.516**
23	126	0.0526	0.1	0.342	0.599	0.0527	0.106	0.0535	0.0772	0.0526	0.095	0.0525	0.114	**0.101**	**0.182**	**1.801**
24	140	0.0531	0.0791	0.343	0.467	0.053	0.0745	0.0531	0.0607	0.0528	0.0717	0.0528	0.0819	**0.101**	**0.139**	**1.373**
25	150	0.0527	0.0622	0.342	0.364	0.0528	0.0552	0.0528	0.0485	0.0525	0.0557	0.0527	0.0631	**0.101**	**0.108**	**1.071**

[¥] Rheological analyses were conducted at day 1 after samples production.

Table B-8: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of experimental sample 5 manufactured by production method 1 [¥]

Meas. Pts.	Stress Amp. (μNm)	5-1-1		5-1-2		5-2-1		5-2-2		5-3-1		5-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	14.2	3.85	15.8	4.32	15.3	4.04	14.3	3.79	14.8	4.05	13.7	3.75	**14.683**	**3.967**	**0.270**
2	16.7	14.3	3.89	15.8	4.33	15.3	4.03	14.3	3.78	14.8	4.06	13.7	3.75	**14.700**	**3.973**	**0.270**
3	18.4	14.3	3.89	15.8	4.33	15.4	4.06	14.3	3.79	14.9	4.09	13.8	3.78	**14.750**	**3.990**	**0.271**
4	20.2	14.3	3.88	15.9	4.36	15.4	4.06	14.4	3.8	14.9	4.1	13.8	3.78	**14.783**	**4.000**	**0.271**
5	22.2	14.2	3.86	15.9	4.36	15.4	4.07	14.4	3.82	14.9	4.1	13.9	3.81	**14.783**	**4.003**	**0.271**
6	24.5	14.3	3.89	15.9	4.36	15.4	4.07	14.4	3.82	14.9	4.1	13.9	3.82	**14.800**	**4.010**	**0.271**
7	26.9	14.3	3.9	15.9	4.37	15.5	4.1	14.4	3.83	14.9	4.1	13.9	3.82	**14.817**	**4.020**	**0.271**
8	29.7	14.3	3.89	15.9	4.37	15.5	4.11	14.4	3.83	14.9	4.11	14	3.85	**14.833**	**4.027**	**0.271**
9	32.7	14.3	3.9	15.9	4.36	15.5	4.1	14.4	3.84	14.9	4.11	14	3.85	**14.833**	**4.027**	**0.271**
10	35.9	14.3	3.9	15.9	4.37	15.5	4.11	14.4	3.83	14.9	4.11	14	3.85	**14.833**	**4.028**	**0.272**
11	39.6	14.3	3.91	15.8	4.35	15.6	4.14	14.4	3.84	14.9	4.12	14	3.86	**14.833**	**4.037**	**0.272**
12	43.6	14.3	3.91	15.8	4.36	15.6	4.15	14.3	3.82	14.9	4.12	14.1	3.89	**14.833**	**4.042**	**0.272**
13	48	14.3	3.91	15.8	4.36	15.7	4.18	14.3	3.83	14.9	4.13	14.1	3.9	**14.850**	**4.052**	**0.273**
14	52.8	14.2	3.9	15.7	4.34	15.6	4.16	14.2	3.81	14.9	4.14	14.1	3.91	**14.783**	**4.043**	**0.274**
15	58.1	14.2	3.91	15.7	4.35	15.7	4.20	14.1	3.8	14.8	4.13	14	3.89	**14.750**	**4.047**	**0.274**
16	64	14.2	3.91	15.6	4.33	15.7	4.21	14.1	3.8	14.8	4.14	14	3.91	**14.733**	**4.050**	**0.275**
17	70.5	14.2	3.92	15.6	4.34	15.6	4.19	14	3.78	14.8	4.15	14	3.92	**14.700**	**4.050**	**0.276**
18	77.6	14.1	3.90	15.6	4.35	15.6	4.20	13.9	3.76	14.7	4.14	13.9	3.9	**14.633**	**4.042**	**0.276**
19	85.5	14.1	3.91	15.5	4.33	15.5	4.18	13.8	3.74	14.7	4.15	13.9	3.91	**14.583**	**4.037**	**0.277**
20	94.1	14	3.91	15.5	4.35	15.4	4.16	13.8	3.75	14.6	4.13	13.8	3.90	**14.517**	**4.033**	**0.278**
21	104	13.9	3.9	15.4	4.34	15.3	4.15	13.7	3.74	14.5	4.1	13.7	3.89	**14.417**	**4.022**	**0.279**
22	114	13.8	3.88	15.3	4.33	15.1	4.12	13.6	3.73	14.4	4.1	13.6	3.89	**14.300**	**4.008**	**0.280**
23	126	13.7	3.86	15.2	4.32	14.9	4.08	13.5	3.72	14.3	4.09	13.5	3.89	**14.183**	**3.993**	**0.282**
24	140	13.5	3.83	15.1	4.3	14.8	4.07	13.4	3.71	14.1	4.07	13.3	3.88	**14.033**	**3.977**	**0.283**
25	150	13.4	3.82	14.9	4.27	14.6	4.04	13.2	3.68	14	4.07	13.2	3.87	**13.883**	**3.958**	**0.285**

[¥] Rheological analyses were conducted at day 1 after samples production.

Table B-9: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of experimental sample 6 manufactured by production method 1 [¥]

Meas. Pts.	Stress Amp. (μNm)	6-1-1		6-1-2		6-2-1		6-2-2		6-3-1		6-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	11.7	3.13	12.2	3.25	11.8	3.16	10.9	2.93	11.9	3.25	11.2	3.04	**11.617**	**3.127**	**0.269**
2	16.7	11.7	3.12	12.2	3.25	11.8	3.17	11	2.96	11.9	3.26	11.2	3.05	**11.633**	**3.135**	**0.269**
3	18.4	11.7	3.13	12.2	3.26	11.9	3.19	11	2.96	12	3.29	11.3	3.08	**11.683**	**3.152**	**0.270**
4	20.2	11.7	3.13	12.2	3.26	11.9	3.2	11	2.97	12	3.3	11.2	3.06	**11.667**	**3.153**	**0.270**
5	22.2	11.8	3.16	12.2	3.26	11.9	3.21	11	2.97	12	3.31	11.3	3.1	**11.700**	**3.168**	**0.271**
6	24.5	11.8	3.17	12.3	3.3	12	3.23	11	2.98	12	3.31	11.3	3.11	**11.733**	**3.183**	**0.271**
7	26.9	11.8	3.18	12.3	3.3	12	3.24	11	2.99	12	3.32	11.3	3.11	**11.733**	**3.190**	**0.272**
8	29.7	11.8	3.19	12.3	3.31	12	3.26	11	2.99	12	3.33	11.3	3.13	**11.733**	**3.202**	**0.273**
9	32.7	11.8	3.2	12.3	3.32	12	3.26	11	3	12	3.35	11.3	3.14	**11.733**	**3.212**	**0.274**
10	35.9	11.8	3.21	12.3	3.33	12	3.27	11	3	12	3.35	11.3	3.15	**11.733**	**3.218**	**0.274**
11	39.6	11.8	3.22	12.3	3.34	12	3.28	11	3.01	12	3.36	11.3	3.16	**11.733**	**3.228**	**0.275**
12	43.6	11.8	3.24	12.2	3.33	12	3.28	10.9	2.99	12	3.37	11.2	3.15	**11.683**	**3.227**	**0.276**
13	48	11.8	3.25	12.2	3.34	12	3.28	10.9	3	11.9	3.35	11.2	3.16	**11.667**	**3.230**	**0.277**
14	52.8	11.7	3.24	12.2	3.35	11.9	3.27	10.8	2.98	11.9	3.37	11.2	3.18	**11.617**	**3.232**	**0.278**
15	58.1	11.7	3.25	12.2	3.36	11.9	3.29	10.7	2.97	11.9	3.38	11.2	3.19	**11.600**	**3.240**	**0.279**
16	64	11.6	3.24	12.1	3.35	11.8	3.27	10.7	2.99	11.8	3.39	11.1	3.18	**11.517**	**3.237**	**0.281**
17	70.5	11.6	3.25	12.1	3.35	11.8	3.29	10.6	2.98	11.7	3.39	11	3.18	**11.467**	**3.240**	**0.283**
18	77.6	11.5	3.24	12	3.35	11.7	3.29	10.6	3	11.6	3.4	10.9	3.18	**11.383**	**3.243**	**0.285**
19	85.5	11.4	3.22	11.9	3.34	11.6	3.29	10.5	3	11.5	3.39	10.9	3.20	**11.300**	**3.240**	**0.287**
20	94.1	11.3	3.21	11.8	3.34	11.5	3.29	10.4	2.99	11.4	3.39	10.7	3.16	**11.183**	**3.230**	**0.289**
21	104	11.2	3.2	11.7	3.33	11.4	3.28	10.3	2.98	11.3	3.39	10.6	3.16	**11.083**	**3.223**	**0.291**
22	114	11.1	3.19	11.5	3.31	11.3	3.28	10.1	2.96	11.2	3.39	10.5	3.16	**10.950**	**3.215**	**0.294**
23	126	10.9	3.17	11.4	3.31	11.2	3.28	9.97	2.95	11	3.39	10.3	3.15	**10.795**	**3.208**	**0.297**
24	140	10.7	3.15	11.2	3.29	11	3.27	9.77	2.93	10.8	3.39	10.1	3.14	**10.595**	**3.195**	**0.302**
25	150	10.6	3.16	11	3.27	10.8	3.26	9.55	2.91	10.5	3.4	9.89	3.14	**10.390**	**3.190**	**0.307**

[¥] Rheological analyses were conducted at day 1 after samples production.

Table B-10: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of experimental sample 7 manufactured by production method 1 [¥]

Meas. Pts.	Stress Amp. (μNm)	7-1-1		7-1-2		7-2-1		7-2-2		7-3-1		7-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	8.85	2.47	9.05	2.53	9.61	2.69	10.1	2.8	9.02	2.52	9.37	2.59	**9.333**	**2.600**	**0.279**
2	16.7	8.84	2.47	9.04	2.53	9.59	2.68	10.1	2.84	9.04	2.52	9.33	2.58	**9.323**	**2.603**	**0.279**
3	18.4	8.84	2.48	9.02	2.53	9.59	2.69	10.1	2.83	9.05	2.5	9.29	2.59	**9.315**	**2.607**	**0.280**
4	20.2	8.82	2.47	9.02	2.54	9.6	2.71	10.1	2.83	9.06	2.5	9.23	2.6	**9.305**	**2.610**	**0.280**
5	22.2	8.8	2.48	9	2.54	9.6	2.72	10.1	2.84	9.06	2.53	9.19	2.58	**9.292**	**2.615**	**0.281**
6	24.5	8.78	2.49	8.96	2.55	9.59	2.73	10.1	2.84	9.07	2.54	9.16	2.58	**9.277**	**2.622**	**0.283**
7	26.9	8.76	2.49	8.93	2.55	9.59	2.73	10.1	2.85	9.06	2.54	9.08	2.6	**9.253**	**2.627**	**0.284**
8	29.7	8.73	2.5	8.89	2.56	9.58	2.73	10.1	2.86	9.04	2.55	9.05	2.6	**9.232**	**2.633**	**0.285**
9	32.7	8.68	2.5	8.84	2.55	9.57	2.74	10.1	2.85	9.02	2.54	9	2.6	**9.202**	**2.630**	**0.286**
10	35.9	8.64	2.51	8.79	2.6	9.52	2.74	9.99	2.86	8.97	2.6	8.94	2.61	**9.142**	**2.638**	**0.289**
11	39.6	8.6	2.51	8.72	2.56	9.52	2.74	9.99	2.87	8.93	2.6	8.88	2.61	**9.107**	**2.640**	**0.290**
12	43.6	8.55	2.52	8.63	2.56	9.51	2.75	9.99	2.87	8.88	2.55	8.8	2.6	**9.060**	**2.643**	**0.292**
13	48	8.48	2.52	8.53	2.56	9.4	2.74	9.88	2.86	8.82	2.55	8.7	2.61	**8.968**	**2.640**	**0.294**
14	52.8	8.4	2.52	8.43	2.57	9.39	2.75	9.88	2.87	8.74	2.55	8.57	2.6	**8.902**	**2.643**	**0.297**
15	58.1	8.28	2.52	8.31	2.57	9.3	2.75	9.8	2.87	8.64	2.55	8.44	2.59	**8.795**	**2.642**	**0.300**
16	64	8.15	2.5	8.18	2.57	9.22	2.75	9.69	2.87	8.52	2.55	8.29	2.59	**8.675**	**2.638**	**0.304**
17	70.5	8.01	2.5	8.02	2.57	9.1	2.74	9.61	2.86	8.37	2.55	8.13	2.58	**8.540**	**2.633**	**0.308**
18	77.6	7.74	2.5	7.83	2.56	8.91	2.73	9.4	2.84	8.2	2.54	7.94	2.57	**8.337**	**2.623**	**0.315**
19	85.5	7.57	2.48	7.6	2.56	8.73	2.71	9.31	2.83	8	2.54	7.7	2.56	**8.152**	**2.613**	**0.321**
20	94.1	7.34	2.46	7.32	2.55	8.5	2.68	9.1	2.81	7.75	3	7.42	2.53	**7.905**	**2.593**	**0.328**
21	104	7.05	2.45	6.94	2.54	8.32	2.66	8.92	2.79	7.41	2.52	7.08	2.51	**7.620**	**2.578**	**0.338**
22	114	6.65	2.42	6.42	2.53	8.01	2.63	8.57	2.76	6.95	2.51	6.63	2.46	**7.205**	**2.552**	**0.354**
23	126	5.99	2.37	5.65	2.48	7.61	2.58	8.2	2.71	6.27	2.5	6.06	2.39	**6.630**	**2.498**	**0.377**
24	140	5.02	2.25	4.42	2.35	7.09	2.51	7.69	2.66	5.17	2.33	5.23	2.3	**5.770**	**2.400**	**0.416**
25	150	3.3	1.95	0.0268	0.228	6.38	2.39	6.94	2.57	0.87	0.228	3.92	2.11	**3.573**	**1.579**	**0.442**

[¥] Rheological analyses were conducted at day 1 after samples production.

