International Food Research Journal 27(6): 1076 - 1086 (December 2020)

Journal homepage: *http://www.ifrj.upm.edu.my*

Optimisation of yogurt mousse dairy protein levels: a rheological, sensory, and microstructural study

[1]*Gomez-Betancur, A. M., [1]Carmona-Tamayo, R., [2]Jaimes-Jaimes, J., [2]Casanova-Yepes, H. and [1,2]Torres-Oquendo, J. D.

[1]Grupo de Investigación en Análisis Sensorial, Facultad de Ciencias Farmacéuticas y Alimentarias, Universidad de Antioquia, Cl. 67 # 53 - 108, Medellín, Antioquia, Colombia
[2]Grupo de Coloides, Facultad de Ciencias Exactas y Naturales, Universidad de Antioquia, Cl. 67 # 53 - 108, Laboratorio 2-328, Medellín, Antioquia, Colombia

Article history

Received: 6 December 2019
Received in revised form: 12 July 2020
Accepted: 18 August 2020

Keywords

yogurt mousse,
response surface methodology,
rheology,
sensory analysis,
microstructural analysis

Abstract

The present work aimed to optimise the skimmed milk powder (SMP) and whey protein concentrate (WPC) levels in order to obtain the best yogurt mousse in terms of rheological, sensory, and microstructural properties by using the response surface methodology. The methods consisted of rheological tests (large and small deformations), sensory profiles, particle size distributions, and differential interference contrast microscopy. The factors were SMP (5.3 - 10.7%) and WPC (2 - 4%), and the responses of the central composite design were the rheological parameters. A non-fermented mousse was employed as a standard. The results indicated that both SMP and WPC had positive and significant influence over the storage, loss, elastic moduli, and over the creep viscosity of yogurt mousses. A formulation containing 10.7% SMP and 2% WPC presented similar rheological and sensory characteristics to the standard mousse. There were microstructural differences between the optimal and standard samples, thus suggesting that the fermentation process influences the microstructure and texture of the product. We suggest more studies related to the variation of other nutrients (*i.e.,* fat and sugar) of this product in order to fully understand its rheological, sensory, and microstructural behaviour.

© All Rights Reserved

Introduction

Aerated desserts such as ice cream and mousses are attractive to consumers due to their foamy texture and pleasant feel (Menéndez *et al.,* 2006; Duquenne *et al.,* 2016). Consumers also consider them as healthier or "light" products (Patel *et al.,* 2008). These perceptions favour aerated desserts as vehicles for the inclusion of health-promoting agents like prebiotics and probiotics (Komatsu *et al.,* 2013; Xavier-Santos *et al.,* 2019). Overall, these products have a similar composition and structure, which is stable foam structure (Miquelim *et al.,* 2010). Regarding mousses, they were conventionally produced in pastry shops; but nowadays, they are being produced at an industrial level. They have earned an essential place in the dessert market (Patel *et al.,* 2008; Buriti *et al.,* 2010). However, industrial production of these products requires significant knowledge about the formation and stabilisation of foams through the usage of emulsifiers, stabilisers, and foaming agents (Buriti *et al.,* 2010).

Some of the most commonly used foaming agents in foods are proteins (Miquelim *et al.,* 2010). The use of milk proteins, especially those from whey, are very popular due to their foaming forming and stabilising properties (Martínez-Padilla *et al.,* 2015). But not only milk proteins are known for their foaming effects. Some authors have mentioned that dairy proteins contribute to the microstructure of food products in the continuous phase, especially in the increase of viscosity (Gallardo-Escamilla *et al.,* 2007). In this case, a gel forms due to the precipitation of dairy proteins (caseins) as a consequence of the pH decrease. This three-dimensional web retains water and avoids syneresis of the product (Parra-Huertas *et al.,* 2015; Marulanda *et al.,* 2016). Besides, other authors (Sandoval-Castilla *et al.,* 2004) state that whey proteins could improve the gel strength in skimmed yogurts.

There are several studies involving the nutritional, instrumental textural, and sensorial characteristics of non-fermented mousses such as chocolate mousses (Aragon-Alegro *et al.,* 2007; Cardarelli *et al.,* 2008), synbiotic mousses (Xavier-Santos *et al.,* 2019), and frozen mousses (Buriti *et al.,* 2010; Komatsu e*t al.,* 2013; Duquenne *et al.,* 2016). Aragon-Alegro *et al.* (2007) studied the addition *of Lactobacillus paracasei*

---

*Corresponding author.
Email: ana.gomez10@udea.edu.co

and inulin in chocolate mousse, obtaining a product with acceptable sensory characteristics. Later, Cardarelli *et al.* (2008) found that the addition of *L. paracasei* increased the firmness and hardness of the chocolate mousse, and these instrumental variables were even higher with the addition of *L. paracasei* and inulin. The sensory judges also perceived that the probiotic and synbiotic mousses were firmer as compared to the standard chocolate mousse. Xavier-Santos *et al.* (2019) obtained similar results by using *L. acidophilus*, inulin, and fructooligosaccharides in synbiotic mousses. On the other hand, Buriti *et al.* (2010) and Komatsu *et al.* (2013) used inulin and whey protein concentrate (WPC) in order to reduce the fat content of guava mousses (both refrigerated and frozen). In both studies, the products with 1.5% fat, 1.3% inulin, and 1.3% WPC had better storage stability. They also had similar texture and sensory attributes as compared to a standard (4.6% fat). Duquenne *et al.* (2016) used gelatine peptides as stabilisers for frozen mousses, obtaining mousses that did not shrink after the thawing process as compared to regular gelatine control.