Table B-11: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of experimental sample 8 manufactured by production method 1 [¥]

Meas. Pts.	Stress Amp. (μNm)	8-1-1		8-1-2		8-2-1		8-2-2		8-3-1		8-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	7.81	2.24	8.13	2.31	7.79	2.21	7.56	2.15	7.27	2.13	7.42	2.17	**7.663**	**2.202**	**0.287**
2	16.7	7.78	2.23	8.11	2.32	7.77	2.21	7.55	2.16	7.24	2.13	7.4	2.17	**7.642**	**2.203**	**0.288**
3	18.4	7.75	2.24	8.1	2.32	7.76	2.22	7.55	2.16	7.22	2.13	7.38	2.17	**7.627**	**2.207**	**0.289**
4	20.2	7.73	2.24	8.1	2.33	7.74	2.22	7.53	2.17	7.2	2.13	7.35	2.18	**7.608**	**2.212**	**0.291**
5	22.2	7.71	2.24	8.09	2.34	7.72	2.22	7.52	2.17	7.19	2.15	7.33	2.19	**7.593**	**2.218**	**0.292**
6	24.5	7.68	2.25	8.09	2.35	7.7	2.23	7.5	2.18	7.17	2.16	7.29	2.19	**7.572**	**2.227**	**0.294**
7	26.9	7.65	2.25	8.06	2.36	7.68	2.23	7.47	2.18	7.13	2.16	7.26	2.19	**7.542**	**2.228**	**0.295**
8	29.7	7.62	2.26	8.04	2.36	7.65	2.24	7.43	2.18	7.1	2.17	7.22	2.19	**7.510**	**2.233**	**0.297**
9	32.7	7.58	2.27	8	2.38	7.61	2.25	7.39	2.19	7.05	2.18	7.17	2.2	**7.467**	**2.245**	**0.301**
10	35.9	7.53	2.27	7.95	2.37	7.57	2.25	7.34	2.19	6.99	2.19	7.11	2.21	**7.415**	**2.247**	**0.303**
11	39.6	7.46	2.27	7.9	2.38	7.52	2.25	7.28	2.19	6.9	2.19	7.05	2.21	**7.352**	**2.248**	**0.306**
12	43.6	7.38	2.28	7.82	2.39	7.46	2.26	7.22	2.19	6.82	2.18	6.97	2.2	**7.278**	**2.253**	**0.310**
13	48	7.28	2.27	7.73	2.39	7.38	2.26	7.14	2.19	6.72	2.19	6.87	2.23	**7.187**	**2.255**	**0.314**
14	52.8	7.17	2.27	7.62	2.39	7.28	2.26	7.04	2.19	6.59	2.2	6.75	2.24	**7.075**	**2.260**	**0.319**
15	58.1	7.03	2.27	7.48	2.39	7.16	2.26	6.92	2.19	6.43	2.2	6.6	2.24	**6.937**	**2.260**	**0.326**
16	64	6.87	2.27	7.32	2.38	7.02	2.25	6.79	2.19	6.22	2.22	6.38	2.26	**6.767**	**2.262**	**0.334**
17	70.5	6.63	2.28	7.12	2.39	6.85	2.25	6.62	2.19	5.93	2.25	6.1	2.28	**6.542**	**2.273**	**0.348**
18	77.6	6.36	2.28	6.87	2.39	6.62	2.25	6.39	2.19	5.49	2.27	5.71	2.31	**6.240**	**2.282**	**0.366**
19	85.5	5.99	2.28	6.55	2.39	6.3	2.24	6.08	2.18	4.84	2.31	5.09	2.36	**5.808**	**2.293**	**0.395**
20	94.1	5.45	2.27	6.09	2.4	5.9	2.22	5.62	2.18	0.303	0.714	3.06	2.24	**4.404**	**2.004**	**0.455**
21	104	4.64	2.22	5.39	2.37	5.36	2.19	4.86	2.13	0.0494	0.11	0.0487	0.105	**3.391**	**1.521**	**0.448**
22	114	3.34	2.03	4.16	2.28	4.5	2.12	3.41	1.97	0.0519	0.0868	0.0514	0.086	**2.586**	**1.429**	**0.553**
23	126	0.0381	0.204	0.0351	0.198	0.0438	0.115	0.046	0.116	0.0525	0.0692	0.0519	0.0711	**0.045**	**0.129**	**2.892**
24	140	0.0491	0.126	0.0478	0.134	0.0484	0.0946	0.0499	0.0872	0.0526	0.0564	0.0523	0.0581	**0.050**	**0.093**	**1.854**
25	150	0.0516	0.0821	0.0511	0.0925	0.0503	0.0749	0.0506	0.0689	0.0523	0.0473	0.0523	0.048	**0.051**	**0.069**	**1.342**

[¥] Rheological analyses were conducted at day 1 after samples production.

Table B-12: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of experimental sample 1 manufactured by production method 2 [¥]

Meas. Pts.	Stress Amp. (μNm)	1-1-1		1-1-2		1-2-1		1-2-2		1-3-1		1-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	15.2	3.92	15.6	3.99	15.3	3.94	15.1	3.86	15.3	3.96	15.7	4.03	**15.367**	**3.950**	**0.257**
2	16.7	15.3	3.95	15.7	4.01	15.3	3.94	15.1	3.86	15.4	3.99	15.8	4.05	**15.433**	**3.967**	**0.257**
3	18.4	15.4	3.98	15.8	4.03	15.4	3.97	15.1	3.86	15.5	4.02	15.8	4.05	**15.500**	**3.985**	**0.257**
4	20.2	15.5	4.0	15.9	4.06	15.4	3.97	15.2	3.89	15.5	4.02	15.9	4.08	**15.567**	**4.005**	**0.257**
5	22.2	15.5	4.02	15.9	4.07	15.5	4	15.3	3.91	15.5	4.03	15.8	4.05	**15.583**	**4.013**	**0.258**
6	24.5	15.6	4.04	15.9	4.08	15.5	4.01	15.4	3.94	15.6	4.06	15.9	4.08	**15.650**	**4.035**	**0.258**
7	26.9	15.7	4.07	16	4.11	15.6	4.04	15.4	3.95	15.6	4.06	16	4.11	**15.717**	**4.057**	**0.258**
8	29.7	15.8	4.1	16	4.12	15.6	4.04	15.5	3.98	15.7	4.09	16.1	4.14	**15.783**	**4.078**	**0.258**
9	32.7	15.8	4.11	16	4.13	15.6	4.05	15.5	3.98	15.7	4.1	16.1	4.15	**15.783**	**4.087**	**0.259**
10	35.9	15.8	4.11	16	4.14	15.6	4.05	15.4	3.96	15.7	4.1	16.2	4.18	**15.783**	**4.090**	**0.259**
11	39.6	15.8	4.11	16	4.14	15.7	4.08	15.5	3.99	15.7	4.1	16.3	4.21	**15.833**	**4.107**	**0.259**
12	43.6	15.9	4.14	16.1	4.17	15.7	4.1	15.5	4	15.8	4.13	16.3	4.22	**15.883**	**4.125**	**0.260**
13	48	15.9	4.15	16	4.15	15.7	4.1	15.5	4.01	15.8	4.14	16.3	4.23	**15.867**	**4.130**	**0.260**
14	52.8	15.9	4.16	16.1	4.19	15.7	4.11	15.5	4.02	15.8	4.14	16.3	4.23	**15.883**	**4.142**	**0.261**
15	58.1	15.9	4.17	16.1	4.2	15.7	4.12	15.5	4.03	15.9	4.17	16.3	4.24	**15.900**	**4.155**	**0.261**
16	64	15.9	4.18	16.1	4.21	15.7	4.13	15.5	4.04	15.9	4.17	16.4	4.27	**15.917**	**4.167**	**0.262**
17	70.5	15.9	4.18	16.1	4.22	15.7	4.14	15.6	4.08	15.9	4.17	16.3	4.25	**15.917**	**4.173**	**0.262**
18	77.6	15.9	4.19	16.1	4.23	15.7	4.16	15.6	4.1	15.8	4.15	16.3	4.26	**15.900**	**4.182**	**0.263**
19	85.5	15.9	4.21	16.1	4.25	15.7	4.17	15.5	4.08	15.7	4.13	16.3	4.27	**15.867**	**4.185**	**0.264**
20	94.1	15.9	4.23	16.1	4.28	15.7	4.18	15.4	4.07	15.7	4.14	16.2	4.25	**15.833**	**4.192**	**0.265**
21	104	15.9	4.25	16	4.3	15.6	4.17	15.4	4.09	15.6	4.13	16.2	4.26	**15.783**	**4.197**	**0.266**
22	114	15.8	4.24	16	4.3	15.6	4.18	15.3	4.07	15.5	4.13	16.1	4.25	**15.717**	**4.195**	**0.267**
23	126	15.8	4.27	15.9	4.29	15.5	4.17	15.3	4.09	15.5	4.17	15.9	4.23	**15.650**	**4.203**	**0.269**
24	140	15.7	4.27	15.8	4.29	15.5	4.20	15.2	4.09	15.4	4.19	15.6	4.2	**15.533**	**4.207**	**0.271**
25	150	15.5	4.25	15.7	4.3	15.3	4.17	15.1	4.09	15.2	4.2	15.5	4.21	**15.383**	**4.202**	**0.273**

[¥] Rheological analyses were conducted at day 1 after samples production.

Table B-13: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of experimental sample 2 manufactured by production method 2 [¥]

Meas. Pts.	Stress Amp. (μNm)	2-1-1		2-1-2		2-2-1		2-2-2		2-3-1		2-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	19.9	4.98	20.5	5.04	20.1	5.07	20.6	5.13	19.7	4.94	19.4	4.95	**20.033**	**5.018**	**0.250**
2	16.7	20	5	20.5	5.05	20.2	5.1	20.5	5.11	19.8	4.97	19.4	4.95	**20.067**	**5.030**	**0.251**
3	18.4	20	5.01	20.6	5.08	20.1	5.08	20.5	5.12	19.8	4.96	19.4	4.96	**20.067**	**5.035**	**0.251**
4	20.2	20.1	5.04	20.6	5.09	20.1	5.1	20.6	5.15	19.8	4.97	19.5	4.99	**20.117**	**5.057**	**0.251**
5	22.2	20.1	5.04	20.6	5.10	20.2	5.14	20.6	5.16	19.9	5	19.5	5.01	**20.150**	**5.075**	**0.252**
6	24.5	20.1	5.05	20.7	5.13	20.2	5.15	20.6	5.17	19.9	5.01	19.5	5.03	**20.167**	**5.090**	**0.252**
7	26.9	20.1	5.05	20.7	5.14	20.2	5.16	20.6	5.19	20	5.05	19.6	5.07	**20.200**	**5.110**	**0.253**
8	29.7	20.2	5.08	20.8	5.17	20.2	5.17	20.7	5.22	19.9	5.04	19.6	5.09	**20.233**	**5.128**	**0.253**
9	32.7	20.1	5.07	20.7	5.16	20.2	5.17	20.7	5.23	19.9	5.06	19.7	5.13	**20.217**	**5.137**	**0.254**
10	35.9	20.2	5.1	20.8	5.19	20.3	5.21	20.8	5.26	20	5.1	19.6	5.11	**20.283**	**5.162**	**0.254**
11	39.6	20.2	5.11	20.8	5.20	20.3	5.21	20.7	5.25	20	5.11	19.7	5.15	**20.283**	**5.172**	**0.255**
12	43.6	20.2	5.12	20.8	5.21	20.3	5.22	20.8	5.28	20	5.12	19.7	5.16	**20.300**	**5.185**	**0.255**
13	48	20.3	5.15	20.9	5.24	20.3	5.23	20.8	5.29	20.1	5.16	19.7	5.17	**20.350**	**5.207**	**0.256**
14	52.8	20.3	5.17	20.8	5.23	20.4	5.26	20.8	5.30	20.1	5.16	19.8	5.2	**20.367**	**5.220**	**0.256**
15	58.1	20.4	5.21	20.9	5.27	20.4	5.27	20.8	5.3	20.2	5.20	19.7	5.18	**20.400**	**5.238**	**0.257**
16	64	20.3	5.20	20.9	5.28	20.4	5.29	20.9	5.33	20.1	5.16	19.8	5.21	**20.400**	**5.245**	**0.257**
17	70.5	20.3	5.22	20.9	5.3	20.4	5.3	20.9	5.35	20.1	5.19	19.8	5.2	**20.400**	**5.263**	**0.258**
18	77.6	20.4	5.26	20.9	5.31	20.5	5.33	20.9	5.36	20	5.17	19.8	5.23	**20.417**	**5.277**	**0.258**
19	85.5	20.3	5.25	20.9	5.32	20.4	5.31	21	5.39	20.1	5.2	19.9	5.26	**20.433**	**5.288**	**0.259**
20	94.1	20.3	5.28	20.9	5.34	20.5	5.35	21	5.4	20	5.21	19.8	5.26	**20.417**	**5.307**	**0.260**
21	104	20.3	5.30	20.8	5.33	20.5	5.36	20.9	5.4	20.1	5.26	19.7	5.26	**20.383**	**5.318**	**0.261**
22	114	20.3	5.33	20.8	5.36	20.4	5.35	20.9	5.42	20	5.3	19.7	5.28	**20.350**	**5.337**	**0.262**
23	126	20.2	5.33	20.8	5.37	20.4	5.37	20.9	5.44	20	5.3	19.6	5.29	**20.317**	**5.350**	**0.263**
24	140	20.2	5.35	20.7	5.35	20.4	5.4	20.8	5.45	19.9	5.32	19.6	5.33	**20.267**	**5.367**	**0.265**
25	150	20.2	5.4	20.7	5.37	20.3	5.4	20.8	5.47	19.8	5.36	19.5	5.38	**20.217**	**5.390**	**0.267**

[¥] Rheological analyses were conducted at day 1 after samples production.

Table B-14: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of experimental sample 3 manufactured by production method 2 [¥]

Meas. Pts.	Stress Amp. (μNm)	3-1-1		3-1-2		3-2-1		3-2-2		3-3-1		3-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	21.8	5.43	21	5.31	22	5.45	21.7	5.43	21.6	5.44	20.4	5.16	**21.417**	**5.370**	**0.251**
2	16.7	22	5.49	21.1	5.34	21.9	5.44	21.8	5.47	21.7	5.46	20.4	5.17	**21.483**	**5.395**	**0.251**
3	18.4	22	5.48	21	5.32	22	5.45	21.8	5.48	21.7	5.45	20.5	5.19	**21.500**	**5.395**	**0.251**
4	20.2	22.1	5.50	21.1	5.35	22.1	5.49	21.8	5.49	21.8	5.47	20.6	5.22	**21.583**	**5.420**	**0.251**
5	22.2	22.2	5.54	21.1	5.35	22.2	5.54	21.9	5.52	21.8	5.48	20.6	5.23	**21.633**	**5.443**	**0.252**
6	24.5	22.1	5.51	21.2	5.39	22.2	5.54	21.9	5.55	21.9	5.54	20.7	5.27	**21.667**	**5.467**	**0.252**
7	26.9	22.2	5.54	21.3	5.42	22.3	5.57	22	5.57	21.9	5.56	20.7	5.28	**21.733**	**5.490**	**0.253**
8	29.7	22.2	5.56	21.2	5.41	22.4	5.61	22.1	5.62	22	5.61	20.7	5.31	**21.767**	**5.520**	**0.254**
9	32.7	22.3	5.59	21.3	5.44	22.4	5.6	22	5.61	22	5.61	20.7	5.32	**21.783**	**5.528**	**0.254**
10	35.9	22.3	5.60	21.3	5.45	22.4	5.6	22.1	5.65	22.1	5.63	20.8	5.36	**21.833**	**5.548**	**0.254**
11	39.6	22.4	5.61	21.4	5.49	22.5	5.65	22.1	5.65	22.2	5.66	20.8	5.36	**21.900**	**5.570**	**0.254**
12	43.6	22.5	5.65	21.5	5.53	22.5	5.67	22.2	5.67	22.1	5.64	20.9	5.4	**21.950**	**5.593**	**0.255**
13	48	22.6	5.69	21.5	5.54	22.6	5.69	22.3	5.7	22.2	5.67	20.8	5.38	**22.000**	**5.612**	**0.255**
14	52.8	22.6	5.71	21.6	5.58	22.7	5.75	22.3	5.71	22.3	5.73	20.9	5.42	**22.067**	**5.650**	**0.256**
15	58.1	22.7	5.74	21.7	5.6	22.8	5.78	22.4	5.74	22.3	5.76	21	5.44	**22.150**	**5.677**	**0.256**
16	64	22.7	5.74	21.7	5.61	22.9	5.81	22.4	5.75	22.4	5.78	20.9	5.44	**22.167**	**5.688**	**0.257**
17	70.5	22.8	5.79	21.7	5.62	22.8	5.81	22.5	5.8	22.3	5.78	21	5.48	**22.183**	**5.713**	**0.258**
18	77.6	22.8	5.81	21.8	5.65	22.9	5.84	22.6	5.84	22.4	5.83	21	5.52	**22.250**	**5.748**	**0.258**
19	85.5	22.8	5.81	21.8	5.67	23	5.87	22.6	5.85	22.4	5.84	21.1	5.56	**22.283**	**5.767**	**0.259**
20	94.1	22.9	5.86	21.9	5.7	23	5.88	22.7	5.88	22.5	5.89	21.1	5.57	**22.350**	**5.797**	**0.259**
21	104	23	5.90	22	5.75	23.1	5.9	22.8	5.93	22.5	5.9	21.2	5.62	**22.433**	**5.833**	**0.260**
22	114	23	5.93	22	5.78	23.2	5.95	22.7	5.92	22.6	5.93	21.2	5.63	**22.450**	**5.857**	**0.261**
23	126	23.1	5.98	22.1	5.82	23.2	5.97	22.8	5.96	22.7	5.98	21.3	5.66	**22.533**	**5.895**	**0.262**
24	140	23.1	6.03	22	5.83	23.3	6	22.9	6	22.6	6	21.4	5.7	**22.550**	**5.927**	**0.263**
25	150	23.2	6.09	22.1	5.89	23.3	6.06	22.9	6.03	22.7	6.05	21.4	5.71	**22.600**	**5.972**	**0.264**

[¥] Rheological analyses were conducted at day 1 after samples production.

Table B-15: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of experimental sample 4 manufactured by production method 2 [¥]

Meas. Pts.	Stress Amp. (μNm)	4-1-1		4-1-2		4-2-1		4-2-2		4-3-1		4-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	18.7	4.66	18.9	4.72	18.8	4.79	17.9	4.52	18.6	4.67	19.3	4.82	**18.700**	**4.697**	**0.251**
2	16.7	18.8	4.67	18.9	4.72	18.9	4.82	18	4.55	18.7	4.70	19.4	4.83	**18.783**	**4.715**	**0.251**
3	18.4	18.8	4.69	19	4.75	19	4.85	18.1	4.58	18.8	4.7	19.4	4.84	**18.850**	**4.738**	**0.251**
4	20.2	18.9	4.72	18.9	4.72	19.1	4.89	18.1	4.59	18.8	4.72	19.4	4.85	**18.867**	**4.748**	**0.252**
5	22.2	18.9	4.72	19	4.75	19.1	4.89	18.1	4.6	18.8	4.7	19.5	4.88	**18.900**	**4.762**	**0.252**
6	24.5	19	4.74	19.1	4.78	19.1	4.9	18.2	4.64	18.8	4.73	19.5	4.88	**18.950**	**4.778**	**0.252**
7	26.9	19	4.77	19.1	4.79	19.2	4.93	18.2	4.65	18.9	4.77	19.5	4.89	**18.983**	**4.800**	**0.253**
8	29.7	19	4.75	19.2	4.81	19.2	4.95	18.3	4.68	18.9	4.77	19.4	4.87	**19.000**	**4.805**	**0.253**
9	32.7	19	4.76	19.1	4.80	19.2	4.95	18.4	4.7	18.9	4.78	19.5	4.90	**19.017**	**4.817**	**0.253**
10	35.9	19.1	4.78	19.1	4.81	19.3	4.98	18.4	4.72	18.9	4.78	19.5	4.91	**19.050**	**4.830**	**0.254**
11	39.6	19.1	4.81	19.1	4.82	19.4	5	18.4	4.72	18.9	4.8	19.5	4.92	**19.067**	**4.845**	**0.254**
12	43.6	19.1	4.82	19.2	4.86	19.4	5.02	18.4	4.73	18.8	4.79	19.5	4.92	**19.067**	**4.857**	**0.255**
13	48	19.1	4.84	19.2	4.88	19.4	5.02	18.4	4.74	18.9	4.82	19.6	4.96	**19.100**	**4.877**	**0.255**
14	52.8	19.1	4.85	19.2	4.9	19.4	5.03	18.5	4.78	18.9	4.83	19.6	4.97	**19.117**	**4.890**	**0.256**
15	58.1	19.1	4.85	19.2	4.89	19.3	5.03	18.5	4.8	19	4.86	19.6	4.99	**19.117**	**4.903**	**0.256**
16	64	19.1	4.87	19.2	4.92	19.4	5.05	18.5	4.81	19	4.87	19.6	5	**19.133**	**4.920**	**0.257**
17	70.5	19.1	4.88	19.1	4.9	19.4	5.05	18.5	4.82	19	4.88	19.6	5.01	**19.117**	**4.923**	**0.258**
18	77.6	19.1	4.89	19.1	4.91	19.4	5.06	18.5	4.82	19	4.89	19.6	5.02	**19.117**	**4.932**	**0.258**
19	85.5	19.1	4.89	19.1	4.93	19.4	5.06	18.6	4.85	18.9	4.89	19.5	5.02	**19.100**	**4.940**	**0.259**
20	94.1	19.1	4.92	19.1	4.96	19.5	5.09	18.6	4.85	18.9	4.92	19.5	5.05	**19.117**	**4.965**	**0.260**
21	104	19.1	4.92	19.1	4.97	19.5	5.10	18.5	4.84	18.9	4.95	19.4	5.07	**19.083**	**4.975**	**0.261**
22	114	19.1	4.95	19.1	4.99	19.5	5.13	18.4	4.85	18.9	4.99	19.4	5.10	**19.067**	**5.002**	**0.262**
23	126	19.1	4.97	19.1	5	19.4	5.14	18.4	4.88	18.8	5.01	19.4	5.15	**19.033**	**5.025**	**0.264**
24	140	19	5.01	19.1	5.04	19.4	5.17	18.3	4.88	18.8	5.06	19.3	5.18	**18.983**	**5.057**	**0.266**
25	150	18.9	5.05	19	5.07	19.4	5.2	18.3	4.9	18.7	5.08	19.2	5.20	**18.917**	**5.087**	**0.269**

[¥] Rheological analyses were conducted at day 1 after samples production.

Table B-16: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of experimental sample 5 manufactured by production method 2 [¥]

Meas. Pts.	Stress Amp. (μNm)	5-1-1		5-1-2		5-2-1		5-2-2		5-3-1		5-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	16.2	4.13	16.9	4.32	17.4	4.45	16.5	4.23	15.7	4.05	16.4	4.2	**16.517**	**4.230**	**0.256**
2	16.7	16.2	4.14	16.9	4.33	17.3	4.43	16.5	4.24	15.7	4.06	16.5	4.22	**16.517**	**4.237**	**0.257**
3	18.4	16.2	4.14	16.9	4.34	17.4	4.45	16.6	4.26	15.8	4.08	16.5	4.23	**16.567**	**4.250**	**0.257**
4	20.2	16.3	4.16	16.9	4.34	17.4	4.46	16.6	4.26	15.8	4.09	16.6	4.26	**16.600**	**4.262**	**0.257**
5	22.2	16.3	4.18	16.9	4.35	17.4	4.46	16.6	4.27	15.8	4.09	16.5	4.24	**16.583**	**4.265**	**0.257**
6	24.5	16.2	4.16	17	4.39	17.4	4.47	16.7	4.29	15.9	4.13	16.6	4.27	**16.633**	**4.285**	**0.258**
7	26.9	16.3	4.19	17	4.39	17.5	4.49	16.7	4.29	15.8	4.12	16.6	4.28	**16.650**	**4.293**	**0.258**
8	29.7	16.3	4.2	17	4.4	17.5	4.49	16.7	4.29	15.9	4.15	16.6	4.28	**16.667**	**4.302**	**0.258**
9	32.7	16.3	4.2	17	4.41	17.5	4.5	16.7	4.29	15.9	4.16	16.6	4.29	**16.667**	**4.308**	**0.259**
10	35.9	16.3	4.22	17	4.41	17.5	4.51	16.8	4.32	15.9	4.16	16.6	4.29	**16.683**	**4.318**	**0.259**
11	39.6	16.3	4.22	17	4.42	17.5	4.51	16.7	4.30	15.9	4.17	16.5	4.27	**16.650**	**4.315**	**0.259**
12	43.6	16.3	4.22	17	4.43	17.5	4.52	16.7	4.31	15.9	4.17	16.5	4.28	**16.650**	**4.322**	**0.260**
13	48	16.2	4.21	17	4.43	17.6	4.55	16.8	4.34	15.9	4.18	16.5	4.30	**16.667**	**4.335**	**0.260**
14	52.8	16.2	4.21	17	4.44	17.6	4.56	16.8	4.35	15.9	4.19	16.4	4.27	**16.650**	**4.337**	**0.260**
15	58.1	16.2	4.22	17	4.45	17.6	4.57	16.8	4.35	15.9	4.20	16.5	4.30	**16.667**	**4.348**	**0.261**
16	64	16.2	4.23	17.1	4.49	17.6	4.57	16.8	4.36	15.9	4.21	16.5	4.31	**16.683**	**4.362**	**0.261**
17	70.5	16.2	4.26	17.1	4.51	17.6	4.58	16.8	4.36	15.9	4.22	16.5	4.32	**16.683**	**4.375**	**0.262**
18	77.6	16.2	4.27	17.1	4.52	17.6	4.59	16.8	4.37	15.9	4.22	16.5	4.33	**16.683**	**4.383**	**0.263**
19	85.5	16.2	4.28	17.1	4.54	17.6	4.6	16.8	4.38	15.9	4.23	16.5	4.34	**16.683**	**4.395**	**0.263**
20	94.1	16.2	4.31	17.1	4.56	17.6	4.6	16.9	4.42	15.9	4.25	16.5	4.34	**16.700**	**4.413**	**0.264**
21	104	16.2	4.34	17.1	4.57	17.5	4.59	16.8	4.41	15.9	4.26	16.5	4.36	**16.667**	**4.422**	**0.265**
22	114	16.1	4.33	17	4.56	17.4	4.59	16.7	4.41	15.8	4.25	16.5	4.38	**16.583**	**4.420**	**0.267**
23	126	16.1	4.35	16.9	4.55	17.4	4.62	16.7	4.43	15.7	4.24	16.4	4.38	**16.533**	**4.428**	**0.268**
24	140	16	4.34	16.9	4.56	17.3	4.62	16.6	4.43	15.6	4.23	16.3	4.38	**16.450**	**4.427**	**0.269**
25	150	15.9	4.34	16.8	4.56	17.2	4.63	16.5	4.44	15.6	4.24	16.2	4.38	**16.367**	**4.432**	**0.271**

[¥] Rheological analyses were conducted at day 1 after samples production.

Table B-17: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of experimental sample 6 manufactured by production method 2 [¥]

Meas. Pts.	Stress Amp. (μNm)	6-1-1		6-1-2		6-2-1		6-2-2		6-3-1		6-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	20.5	5.17	20.1	5.05	21	5.31	22.1	5.5	22.9	5.60	22.2	5.46	**21.467**	**5.348**	**0.249**
2	16.7	20.6	5.2	20.1	5.06	21	5.3	22.2	5.51	22.8	5.57	22.2	5.47	**21.483**	**5.352**	**0.249**
3	18.4	20.5	5.19	20.2	5.08	21	5.3	22.2	5.51	22.8	5.57	22.3	5.49	**21.500**	**5.357**	**0.249**
4	20.2	20.6	5.22	20.2	5.09	21.1	5.33	22.3	5.53	22.9	5.6	22.3	5.50	**21.567**	**5.378**	**0.249**
5	22.2	20.5	5.21	20.3	5.12	21.1	5.34	22.2	5.52	22.9	5.6	22.4	5.52	**21.567**	**5.385**	**0.250**
6	24.5	20.6	5.23	20.3	5.12	21.2	5.36	22.3	5.55	23	5.61	22.3	5.50	**21.617**	**5.395**	**0.250**
7	26.9	20.6	5.24	20.3	5.13	21.2	5.37	22.3	5.55	23	5.64	22.4	5.53	**21.633**	**5.410**	**0.250**
8	29.7	20.6	5.24	20.4	5.16	21.3	5.39	22.4	5.58	23.1	5.67	22.4	5.53	**21.700**	**5.428**	**0.250**
9	32.7	20.6	5.24	20.3	5.14	21.2	5.37	22.4	5.58	23.1	5.67	22.5	5.56	**21.683**	**5.427**	**0.250**
10	35.9	20.7	5.27	20.4	5.16	21.3	5.4	22.5	5.61	23.1	5.66	22.5	5.56	**21.750**	**5.443**	**0.250**
11	39.6	20.7	5.27	20.4	5.17	21.3	5.41	22.5	5.62	23.2	5.7	22.6	5.58	**21.783**	**5.458**	**0.251**
12	43.6	20.8	5.31	20.4	5.18	21.4	5.44	22.5	5.62	23.2	5.71	22.6	5.59	**21.817**	**5.475**	**0.251**
13	48	20.9	5.34	20.5	5.21	21.5	5.47	22.6	5.65	23.3	5.73	22.7	5.61	**21.917**	**5.502**	**0.251**
14	52.8	20.9	5.34	20.5	5.21	21.4	5.45	22.5	5.63	23.3	5.74	22.8	5.64	**21.900**	**5.502**	**0.251**
15	58.1	20.9	5.35	20.5	5.22	21.5	5.48	22.6	5.66	23.3	5.75	22.7	5.62	**21.917**	**5.513**	**0.252**
16	64	21	5.38	21	5.23	21.5	5.49	22.6	5.67	23.4	5.78	22.8	5.66	**21.967**	**5.535**	**0.252**
17	70.5	20.9	5.36	20.6	5.27	21.5	5.49	22.6	5.68	23.4	5.78	22.8	5.65	**21.967**	**5.538**	**0.252**
18	77.6	20.9	5.38	20.5	5.25	21.6	5.52	22.7	5.71	23.5	5.81	22.8	5.67	**22.000**	**5.557**	**0.253**
19	85.5	20.9	5.39	20.5	5.27	21.5	5.5	22.6	5.69	23.6	5.84	22.9	5.70	**22.000**	**5.565**	**0.253**
20	94.1	20.8	5.38	20.5	5.28	21.5	5.51	22.6	5.7	23.5	5.82	22.9	5.72	**21.967**	**5.568**	**0.253**
21	104	20.8	5.39	20.5	5.3	21.5	5.52	22.5	5.68	23.5	5.83	22.8	5.70	**21.933**	**5.570**	**0.254**
22	114	20.8	5.4	20.5	5.31	21.5	5.53	22.5	5.69	23.5	5.86	22.8	5.72	**21.933**	**5.585**	**0.255**
23	126	20.7	5.42	20.4	5.3	21.5	5.55	22.5	5.71	23.4	5.86	22.8	5.75	**21.883**	**5.598**	**0.256**
24	140	20.8	5.47	20.4	5.32	21.5	5.57	22.5	5.74	23.5	5.92	22.7	5.76	**21.900**	**5.630**	**0.257**
25	150	20.7	5.46	20.4	5.35	21.4	5.58	22.5	5.77	23.4	5.95	22.7	5.81	**21.850**	**5.653**	**0.259**

[¥] Rheological analyses were conducted at day 1 after samples production.

Table B-18: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of experimental sample 7 manufactured by production method 2 [¥]

Meas. Pts.	Stress Amp. (μNm)	7-1-1		7-1-2		7-2-1		7-2-2		7-3-1		7-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	22.7	5.63	23	5.68	23.5	5.75	22.9	5.66	21.4	5.43	22.2	5.60	**22.617**	**5.625**	**0.249**
2	16.7	22.8	5.66	23	5.69	23.6	5.78	23	5.68	21.4	5.43	22.3	5.63	**22.683**	**5.645**	**0.249**
3	18.4	22.9	5.7	23.1	5.71	23.7	5.81	23.1	5.71	21.5	5.46	22.3	5.63	**22.767**	**5.667**	**0.249**
4	20.2	22.9	5.68	23.1	5.71	23.7	5.81	23.2	5.74	21.5	5.46	22.3	5.64	**22.783**	**5.673**	**0.249**
5	22.2	23	5.71	23.2	5.74	23.8	5.85	23.3	5.77	21.6	5.49	22.4	5.67	**22.883**	**5.705**	**0.249**
6	24.5	23	5.71	23.3	5.77	23.9	5.89	23.3	5.78	21.5	5.5	22.4	5.68	**22.900**	**5.717**	**0.250**
7	26.9	23.1	5.74	23.3	5.77	23.9	5.9	23.4	5.81	21.6	5.5	22.5	5.71	**22.967**	**5.738**	**0.250**
8	29.7	23.2	5.76	23.4	5.80	24	5.92	23.5	5.84	21.5	5.49	22.4	5.69	**23.000**	**5.750**	**0.250**
9	32.7	23.2	5.77	23.5	5.82	24	5.93	23.4	5.83	21.6	5.51	22.5	5.73	**23.033**	**5.765**	**0.250**
10	35.9	23.3	5.80	23.5	5.8	24.1	5.97	23.5	5.86	21.6	5.52	22.6	5.76	**23.100**	**5.788**	**0.251**
11	39.6	23.4	5.82	23.6	5.85	24.1	5.97	23.5	5.87	21.6	5.52	22.6	5.77	**23.133**	**5.800**	**0.251**
12	43.6	23.4	5.83	23.6	5.86	24.2	6.01	23.6	5.91	21.7	5.55	22.7	5.80	**23.200**	**5.827**	**0.251**
13	48	23.5	5.86	23.7	5.89	24.3	6.03	23.6	5.92	21.7	5.6	22.7	5.81	**23.250**	**5.845**	**0.251**
14	52.8	23.5	5.86	23.7	5.9	24.3	6.04	23.7	5.96	21.8	5.59	22.7	5.82	**23.283**	**5.862**	**0.252**
15	58.1	23.5	5.9	23.7	5.91	24.4	6.07	23.7	5.97	21.8	5.6	22.8	5.86	**23.317**	**5.880**	**0.252**
16	64	23.6	5.9	23.8	5.94	24.4	6.09	23.8	6.01	21.8	5.62	22.8	5.87	**23.367**	**5.905**	**0.253**
17	70.5	23.7	5.93	23.8	5.95	24.5	6.13	23.8	6.02	21.9	5.64	22.9	5.91	**23.433**	**5.930**	**0.253**
18	77.6	23.6	5.92	23.9	5.99	24.5	6.14	23.9	6.07	21.9	5.65	22.8	5.90	**23.433**	**5.945**	**0.254**
19	85.5	23.7	5.96	23.9	6	24.6	6.18	24	6.10	22	5.69	22.9	5.94	**23.517**	**5.978**	**0.254**
20	94.1	23.7	5.97	23.9	6.02	24.6	6.19	24.1	6.14	21.9	5.67	22.9	5.95	**23.517**	**5.990**	**0.255**
21	104	23.7	6.00	23.9	6.05	24.6	6.21	24.1	6.16	22	5.71	23	6	**23.550**	**6.022**	**0.256**
22	114	23.8	6.05	24	6.09	24.8	6.28	24.1	6.18	22.1	5.75	23.1	6.03	**23.650**	**6.063**	**0.256**
23	126	23.8	6.1	24	6.13	24.7	6.28	24.2	6.24	22.1	5.77	23.1	6.05	**23.650**	**6.095**	**0.258**
24	140	23.9	6.17	24.1	6.2	24.8	6.33	24.2	6.27	22.2	5.81	23.1	6.06	**23.717**	**6.140**	**0.259**
25	150	24	6.23	24.1	6.26	24.8	6.37	24.3	6.31	22.3	5.86	23.2	6.11	**23.783**	**6.190**	**0.260**

[¥] Rheological analyses were conducted at day 1 after samples production.

Table B-19: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of experimental sample 8 manufactured by production method 2 [¥]

Meas. Pts.	Stress Amp. (μNm)	8-1-1		8-1-2		8-2-1		8-2-2		8-3-1		8-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	19.6	4.88	18.8	4.76	20.7	5.09	20.3	5.01	20.3	5.03	20.6	5.05	**20.050**	**4.970**	**0.248**
2	16.7	19.7	4.91	18.8	4.77	20.9	5.13	20.3	5.02	20.4	5.05	20.8	5.1	**20.150**	**4.997**	**0.248**
3	18.4	19.7	4.92	18.9	4.79	20.9	5.14	20.4	5.04	20.5	5.08	20.7	5.1	**20.183**	**5.008**	**0.248**
4	20.2	19.7	4.92	19	4.82	21	5.16	20.4	5.04	20.5	5.08	20.7	5.09	**20.217**	**5.018**	**0.248**
5	22.2	19.8	4.95	18.9	4.8	21.1	5.18	20.4	5.05	20.5	5.09	20.8	5.11	**20.250**	**5.030**	**0.248**
6	24.5	19.9	4.98	18.9	4.8	21.1	5.19	20.5	5.08	20.5	5.11	20.8	5.13	**20.283**	**5.048**	**0.249**
7	26.9	19.9	4.97	19	4.82	21.1	5.19	20.5	5.08	20.5	5.11	20.9	5.15	**20.317**	**5.053**	**0.249**
8	29.7	19.9	4.98	19	4.83	21.1	5.2	20.6	5.11	20.6	5.14	20.9	5.16	**20.350**	**5.070**	**0.249**
9	32.7	20	5	19	4.84	21.2	5.23	20.6	5.12	20.5	5.12	20.9	5.16	**20.367**	**5.078**	**0.249**
10	35.9	20	5.01	19.1	4.86	21.1	5.22	20.6	5.13	20.5	5.14	21	5.19	**20.383**	**5.092**	**0.250**
11	39.6	20	5.02	19.1	4.88	21.1	5.22	20.5	5.11	20.6	5.17	21	5.2	**20.383**	**5.100**	**0.250**
12	43.6	19.9	4.99	19.1	4.89	21.1	5.23	20.6	5.14	20.6	5.18	21	5.21	**20.383**	**5.107**	**0.251**
13	48	19.9	5	19.1	4.9	21.2	5.25	20.6	5.15	20.6	5.19	21	5.22	**20.400**	**5.118**	**0.251**
14	52.8	20	5.03	19.2	4.92	21.2	5.25	20.6	5.15	20.6	5.2	21	5.23	**20.433**	**5.130**	**0.251**
15	58.1	20	5.05	19.2	4.95	21.2	5.26	20.6	5.16	20.7	5.23	21.1	5.27	**20.467**	**5.153**	**0.252**
16	64	20	5.07	19.2	4.96	21.3	5.28	20.7	5.19	20.7	5.24	21	5.25	**20.483**	**5.165**	**0.252**
17	70.5	19.9	5.05	19.3	4.99	21.3	5.29	20.7	5.2	20.7	5.24	21.1	5.28	**20.500**	**5.175**	**0.252**
18	77.6	19.9	5.06	19.2	4.98	21.2	5.29	20.6	5.19	20.8	5.29	21.1	5.29	**20.467**	**5.183**	**0.253**
19	85.5	19.9	5.07	19.2	4.98	21.2	5.3	20.7	5.23	20.8	5.28	21.1	5.31	**20.483**	**5.195**	**0.254**
20	94.1	19.8	5.08	19.2	5	21.2	5.33	20.7	5.24	20.8	5.3	21.2	5.35	**20.483**	**5.217**	**0.255**
21	104	19.8	5.09	19.1	5	21.2	5.34	20.6	5.23	20.9	5.34	21.2	5.36	**20.467**	**5.227**	**0.255**
22	114	19.7	5.1	19.1	5.01	21.1	5.34	20.6	5.26	20.8	5.33	21.1	5.34	**20.400**	**5.230**	**0.256**
23	126	19.7	5.13	19.1	5.01	21.1	5.37	20.5	5.27	20.8	5.35	21.1	5.36	**20.383**	**5.248**	**0.257**
24	140	19.7	5.15	19	5.01	21	5.38	20.4	5.28	20.7	5.35	21.1	5.36	**20.317**	**5.255**	**0.259**
25	150	19.6	5.15	19	5.02	20.9	5.43	20.4	5.31	20.6	5.36	21	5.39	**20.250**	**5.277**	**0.261**

[¥] Rheological analyses were conducted at day 1 after samples production.

Table B-20: Mass of ingredients needed for the production of the proposed formulation

Ingredient	Amount g $100g^{-1}$
MPC-85	10.39
NaCN	2.22
MPP	1.85
H_2O	85.54
SC	0.007

Appendix C – Physicochemical and rheological stability of the recombined yogurt obtained from the dry formulation proposed, and the application of a simplified method for the hydration of the yogurt powder

Table C-1: Mass of ingredients used to produce experimental samples by means of production method A [¥]

Sample No.[†]	Ingredients				
	MPC-85 (g)	NaCN (g)	MPP (g)	H_2O (g)	SC (mg)
1D-1	93.51	20.02	16.64	769.83	65.02
1D-2	93.51	20.02	16.64	769.85	65.09
1D-3	93.51	20.02	16.64	769.82	64.98
4D-1	93.51	20.02	16.64	769.84	65.09
4D-2	93.51	20.02	16.64	769.82	65.13
4D-3	93.51	20.02	16.64	769.82	65.08
8D-1	93.51	20.02	16.64	769.83	65.22
8D-2	93.51	20.02	16.64	769.84	65.08
8D-3	93.51	20.02	16.64	769.83	65.11
12D-1	93.51	20.02	16.64	769.83	65.04
12D-2	93.51	20.02	16.64	769.81	65.13
12D-3	93.51	20.02	16.64	769.82	65.06

[¥]Presented amounts were used to prepare 900g of recombined and inoculated milks. [†] 1D: samples stored for1 Day; 4D: samples stored for 4 days; 8D: samples stored for 8 days; 12D: samples stored for 12 days.

Table C-2: Mass of ingredients used to produce experimental samples by means of production method B [¥]

Sample No.[†]	Ingredients				
	MPC-85 (g)	NaCN (g)	MPP (g)	H_2O (g)	SC (mg)
1D-1	72.73	15.57	12.94	598.75	50.09
1D-2	72.73	15.57	12.94	598.76	50.16
1D-3	72.73	15.57	12.94	598.75	50.04
8D-1	72.73	15.57	12.94	598.74	50.05
8D-2	72.73	15.57	12.94	598.76	50.11
8D-3	72.73	15.57	12.94	598.77	50.19

[¥]Presented amounts were used to prepare 700g of recombined and inoculated milks. [†] 1D: samples stored for1 Day; 8D: samples stored for 8 days.

Table C-3: Physicochemical analyses of experimental samples manufactured by production method A [¥]

Sample No.[†]	Incubation time (hours)	Surface whey-off before homogenization (%m/m)	Surface whey-off after homogenization (%m/m)	Whey drainage (Ordinal scale: 0;1;2)	Size of visible clusters (Ordinal scale: 0;1;2;3)
1D-1	10.8	0.00	0.00	0	1
1D-2	10.7	0.00	0.00	0	1
1D-3	11.0	0.00	0.00	0	1
Mean ± SD	**10.833 ± 0.153**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**1.000 ± 0.000**
4D-1	10.8	0.00	0.00	0	1
4D-2	10.9	0.00	0.00	0	1
4D-3	10.9	0.00	0.00	0	1
Mean ± SD	**10.867 ± 0.058**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**1.000 ± 0.000**
8D-1	10.9	0.00	0.00	0	1
8D-2	10.8	0.00	0.00	0	1
8D-3	11.1	0.00	0.00	0	1
Mean ± SD	**10.933 ± 0.153**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**1.000 ± 0.000**
12D-1	11.0	0.00	0.00	0	1
12D-2	10.8	0.00	0.00	1	1
12D-3	10.7	0.00	0.00	1	1
Mean ± SD	**10.833 ± 0.153**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.667 ± 0.577**	**1.000 ± 0.000**

[¥] Physicochemical analyses were conducted after the corresponding storage time of each sample. [†] 1D: samples stored for1 Day; 4D: samples stored for 4 days; 8D: samples stored for 8 days; 12D: samples stored for 12 days.

Table C-4: Physicochemical analyses of experimental samples manufactured by production method B [¥]

Sample No.[†]	Incubation time (hours)	Surface whey-off before homogenization (%m/m)	Surface whey-off after homogenization (%m/m)	Whey drainage (Ordinal scale: 0;1;2)	Size of visible clusters (Ordinal scale: 0;1;2;3)
1D-1	12.2	0.00	0.00	0	1
1D-2	12.4	0.00	0.00	0	1
1D-3	12.5	0.00	0.00	0	1
Mean ± SD	**12.367 ± 0.153**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**1.000 ± 0.000**
8D-1	12.3	0.00	0.00	0	1
8D-2	12.3	0.00	0.00	0	1
8D-3	12.6	0.00	0.00	0	1
Mean ± SD	**12.400 ± 0.173**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**1.000 ± 0.000**

[¥] Physicochemical analyses were conducted after the corresponding storage time of each sample. [†] 1D: samples stored for1 Day; 8D: samples stored for 8 days.

Table C-5: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of samples produced by method A and stored for 1 day [¥]

Meas. Pts.	Stress Amp. (μNm)	1D-1-1		1D-1-2		1D-2-1		1D-2-2		1D-3-1		1D-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	21.3	5.35	21.7	5.45	20.7	5.24	21.1	5.3	21.6	5.4	21	5.29	**21.233**	**5.338**	**0.251**
2	16.7	21.4	5.39	21.7	5.46	20.7	5.25	21	5.3	21.7	5.44	21.1	5.32	**21.267**	**5.360**	**0.252**
3	18.4	21.3	5.38	21.8	5.5	20.7	5.27	21.1	5.33	21.7	5.45	21.2	5.34	**21.300**	**5.378**	**0.253**
4	20.2	21.4	5.4	21.8	5.5	20.8	5.31	21.1	5.35	21.8	5.47	21.2	5.35	**21.350**	**5.397**	**0.253**
5	22.2	21.4	5.42	21.9	5.54	20.8	5.31	21.2	5.38	21.9	5.5	21.3	5.38	**21.417**	**5.422**	**0.253**
6	24.5	21.5	5.45	22	5.57	20.9	5.34	21.1	5.36	21.9	5.49	21.3	5.37	**21.450**	**5.430**	**0.253**
7	26.9	21.5	5.45	22	5.57	20.9	5.34	21.2	5.39	22	5.52	21.4	5.41	**21.500**	**5.447**	**0.253**
8	29.7	21.6	5.48	22	5.58	20.9	5.35	21.2	5.4	22	5.54	21.4	5.43	**21.517**	**5.463**	**0.254**
9	32.7	21.7	5.51	22.1	5.61	20.9	5.36	21.2	5.41	22.1	5.56	21.5	5.46	**21.583**	**5.485**	**0.254**
10	35.9	21.6	5.49	22.1	5.63	20.9	5.36	21.3	5.44	22	5.55	21.4	5.44	**21.550**	**5.485**	**0.255**
11	39.6	21.7	5.51	22.2	5.65	21	5.4	21.3	5.44	22.1	5.57	21.5	5.46	**21.633**	**5.505**	**0.254**
12	43.6	21.7	5.53	22.1	5.65	21	5.41	21.3	5.45	22.1	5.59	21.6	5.5	**21.633**	**5.522**	**0.255**
13	48	21.7	5.51	22.2	5.69	21.1	5.44	21.4	5.49	22.2	5.63	21.7	5.55	**21.717**	**5.552**	**0.256**
14	52.8	21.8	5.56	22.2	5.70	21.1	5.46	21.5	5.52	22.2	5.63	21.8	5.58	**21.767**	**5.575**	**0.256**
15	58.1	21.8	5.58	22.3	5.74	21.1	5.46	21.5	5.5	22.3	5.67	21.7	5.57	**21.783**	**5.592**	**0.257**
16	64	21.8	5.59	22.3	5.75	21.2	5.49	21.5	5.53	22.4	5.72	21.8	5.6	**21.833**	**5.613**	**0.257**
17	70.5	21.9	5.62	22.4	5.79	21.2	5.51	21.6	5.56	22.3	5.70	21.8	5.63	**21.867**	**5.635**	**0.258**
18	77.6	21.9	5.65	22.4	5.81	21.2	5.53	21.5	5.56	22.4	5.74	21.8	5.64	**21.867**	**5.655**	**0.259**
19	85.5	21.9	5.67	22.5	5.87	21.3	5.57	21.6	5.61	22.5	5.78	21.9	5.69	**21.950**	**5.698**	**0.260**
20	94.1	22	5.71	22.5	5.88	21.3	5.57	21.6	5.62	22.5	5.79	21.9	5.7	**21.967**	**5.712**	**0.260**
21	104	22	5.74	22.5	5.9	21.4	5.62	21.7	5.66	22.6	5.84	21.9	5.71	**22.017**	**5.745**	**0.261**
22	114	22.1	5.78	22.5	5.93	21.4	5.64	21.7	5.67	22.6	5.86	22	5.77	**22.050**	**5.775**	**0.262**
23	126	22.2	5.83	22.6	5.98	21.5	5.69	21.8	5.72	22.7	5.91	22.1	5.81	**22.150**	**5.823**	**0.263**
24	140	22.2	5.84	22.6	6.01	21.4	5.67	21.8	5.74	22.7	5.96	22.1	5.82	**22.133**	**5.840**	**0.264**
25	150	22.2	5.87	22.7	6.07	21.5	5.7	21.8	5.76	22.8	6.01	22.1	5.84	**22.183**	**5.875**	**0.265**

[¥] Rheological analyses were conducted after the corresponding storage time.

Table C-6: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of samples produced by method A and stored for 4 days [¥]

Meas. Pts.	Stress Amp. (μNm)	4D-1-1		4D-1-2		4D-2-1		4D-2-2		4D-3-1		4D-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	20.1	5.06	19.4	4.93	21.7	5.49	21.8	5.5	19.9	5.03	20.4	5.16	**20.550**	**5.195**	**0.253**
2	16.7	20.1	5.05	19.5	4.95	21.6	5.5	21.9	5.54	19.9	5.05	20.5	5.2	**20.583**	**5.215**	**0.253**
3	18.4	20.2	5.08	19.5	4.96	21.7	5.53	21.9	5.56	20	5.09	20.5	5.21	**20.633**	**5.238**	**0.254**
4	20.2	20.2	5.1	19.6	4.99	21.8	5.56	22	5.58	20	5.11	20.5	5.24	**20.683**	**5.263**	**0.254**
5	22.2	20.3	5.14	19.6	5	21.8	5.57	22	5.59	20.1	5.15	20.6	5.27	**20.733**	**5.287**	**0.255**
6	24.5	20.4	5.16	19.7	5.03	21.9	5.6	22	5.6	20.2	5.19	20.6	5.28	**20.800**	**5.310**	**0.255**
7	26.9	20.3	5.14	19.8	5.06	21.9	5.6	22.1	5.64	20.2	5.2	20.7	5.32	**20.833**	**5.327**	**0.256**
8	29.7	20.4	5.16	19.8	5.07	22	5.62	22.1	5.63	20.3	5.22	20.8	5.36	**20.900**	**5.343**	**0.256**
9	32.7	20.4	5.17	19.9	5.1	22	5.63	22.2	5.67	20.4	5.25	20.7	5.34	**20.933**	**5.360**	**0.256**
10	35.9	20.5	5.21	19.9	5.11	22	5.65	22.2	5.69	20.5	5.3	20.7	5.36	**20.967**	**5.387**	**0.257**
11	39.6	20.5	5.21	19.9	5.12	22.1	5.69	22.3	5.71	20.4	5.26	20.8	5.39	**21.000**	**5.397**	**0.257**
12	43.6	20.6	5.24	20	5.15	22.2	5.72	22.4	5.74	20.5	5.29	20.8	5.39	**21.083**	**5.422**	**0.257**
13	48	20.7	5.28	20	5.17	22.2	5.72	22.3	5.74	20.6	5.32	20.9	5.42	**21.117**	**5.442**	**0.258**
14	52.8	20.6	5.26	20	5.18	22.3	5.75	22.4	5.77	20.6	5.35	20.9	5.43	**21.133**	**5.457**	**0.258**
15	58.1	20.7	5.3	20.1	5.21	22.2	5.74	22.5	5.79	20.6	5.37	21	5.47	**21.183**	**5.482**	**0.259**
16	64	20.7	5.32	20.1	5.22	22.2	5.77	22.5	5.81	20.6	5.38	21	5.5	**21.183**	**5.497**	**0.259**
17	70.5	20.8	5.36	20.2	5.26	22.3	5.8	22.6	5.85	20.7	5.42	21	5.49	**21.267**	**5.530**	**0.260**
18	77.6	20.8	5.38	20.1	5.26	22.3	5.83	22.5	5.8	20.7	5.44	21	5.51	**21.233**	**5.542**	**0.261**
19	85.5	20.8	5.4	20.2	5.31	22.4	5.86	22.6	5.89	20.8	5.49	21.1	5.56	**21.317**	**5.587**	**0.262**
20	94.1	20.9	5.45	20.2	5.31	22.4	5.87	22.6	5.91	20.8	5.47	21.2	5.6	**21.350**	**5.602**	**0.262**
21	104	20.9	5.47	20.3	5.34	22.4	5.9	22.6	5.92	20.8	5.5	21.2	5.6	**21.367**	**5.625**	**0.263**
22	114	20.9	5.48	20.3	5.37	22.5	5.94	22.7	5.98	20.9	5.53	21.3	5.67	**21.433**	**5.662**	**0.264**
23	126	20.9	5.50	20.4	5.42	22.5	5.96	22.7	6.02	20.8	5.54	21.4	5.71	**21.450**	**5.692**	**0.265**
24	140	21	5.56	20.5	5.48	22.5	5.97	22.7	6.04	20.8	5.56	21.3	5.71	**21.467**	**5.719**	**0.266**
25	150	21	5.59	20.5	5.52	22.6	5.99	22.8	6.07	20.8	5.59	21.4	5.76	**21.517**	**5.753**	**0.267**

[¥] Rheological analyses were conducted after the corresponding storage time.

Table C-7: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of samples produced by method A and stored for 8 days [¥]

Meas. Pts.	Stress Amp. (μNm)	8D-1-1		8D-1-2		8D-2-1		8D-2-2		8D-3-1		8D-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	21.9	5.56	21.6	5.49	22	5.54	22.4	5.64	22.5	5.65	22.9	5.73	**22.217**	**5.602**	**0.252**
2	16.7	22	5.6	21.8	5.55	22.3	5.62	22.5	5.67	22.6	5.7	23.1	5.8	**22.383**	**5.657**	**0.253**
3	18.4	22.1	5.64	21.8	5.56	22.2	5.60	22.6	5.7	22.7	5.74	23.3	5.86	**22.450**	**5.683**	**0.253**
4	20.2	22.2	5.67	22	5.62	22.3	5.63	22.8	5.76	22.8	5.78	23.4	5.90	**22.583**	**5.727**	**0.254**
5	22.2	22.3	5.7	22.1	5.66	22.4	5.67	22.8	5.77	23	5.85	23.5	5.94	**22.683**	**5.765**	**0.254**
6	24.5	22.3	5.71	22.2	5.69	22.5	5.70	23	5.84	23.1	5.88	23.6	5.99	**22.783**	**5.802**	**0.255**
7	26.9	22.4	5.73	22.3	5.72	22.7	5.77	23	5.84	23.2	5.9	23.8	6.06	**22.900**	**5.837**	**0.255**
8	29.7	22.5	5.77	22.4	5.76	22.9	5.84	23.1	5.87	23.4	5.97	23.9	6.1	**23.033**	**5.885**	**0.255**
9	32.7	22.6	5.79	22.6	5.81	23	5.9	23.2	5.91	23.4	5.96	24	6.13	**23.133**	**5.912**	**0.256**
10	35.9	22.7	5.83	22.7	5.85	23.1	5.92	23.4	5.99	23.5	6	24.1	6.17	**23.250**	**5.960**	**0.256**
11	39.6	22.9	5.89	22.8	5.89	23.2	5.95	23.5	6.03	23.6	6.05	24.2	6.20	**23.367**	**6.002**	**0.257**
12	43.6	23	5.93	22.9	5.92	23.4	6.01	23.6	6.06	23.7	6.09	24.4	6.27	**23.500**	**6.047**	**0.257**
13	48	23.1	5.99	23	5.95	23.5	6.05	23.7	6.1	23.8	6.13	24.4	6.28	**23.583**	**6.083**	**0.258**
14	52.8	23.1	5.99	23.1	5.98	23.7	6.12	23.8	6.14	23.9	6.18	24.5	6.32	**23.683**	**6.122**	**0.258**
15	58.1	23.2	6.03	23.3	6.05	23.7	6.14	24	6.21	23.9	6.21	24.6	6.36	**23.783**	**6.167**	**0.259**
16	64	23.3	6.07	23.2	6.03	23.8	6.17	24	6.23	24	6.25	24.8	6.43	**23.850**	**6.197**	**0.260**
17	70.5	23.4	6.1	23.3	6.06	23.9	6.22	24.1	6.27	24.1	6.31	24.7	6.42	**23.917**	**6.230**	**0.260**
18	77.6	23.3	6.1	23.5	6.13	24	6.25	24.2	6.31	24.1	6.3	24.8	6.47	**23.983**	**6.260**	**0.261**
19	85.5	23.4	6.13	23.5	6.15	24.1	6.29	24.4	6.37	24.2	6.34	24.9	6.52	**24.083**	**6.300**	**0.262**
20	94.1	23.5	6.15	23.6	6.2	24.2	6.33	24.4	6.37	24.3	6.36	25	6.56	**24.167**	**6.328**	**0.262**
21	104	23.5	6.17	23.7	6.23	24.3	6.36	24.5	6.41	24.3	6.4	25.1	6.6	**24.233**	**6.362**	**0.263**
22	114	23.6	6.2	23.7	6.25	24.3	6.4	24.6	6.45	24.4	6.44	25.2	6.63	**24.300**	**6.390**	**0.263**
23	126	23.6	6.19	23.8	6.29	24.4	6.41	24.7	6.5	24.5	6.49	25.2	6.65	**24.367**	**6.422**	**0.264**
24	140	23.7	6.22	23.8	6.30	24.5	6.44	24.8	6.54	24.5	6.5	25.3	6.71	**24.433**	**6.452**	**0.264**
25	150	23.8	6.26	23.9	6.34	24.6	6.47	24.9	6.57	24.6	6.54	25.3	6.7	**24.517**	**6.485**	**0.265**

[¥] Rheological analyses were conducted after the corresponding storage time.

Table C-8: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of samples produced by method A and stored for 12 days [¥]

Meas. Pts.	Stress Amp. (μNm)	12D-1-1		12D-1-2		12D-2-1		12D-2-2		12D-3-1		12D-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	20.3	5.1	20.5	5.13	18.9	4.76	19.4	4.89	19.2	4.85	19	4.82	**19.550**	**4.925**	**0.252**
2	16.7	20.4	5.12	20.5	5.14	18.9	4.75	19.3	4.89	19.2	4.87	19	4.82	**19.550**	**4.932**	**0.252**
3	18.4	20.4	5.15	20.5	5.15	18.9	4.77	19.4	4.91	19.2	4.88	19	4.83	**19.567**	**4.948**	**0.253**
4	20.2	20.4	5.17	20.5	5.16	18.9	4.78	19.4	4.91	19.2	4.89	19	4.84	**19.567**	**4.958**	**0.253**
5	22.2	20.4	5.17	20.5	5.17	19	4.79	19.4	4.9	19.2	4.90	19	4.84	**19.583**	**4.962**	**0.253**
6	24.5	20.4	5.2	20.6	5.21	19	4.8	19.4	4.91	19.2	4.90	19	4.85	**19.600**	**4.978**	**0.254**
7	26.9	20.5	5.24	20.6	5.22	19	4.82	19.5	4.94	19.3	4.93	19.1	4.88	**19.667**	**5.005**	**0.254**
8	29.7	20.4	5.23	20.6	5.24	18.9	4.82	19.5	4.95	19.3	4.94	19	4.87	**19.617**	**5.008**	**0.255**
9	32.7	20.5	5.25	20.6	5.26	18.9	4.84	19.5	4.96	19.3	4.96	19.1	4.91	**19.650**	**5.030**	**0.256**
10	35.9	20.5	5.27	20.6	5.27	18.9	4.85	19.5	4.97	19.3	4.97	19.1	4.91	**19.650**	**5.040**	**0.256**
11	39.6	20.5	5.29	20.7	5.31	18.9	4.85	19.5	4.99	19.2	4.97	19.1	4.92	**19.650**	**5.055**	**0.257**
12	43.6	20.5	5.32	20.6	5.31	18.9	4.86	19.4	4.98	19.2	4.97	19	4.91	**19.600**	**5.058**	**0.258**
13	48	20.4	5.33	20.7	5.36	18.8	4.86	19.4	4.99	19.2	4.98	19	4.92	**19.583**	**5.073**	**0.259**
14	52.8	20.4	5.34	20.7	5.37	18.8	4.87	19.4	5.01	19.1	4.98	19	4.93	**19.567**	**5.083**	**0.260**
15	58.1	20.3	5.33	20.6	5.36	18.8	4.87	19.3	5	19.1	4.98	19	4.94	**19.517**	**5.080**	**0.260**
16	64	20.4	5.37	20.6	5.37	18.7	4.88	19.3	5.02	19	4.97	18.9	4.93	**19.483**	**5.090**	**0.261**
17	70.5	20.3	5.36	20.6	5.4	18.6	4.88	19.2	5.01	18.9	4.97	18.8	4.93	**19.400**	**5.092**	**0.262**
18	77.6	20.3	5.39	20.6	5.42	18.6	4.88	19.2	5.02	18.8	4.96	18.7	4.92	**19.367**	**5.098**	**0.263**
19	85.5	20.3	5.41	20.5	5.42	18.5	4.89	19.1	5.02	18.6	4.93	18.6	4.92	**19.267**	**5.098**	**0.265**
20	94.1	20.2	5.4	20.5	5.42	18.3	4.88	18.9	5.02	18.4	4.92	18.4	4.90	**19.117**	**5.090**	**0.266**
21	104	20.2	5.41	20.5	5.45	18.1	4.89	18.7	5.03	18.1	4.9	18.1	4.86	**18.950**	**5.090**	**0.269**
22	114	20.2	5.42	20.5	5.47	17.9	4.88	18.4	5.04	17.8	4.86	17.9	4.84	**18.783**	**5.085**	**0.271**
23	126	20.2	5.44	20.4	5.46	17.8	4.89	18.2	5.06	17.6	4.85	17.6	4.82	**18.633**	**5.087**	**0.273**
24	140	20.1	5.45	20.4	5.48	17.6	4.9	17.9	5.06	17.3	4.82	17.2	4.79	**18.417**	**5.083**	**0.276**
25	150	20.1	5.45	20.4	5.50	17.5	4.9	17.7	5.06	16.9	4.78	16.8	4.74	**18.233**	**5.072**	**0.278**

[¥] Rheological analyses were conducted after the corresponding storage time.

Table C-9: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of samples produced by method B and stored for 1 day [¥]

Meas. Pts.	Stress Amp. (μNm)	1D-1-1		1D-1-2		1D-2-1		1D-2-2		1D-3-1		1D-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	19.3	4.9	19.6	4.94	21	5.31	20.7	5.23	21.3	5.34	21.2	5.3	**20.517**	**5.165**	**0.252**
2	16.7	19.3	4.88	19.7	4.97	21.1	5.33	20.6	5.21	21.3	5.36	21.3	5.34	**20.550**	**5.182**	**0.252**
3	18.4	19.3	4.88	19.7	4.98	21.2	5.37	20.7	5.24	21.3	5.36	21.4	5.36	**20.600**	**5.198**	**0.252**
4	20.2	19.4	4.92	19.7	5	21.2	5.35	20.7	5.26	21.4	5.38	21.5	5.40	**20.650**	**5.218**	**0.253**
5	22.2	19.4	4.94	19.8	5.04	21.3	5.4	20.8	5.3	21.5	5.42	21.4	5.38	**20.700**	**5.247**	**0.253**
6	24.5	19.5	4.98	19.8	5.04	21.4	5.42	20.8	5.29	21.5	5.43	21.5	5.4	**20.750**	**5.260**	**0.253**
7	26.9	19.5	4.97	19.9	5.08	21.3	5.42	20.9	5.32	21.6	5.46	21.6	5.44	**20.800**	**5.282**	**0.254**
8	29.7	19.5	5	19.9	5.09	21.4	5.46	21	5.36	21.7	5.51	21.6	5.46	**20.850**	**5.313**	**0.255**
9	32.7	19.6	5.03	20	5.13	21.4	5.45	21	5.35	21.8	5.55	21.7	5.49	**20.917**	**5.333**	**0.255**
10	35.9	19.6	5.05	19.9	5.11	21.5	5.49	21.1	5.4	21.7	5.53	21.8	5.51	**20.933**	**5.348**	**0.255**
11	39.6	19.6	5.06	19.9	5.12	22	5.5	21.1	5.41	21.8	5.56	21.9	5.54	**20.967**	**5.365**	**0.256**
12	43.6	19.6	5.08	20	5.16	22	5.53	21.2	5.43	21.9	5.59	22	5.57	**21.050**	**5.393**	**0.256**
13	48	19.7	5.11	20	5.17	21.7	5.58	21.3	5.46	21.9	5.60	22	5.58	**21.100**	**5.417**	**0.257**
14	52.8	19.7	5.14	20	5.19	21.7	5.57	21.3	5.47	21.9	5.61	22.1	5.62	**21.117**	**5.433**	**0.257**
15	58.1	19.8	5.16	20.1	5.23	21.8	5.61	21.4	5.51	22	5.64	22.1	5.62	**21.200**	**5.462**	**0.258**
16	64	19.9	5.21	20.1	5.24	21.9	5.64	21.3	5.49	22	5.66	22.2	5.66	**21.233**	**5.483**	**0.258**
17	70.5	19.8	5.18	20.2	5.27	22	5.69	21.4	5.54	22.1	5.7	22.3	5.7	**21.300**	**5.513**	**0.259**
18	77.6	19.9	5.2	20.2	5.27	22	5.69	21.4	5.55	22.2	5.74	22.3	5.71	**21.333**	**5.527**	**0.259**
19	85.5	19.9	5.21	20.3	5.31	22.1	5.72	21.5	5.6	22.1	5.72	22.4	5.75	**21.383**	**5.552**	**0.260**
20	94.1	20	5.24	20.2	5.3	22	5.7	21.6	5.64	22.2	5.74	22.5	5.79	**21.417**	**5.568**	**0.260**
21	104	19.9	5.24	20.3	5.34	22.1	5.74	21.5	5.63	22.3	5.78	22.6	5.82	**21.450**	**5.592**	**0.261**
22	114	19.9	5.26	20.3	5.36	22.2	5.79	21.6	5.68	22.4	5.81	22.6	5.84	**21.500**	**5.623**	**0.262**
23	126	20	5.32	20.3	5.38	22.2	5.81	21.7	5.71	22.4	5.84	22.7	5.88	**21.550**	**5.657**	**0.262**
24	140	20	5.34	20.4	5.44	22.3	5.84	21.7	5.72	22.5	5.89	22.7	5.9	**21.600**	**5.688**	**0.263**
25	150	20.1	5.41	20.5	5.49	22.4	5.87	21.8	5.77	22.6	5.92	22.8	5.94	**21.700**	**5.733**	**0.264**

[¥] Rheological analyses were conducted after the corresponding storage time.

Table C-10: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of samples produced by method B and stored for 8 days [¥]

Meas. Pts.	Stress Amp. (μNm)	8D-1-1		8D-1-2		8D-2-1		8D-2-2		8D-3-1		8D-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	22.4	5.62	22.3	5.62	21.8	5.52	22.1	5.55	21.5	5.44	21.1	5.36	**21.867**	**5.518**	**0.252**
2	16.7	22.5	5.65	22.5	5.68	21.9	5.56	22.1	5.56	21.6	5.47	21.1	5.37	**21.950**	**5.548**	**0.253**
3	18.4	22.6	5.69	22.5	5.7	22	5.59	22.2	5.60	21.7	5.51	21.2	5.4	**22.033**	**5.582**	**0.253**
4	20.2	22.7	5.73	22.6	5.73	22.1	5.63	22.3	5.63	21.8	5.56	21.3	5.44	**22.133**	**5.620**	**0.254**
5	22.2	22.8	5.76	22.7	5.77	22.3	5.68	22.4	5.67	21.9	5.59	21.4	5.49	**22.250**	**5.660**	**0.254**
6	24.5	22.9	5.79	22.8	5.8	22.3	5.69	22.5	5.7	22	5.63	21.5	5.54	**22.333**	**5.692**	**0.255**
7	26.9	23	5.82	23	5.85	22.4	5.73	22.6	5.74	22.1	5.65	21.6	5.57	**22.450**	**5.727**	**0.255**
8	29.7	23.2	5.89	23	5.85	22.5	5.77	22.7	5.78	22.3	5.72	21.7	5.6	**22.567**	**5.768**	**0.256**
9	32.7	23.2	5.89	23.1	5.89	22.6	5.80	22.8	5.83	22.3	5.73	21.8	5.65	**22.633**	**5.798**	**0.256**
10	35.9	23.3	5.91	23.2	5.92	22.7	5.83	23	5.88	22.4	5.77	21.9	5.69	**22.750**	**5.833**	**0.256**
11	39.6	23.4	5.95	23.3	5.96	22.7	5.85	23	5.89	22.5	5.81	21.9	5.70	**22.800**	**5.860**	**0.257**
12	43.6	23.5	5.98	23.4	6	22.8	5.88	23.1	5.93	22.5	5.83	21.9	5.72	**22.867**	**5.890**	**0.258**
13	48	23.6	6.02	23.5	6.03	22.9	5.92	23.2	5.97	22.6	5.87	22	5.77	**22.967**	**5.930**	**0.258**
14	52.8	23.7	6.06	23.6	6.07	23	5.96	23.3	6.01	22.7	5.89	22.1	5.81	**23.067**	**5.967**	**0.259**
15	58.1	23.8	6.1	23.8	6.13	23	5.97	23.4	6.05	22.7	5.93	22.2	5.86	**23.150**	**6.007**	**0.259**
16	64	24	6.17	23.7	6.13	23.1	6.02	23.5	6.09	22.8	5.96	22.2	5.86	**23.217**	**6.038**	**0.260**
17	70.5	24	6.19	23.8	6.17	23.2	6.05	23.6	6.12	22.8	5.96	22.2	5.88	**23.267**	**6.062**	**0.261**
18	77.6	24.1	6.23	23.9	6.2	23.3	6.08	23.6	6.13	22.8	5.98	22.3	5.91	**23.333**	**6.088**	**0.261**
19	85.5	24.2	6.26	24	6.24	23.3	6.09	23.7	6.17	22.9	6.01	22.3	5.93	**23.400**	**6.117**	**0.261**
20	94.1	24.2	6.27	24.1	6.29	23.4	6.15	23.7	6.18	22.9	6.03	22.4	5.97	**23.450**	**6.148**	**0.262**
21	104	24.3	6.31	24.1	6.3	23.4	6.17	23.8	6.22	23	6.06	22.4	5.98	**23.500**	**6.173**	**0.263**
22	114	24.4	6.35	24.2	6.33	23.4	6.19	23.8	6.25	23	6.08	22.4	5.99	**23.533**	**6.198**	**0.263**
23	126	24.4	6.38	24.2	6.35	23.5	6.24	23.9	6.27	23	6.11	22.5	6.02	**23.583**	**6.228**	**0.264**
24	140	24.5	6.43	24.3	6.39	23.5	6.25	23.9	6.3	23	6.12	22.5	6.03	**23.617**	**6.253**	**0.265**
25	150	24.5	6.44	24.4	6.47	23.6	6.30	23.9	6.31	23.1	6.17	22.5	6.05	**23.667**	**6.290**	**0.266**

[¥] Rheological analyses were conducted after the corresponding storage time.

Table C-11: Total solids content of samples produced by method A [¥]

Sample No.	Dish weight (g)	Initial yogurt weight (g)	Dried sample + dish weight (g)	Total solids (%)	Mean total solids (%) ± SD
1D-1-1	1.38623	3.47572	1.86748	13.846	**13.871 ± 0.023**
1D-1-2	1.3782	2.97085	1.79029	13.871	
1D-2-1	1.38748	3.75813	1.90853	13.865	
1D-2-2	1.37726	3.55125	1.86977	13.869	
1D-3-1	1.38923	2.78982	1.77744	13.915	
1D-3-2	1.3763	3.09743	1.80567	13.862	

[¥]Total solids content analyses were only done on samples stored for 1 day. No significant difference was assumed between the total solids content of samples stored for different times.

Table C-12: Total solids content of samples produced by method B [¥]

Sample No.	Dish weight (g)	Initial yogurt weight (g)	Dried sample + dish weight (g)	Total solids (%)	Mean Total Solids (%) ± SD
1D-1-1	1.37718	3.11538	1.80646	13.779	**13.783 ± 0.011**
1D-1-2	1.38672	3.00639	1.8012	13.787	
1D-2-1	1.38817	3.32011	1.84599	13.789	
1D-2-2	1.3786	2.99056	1.79131	13.800	
1D-3-1	1.37997	3.09024	1.80553	13.771	
1D-3-2	1.38222	3.1688	1.81862	13.772	

[¥]Total solids content analyses were only done on samples stored for 1 day. No significant difference was assumed between the total solids content of samples stored for different times.

Appendix D – Effects of storing the dry formulation on the rheological and physicochemical characteristics of the recombined yogurt

Table D-1: Mass of ingredients used to produce experimental samples [¥]

Sample No.	Ingredients				
	MPC-85 (g)	NaCN (g)	MPP (g)	H_2O (g)	SC (mg)
1-1	72.73	15.57	12.94	598.77	50.02
1-2	72.73	15.57	12.94	598.75	50.11
1-3	72.73	15.57	12.94	598.75	50.09
2-1	72.73	15.57	12.94	598.76	50.07
2-2	72.73	15.57	12.94	598.76	50.14
2-3	72.73	15.57	12.94	598.74	50.09
3-1	72.73	15.57	12.94	598.77	50.02
3-2	72.73	15.57	12.94	598.75	49.97
3-3	72.73	15.57	12.94	598.76	50.10
4-1	72.73	15.57	12.94	598.73	50.16
4-2	72.73	15.57	12.94	598.75	50.09
4-3	72.73	15.57	12.94	598.76	50.04

[¥]Presented amounts were used to prepare 700g of recombined and inoculated milks.

Table D-2: Physicochemical analyses of experimental samples [¥]

Sample No.	Incubation time (hours)	Surface whey-off before homogenization (%m/m)	Surface whey-off after homogenization (%m/m)	Whey drainage (Ordinal scale: 0;1;2)	Size of visible clusters (Ordinal scale: 0;1;2;3)
1-1	12.3	0.00	0.00	0	1
1-2	12.5	0.00	0.00	0	1
1-3	12.3	0.00	0.00	0	1
Mean ± SD	**12.367 ± 0.115**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**1.000 ± 0.000**
2-1	12.4	0.00	0.00	0	1
2-2	12.3	0.00	0.00	0	1
2-3	12.3	0.00	0.00	0	1
Mean ± SD	**12.333 ± 0.058**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**1.000 ± 0.000**
3-1	12.4	0.00	0.00	0	1
3-2	12.2	0.00	0.00	0	1
3-3	12.3	0.00	0.00	0	1
Mean ± SD	**12.300 ± 0.100**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**	**1.000 ± 0.000**
4-1	17.8	0.00	0.00	1	1
4-2	14.7	0.00	0.00	0	1
4-3	15.1	0.00	0.00	0	1
Mean ± SD	**15.867 ± 1.686**	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.333 ± 0.577**	**1.000 ± 0.000**

[¥] Physicochemical analyses were conducted at day 8 after samples production.

Table D-3: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of experimental sample 1 [¥]

Meas. Pts.	Stress Amp. (μNm)	1-1-1		1-1-2		1-2-1		1-2-2		1-3-1		1-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	21.9	5.61	22.1	5.66	22.6	5.74	22.3	5.69	21.2	5.43	21.5	5.55	**21.933**	**5.613**	**0.256**
2	16.7	22.1	5.67	22.2	5.7	22.8	5.82	22.4	5.72	21.4	5.5	21.6	5.61	**22.083**	**5.670**	**0.257**
3	18.4	22.3	5.72	22.3	5.74	23	5.89	22.6	5.8	21.5	5.55	21.8	5.68	**22.250**	**5.730**	**0.258**
4	20.2	22.4	5.77	22.5	5.79	23.1	5.94	22.7	5.84	21.6	5.59	21.9	5.73	**22.367**	**5.777**	**0.258**
5	22.2	22.5	5.81	22.6	5.86	23.2	5.99	22.9	5.91	21.8	5.66	22.1	5.77	**22.517**	**5.833**	**0.259**
6	24.5	22.6	5.86	22.7	5.91	23.4	6.03	23	5.95	21.9	5.71	22.2	5.81	**22.633**	**5.878**	**0.260**
7	26.9	22.8	5.9	22.9	5.95	23.5	6.08	23.1	6.01	22	5.75	22.3	5.86	**22.767**	**5.925**	**0.260**
8	29.7	22.9	5.94	23	6	23.6	6.12	23.2	6.06	22.1	5.79	22.5	5.91	**22.883**	**5.970**	**0.261**
9	32.7	23	5.99	23.2	6.04	23.8	6.18	23.4	6.1	22.3	5.85	22.6	5.95	**23.050**	**6.018**	**0.261**
10	35.9	23.1	6.03	23.3	6.09	23.9	6.22	23.5	6.13	22.4	5.88	22.7	6	**23.150**	**6.058**	**0.262**
11	39.6	23.2	6.07	23.4	6.13	24	6.25	23.6	6.17	22.5	5.91	22.8	6.03	**23.250**	**6.093**	**0.262**
12	43.6	23.3	6.1	23.5	6.16	24.1	6.29	23.7	6.2	22.6	5.95	23	6.1	**23.367**	**6.133**	**0.262**
13	48	23.4	6.14	23.7	6.2	24.3	6.33	23.8	6.25	22.8	6	23.1	6.13	**23.517**	**6.175**	**0.263**
14	52.8	23.6	6.19	23.8	6.23	24.4	6.38	24	6.31	22.9	6.04	23.2	6.17	**23.650**	**6.220**	**0.263**
15	58.1	23.7	6.23	23.8	6.27	24.6	6.44	24.1	6.35	23	6.09	23.3	6.21	**23.750**	**6.265**	**0.264**
16	64	23.7	6.25	23.9	6.3	24.7	6.47	24.2	6.38	23.1	6.13	23.5	6.27	**23.850**	**6.300**	**0.264**
17	70.5	23.8	6.29	24	6.32	24.8	6.49	24.2	6.42	23.2	6.16	23.6	6.31	**23.933**	**6.332**	**0.265**
18	77.6	23.9	6.32	24	6.36	24.9	6.52	24.3	6.45	23.3	6.2	23.7	6.34	**24.017**	**6.365**	**0.265**
19	85.5	23.9	6.34	24.1	6.39	25	6.56	24.4	6.48	23.4	6.22	23.7	6.35	**24.083**	**6.390**	**0.265**
20	94.1	24	6.37	24.1	6.41	25	6.58	24.5	6.5	23.4	6.23	23.8	6.38	**24.133**	**6.412**	**0.266**
21	104	24	6.37	24.2	6.44	25.1	6.61	24.5	6.51	23.5	6.26	23.8	6.38	**24.183**	**6.428**	**0.266**
22	114	24	6.39	24.2	6.46	25.1	6.63	24.6	6.55	23.5	6.28	23.8	6.4	**24.200**	**6.452**	**0.267**
23	126	24.1	6.43	24.2	6.49	25.2	6.67	24.6	6.58	23.6	6.3	23.9	6.43	**24.267**	**6.483**	**0.267**
24	140	24.1	6.44	24.3	6.51	25.3	6.7	24.7	6.6	23.7	6.34	23.9	6.44	**24.333**	**6.505**	**0.267**
25	150	24.1	6.47	24.4	6.54	25.3	6.71	24.8	6.64	23.7	6.37	24	6.47	**24.383**	**6.533**	**0.268**

[¥] Rheological analyses were conducted at day 8 after samples production.

Table D-4: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of experimental sample 2 [¥]

Meas. Pts.	Stress Amp. (μNm)	2-1-1		2-1-2		2-2-1		2-2-2		2-3-1		2-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	20.9	5.35	20.5	5.29	21.8	5.56	22.1	5.64	22.4	5.69	22.1	5.64	**21.633**	**5.528**	**0.256**
2	16.7	21	5.38	20.6	5.33	22	5.62	22.2	5.67	22.5	5.72	22.3	5.7	**21.767**	**5.570**	**0.256**
3	18.4	21.1	5.42	20.6	5.35	22.1	5.66	22.3	5.71	22.7	5.8	22.4	5.73	**21.867**	**5.612**	**0.257**
4	20.2	21.2	5.45	20.7	5.37	22.2	5.71	22.5	5.78	22.8	5.84	22.5	5.77	**21.983**	**5.653**	**0.257**
5	22.2	21.3	5.48	20.8	5.41	22.3	5.76	22.6	5.82	22.9	5.89	22.6	5.81	**22.083**	**5.695**	**0.258**
6	24.5	21.4	5.52	20.8	5.43	22.4	5.8	22.7	5.85	23	5.93	22.7	5.86	**22.167**	**5.732**	**0.259**
7	26.9	21.4	5.53	20.8	5.44	22.5	5.84	22.9	5.91	23.1	5.96	22.8	5.91	**22.250**	**5.765**	**0.259**
8	29.7	21.5	5.56	20.9	5.47	22.6	5.89	23	5.95	23.2	6.01	22.9	5.95	**22.350**	**5.805**	**0.260**
9	32.7	21.6	5.58	20.9	5.49	22.8	5.95	23.1	6	23.3	6.05	23	5.99	**22.450**	**5.843**	**0.260**
10	35.9	21.6	5.59	21	5.52	22.9	5.98	23.2	6.03	23.4	6.1	23.1	6.02	**22.533**	**5.873**	**0.261**
11	39.6	21.6	5.61	21	5.53	23	6.01	23.3	6.07	23.5	6.14	23.1	6.05	**22.583**	**5.902**	**0.261**
12	43.6	21.7	5.65	21	5.53	23.1	6.05	23.4	6.12	23.6	6.18	23.2	6.07	**22.667**	**5.933**	**0.262**
13	48	21.7	5.64	21	5.55	23.2	6.08	23.4	6.14	23.7	6.21	23.3	6.1	**22.717**	**5.953**	**0.262**
14	52.8	21.7	5.65	21	5.57	23.3	6.11	23.5	6.18	23.8	6.25	23.4	6.14	**22.783**	**5.983**	**0.263**
15	58.1	21.7	5.67	21.1	5.6	23.3	6.13	23.6	6.21	23.9	6.28	23.5	6.16	**22.850**	**6.008**	**0.263**
16	64	21.7	5.69	21.1	5.62	23.4	6.16	23.7	6.24	24	6.31	23.5	6.17	**22.900**	**6.032**	**0.263**
17	70.5	21.8	5.72	21.2	5.65	23.5	6.2	23.7	6.26	24.1	6.35	23.6	6.21	**22.983**	**6.065**	**0.264**
18	77.6	21.8	5.73	21.1	5.64	23.5	6.22	23.8	6.3	24.1	6.37	23.7	6.24	**23.000**	**6.083**	**0.264**
19	85.5	21.8	5.75	21.1	5.64	23.6	6.25	23.8	6.28	24.2	6.4	23.7	6.25	**23.033**	**6.095**	**0.265**
20	94.1	21.8	5.77	21.2	5.67	23.6	6.26	23.8	6.31	24.3	6.42	23.7	6.26	**23.067**	**6.115**	**0.265**
21	104	21.9	5.81	21.2	5.68	23.6	6.26	23.9	6.34	24.3	6.44	23.8	6.29	**23.117**	**6.137**	**0.265**
22	114	21.8	5.79	21.2	5.68	23.7	6.28	23.9	6.33	24.3	6.45	23.8	6.28	**23.117**	**6.135**	**0.265**
23	126	21.8	5.8	21.3	5.71	23.7	6.29	24	6.37	24.4	6.47	23.8	6.29	**23.167**	**6.155**	**0.266**
24	140	21.9	5.83	21.3	5.73	23.8	6.33	24	6.38	24.4	6.48	23.9	6.32	**23.217**	**6.178**	**0.266**
25	150	21.9	5.84	21.3	5.74	23.7	6.31	24	6.41	24.5	6.53	23.9	6.34	**23.217**	**6.195**	**0.267**

[¥] Rheological analyses were conducted at day 8 after samples production.

Table D-5: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of experimental sample 3 [¥]

Meas. Pts.	Stress Amp. (μNm)	3-1-1		3-1-2		3-2-1		3-2-2		3-3-1		3-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	22	5.61	21.7	5.53	22.9	5.77	22.4	5.67	21.4	5.48	21.8	5.58	**22.033**	**5.607**	**0.254**
2	16.7	22.2	5.66	21.8	5.57	23	5.82	22.6	5.73	21.5	5.51	21.9	5.63	**22.167**	**5.653**	**0.255**
3	18.4	22.3	5.7	21.9	5.6	23.2	5.87	22.8	5.79	21.6	5.56	22	5.66	**22.300**	**5.697**	**0.255**
4	20.2	22.4	5.74	22.1	5.65	23.3	5.91	22.8	5.8	21.7	5.59	22.1	5.7	**22.400**	**5.732**	**0.256**
5	22.2	22.5	5.79	22.2	5.69	23.4	5.96	22.9	5.84	21.7	5.61	22.2	5.73	**22.483**	**5.770**	**0.257**
6	24.5	22.7	5.85	22.4	5.76	23.6	6	23	5.89	21.8	5.64	22.3	5.77	**22.633**	**5.818**	**0.257**
7	26.9	22.8	5.89	22.5	5.79	23.7	6.03	23.1	5.92	21.9	5.68	22.3	5.8	**22.717**	**5.852**	**0.258**
8	29.7	23	5.94	22.6	5.84	23.8	6.07	23.2	5.96	22	5.72	22.4	5.83	**22.833**	**5.893**	**0.258**
9	32.7	23.1	5.98	22.8	5.89	23.9	6.12	23.3	5.99	22.1	5.75	22.5	5.87	**22.950**	**5.933**	**0.259**
10	35.9	23.2	6.02	22.9	5.93	24	6.16	23.5	6.05	22.1	5.76	22.6	5.91	**23.050**	**5.972**	**0.259**
11	39.6	23.3	6.05	23	5.97	24.1	6.19	23.6	6.08	22.2	5.8	22.6	5.93	**23.133**	**6.003**	**0.260**
12	43.6	23.5	6.11	23.1	6.01	24.2	6.22	23.7	6.12	22.3	5.84	22.7	5.95	**23.250**	**6.042**	**0.260**
13	48	23.6	6.14	23.3	6.07	24.3	6.26	23.8	6.16	22.4	5.87	22.8	5.99	**23.367**	**6.082**	**0.260**
14	52.8	23.7	6.18	23.4	6.11	24.5	6.31	23.9	6.19	22.4	5.89	22.8	6.01	**23.450**	**6.115**	**0.261**
15	58.1	23.8	6.21	23.5	6.14	24.4	6.31	24	6.23	22.5	5.91	22.9	6.04	**23.517**	**6.140**	**0.261**
16	64	23.9	6.25	23.6	6.18	24.5	6.35	24	6.26	22.5	5.93	22.9	6.05	**23.567**	**6.170**	**0.262**
17	70.5	24	6.28	23.7	6.21	24.6	6.38	24.1	6.31	22.5	5.94	23	6.09	**23.650**	**6.202**	**0.262**
18	77.6	24	6.29	23.8	6.24	24.7	6.45	24.2	6.34	22.6	5.98	23	6.1	**23.717**	**6.233**	**0.263**
19	85.5	24.1	6.33	23.8	6.26	24.8	6.48	24.2	6.36	22.6	5.98	23	6.11	**23.750**	**6.253**	**0.263**
20	94.1	24.1	6.36	23.9	6.29	24.9	6.5	24.3	6.39	22.7	6.01	23.1	6.14	**23.833**	**6.282**	**0.264**
21	104	24.2	6.38	24	6.33	24.9	6.53	24.3	6.41	22.6	5.99	23.1	6.15	**23.850**	**6.298**	**0.264**
22	114	24.3	6.41	24	6.35	25	6.57	24.4	6.44	22.6	6.01	23.1	6.16	**23.900**	**6.323**	**0.265**
23	126	24.2	6.41	24.1	6.38	25	6.6	24.5	6.47	22.7	6.04	23.1	6.18	**23.933**	**6.347**	**0.265**
24	140	24.3	6.43	24.1	6.39	25	6.62	24.5	6.51	22.7	6.06	23.2	6.2	**23.967**	**6.368**	**0.266**
25	150	24.4	6.46	24.2	6.41	25.1	6.65	24.6	6.57	22.7	6.09	23.1	6.23	**24.017**	**6.402**	**0.267**

[¥] Rheological analyses were conducted at day 8 after samples production.

Table D-6: Storage modulus (G'), loss modulus (G''), and loss tangent (G''/G') of experimental sample 4 [¥]

Meas. Pts.	Stress Amp. (μNm)	4-1-1		4-1-2		4-2-1		4-2-2		4-3-1		4-3-2		**Total mean value**		
		G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)	**G' (Pa)**	**G'' (Pa)**	**G''/G'**
1	14.6	17.9	4.79	17.4	4.67	19.8	5.13	19.7	5.12	19	4.98	19.3	5.02	**18.850**	**4.952**	**0.263**
2	16.7	17.9	4.8	17.4	4.68	19.9	5.17	19.8	5.15	19.1	5	19.4	5.06	**18.917**	**4.977**	**0.263**
3	18.4	17.8	4.82	17.4	4.67	20	5.2	19.8	5.17	19.1	5.02	19.5	5.09	**18.933**	**4.995**	**0.264**
4	20.2	17.8	4.82	17.4	4.7	20	5.23	19.9	5.21	19.2	5.05	19.6	5.13	**18.983**	**5.023**	**0.265**
5	22.2	17.8	4.84	17.4	4.71	20.1	5.25	20	5.24	19.3	5.08	19.6	5.15	**19.033**	**5.045**	**0.265**
6	24.5	17.8	4.86	17.3	4.71	20.2	5.29	20	5.26	19.3	5.1	19.6	5.16	**19.033**	**5.063**	**0.266**
7	26.9	17.8	4.88	17.3	4.72	20.2	5.31	20.1	5.3	19.3	5.11	19.6	5.18	**19.050**	**5.083**	**0.267**
8	29.7	17.7	4.88	17.3	4.75	20.2	5.32	20.1	5.31	19.3	5.13	19.7	5.2	**19.050**	**5.098**	**0.268**
9	32.7	17.7	4.92	17.2	4.75	20.2	5.34	20.1	5.31	19.4	5.17	19.7	5.22	**19.050**	**5.118**	**0.269**
10	35.9	17.7	4.93	17.2	4.8	20.3	5.37	20.1	5.32	19.4	5.18	19.7	5.23	**19.067**	**5.138**	**0.269**
11	39.6	17.6	4.95	17.1	4.79	20.3	5.37	20.1	5.34	19.4	5.19	19.7	5.25	**19.033**	**5.148**	**0.270**
12	43.6	17.6	4.98	17.1	4.81	20.3	5.39	20.2	5.37	19.4	5.2	19.7	5.25	**19.050**	**5.167**	**0.271**
13	48	17.5	4.98	17	4.81	20.4	5.43	20.2	5.37	19.4	5.21	19.7	5.26	**19.033**	**5.177**	**0.272**
14	52.8	17.4	5.01	16.9	4.84	20.4	5.44	20.2	5.38	19.4	5.23	19.7	5.27	**19.000**	**5.195**	**0.273**
15	58.1	17.3	5.01	16.9	4.89	20.4	5.44	20.2	5.39	19.5	5.26	19.7	5.29	**19.000**	**5.213**	**0.274**
16	64	17.2	5.02	16.7	4.87	20.4	5.45	20.2	5.41	19.5	5.28	19.7	5.33	**18.950**	**5.227**	**0.276**
17	70.5	17.1	5.07	16.6	4.9	20.4	5.47	20.2	5.43	19.5	5.27	19.7	5.31	**18.917**	**5.242**	**0.277**
18	77.6	17	5.09	16.5	4.93	20.4	5.48	20.3	5.45	19.4	5.25	19.7	5.33	**18.883**	**5.255**	**0.278**
19	85.5	16.8	5.12	16.4	4.98	20.4	5.5	20.3	5.47	19.4	5.27	19.7	5.35	**18.833**	**5.282**	**0.280**
20	94.1	16.7	5.14	16.2	5	20.5	5.53	20.3	5.48	19.4	5.28	19.6	5.35	**18.783**	**5.297**	**0.282**
21	104	16.5	5.17	16	5.01	20.4	5.51	20.3	5.5	19.3	5.27	19.6	5.37	**18.683**	**5.305**	**0.284**
22	114	16.3	5.2	15.8	5.06	20.4	5.53	20.2	5.5	19.3	5.28	19.6	5.38	**18.600**	**5.325**	**0.286**
23	126	16	5.18	15.5	5.08	20.5	5.55	20.2	5.49	19.3	5.3	19.6	5.4	**18.517**	**5.333**	**0.288**
24	140	15.7	5.24	15.2	5.17	20.5	5.57	20.3	5.53	19.4	5.33	19.6	5.4	**18.450**	**5.373**	**0.291**
25	150	15.4	5.3	14.8	5.27	20.5	5.6	20.3	5.54	19.4	5.35	19.6	5.43	**18.333**	**5.415**	**0.295**

[¥] Rheological analyses were conducted at day 8 after samples production.