Although these studies focussed on the sensory and texture characterisation of mousses, the study of the rheology of these complex systems is essential because it helps to understand the flow behaviour, structure, and microstructure of these products (Menéndez *et al.,* 2006). Moreover, rheological parameters and sensory attributes are often correlated (Soukoulis *et al.,* 2010). Menéndez *et al.* (2006) studied the effect of ovalbumin addition over the rheological and organoleptic characteristics of yogurt mousses. They stated that yogurt mousses have a viscoelastic matrix that combines structures of foams, gels, and emulsions. They also affirm that the ovalbumin addition is acceptable at a 1.3% incorporation. Attalla and El-Hussieny (2017) studied the substitution of gelatine for chia seeds in yogurt mousse, obtaining a similar yogurt mousse in terms of viscosity, water holding capacity, sensory quality, microstructure, and improved nutritional content (3% chia addition).

Considering that there are very few studies related to yogurt mousse (Menéndez *et al.,* 2006; Attalla and El-Hussieny, 2017), and dairy proteins have great potential as foam and texture modifiers, the present work aimed to use the response surface methodology in order to optimise dairy protein levels of yogurt mousse. We also studied the influence of these variations over the product's rheological, sensory, and microstructural characteristics.

Materials and methods

Materials

The ingredients used for the standard mousse and yogurt mousse samples were water, commercial skimmed milk powder (SMP), 80% whey protein concentrate (WPC) (Tecnas S.A., Itagüí, Antioquia, Colombia), commercial 35% UHT cream, commercial sugar, emulsifying and stabilising agents (Tecnas S.A., Itagüí, Antioquia, Colombia), tween 80 (Protokimica Ltda., Medellín, Antioquia, Colombia), commercial 250° bloom gelatine, and commercial yogurt (as starter culture). The proportions of the ingredients were as follows: 20% UHT cream, 9% commercial sugar, 0.13% emulsifying and stabilising agents, 0.13% tween 80, 0.5% commercial 250° gelatine, and 1.5% commercial yogurt. The SMP and WPC levels varied according to the experimental design.

Samples preparation

We used the modified procedures described by Chandan and O'Rell (2006) and Menéndez *et al.* (2006) to prepare all samples.

Standard mousse (non-fermented) preparation

For the standard mousse, all the ingredients (except for gelatine, 1/10 of water, and 1/5 of sugar from the formulations) were mixed with an Oster® blender (Sunbeam Products Inc., Florida, USA) at a medium speed for 2 min. The standard mixture was pasteurised in an open jar for 15 min at 80°C, until it reached 46.6% of the total solids (Precisa XM-60 Moisture Balance, Dietikon, Switzerland). Then, it was placed in an ice-water bath to reduce the temperature to approximately 25°C. Later, the mousse mixture was refrigerated for 24 h at 10 ± 1°C. The air incorporation process was performed by adding the gelatine and remaining sugar (previously diluted in the 1/10 of water from the formulation) into the mousse mixture while whipping it with a Premium® PHM425 hand mixer (Precision Trading Corp., Florida, USA) at medium-high speed for 3 min. The mousse sample was immediately refrigerated for 24 h at 10 ± 1°C, prior to rheological, structural, or sensorial analyses.

Yogurt mousse samples preparation

We used the previously described procedure for the yogurt mousse sample preparation. The pasteurisation was performed in closed lid jars (no evaporation process) and in water-bath (80°C for 15 min). Following pasteurisation, the yogurt mousse samples were inoculated at 42 ± 2°C with commercial yogurt as a starter culture, and then they were incubated for 6 - 7 h until reaching a 4.7 ± 0.2 pH. We employed the same air incorporation process described in the *Standard mousse preparation.*

Rheological measurements

All the rheological analyses were performed in a Bohlin Gemini HR Nano rheometer (Malvern Ltd., UK), equipped with Bohlin P6.51.0.3 software (Malvern Ltd., UK), using a cone-plate geometry (40 mm diameter, 4° cone angle, and 0.150 µm gap), and the test temperature was set at 10 ± 1°C with a Peltier system in order to assimilate the commercial temperature of refrigerated products (Morell *et al.,* 2015).

Large deformations measurements (flow behaviour)

A flow behaviour analysis was performed in three phases: an ascending curve (0.01 - 100 s^{-1} for 1 min), holding time (100 s^{-1} for 1 min), and a descending curve (100 - 0.01 s^{-1} for 1 min) (Sah *et al.,* 2016). The data from the descending curve fitted the Herschel-Bulkley model, according to Sah *et al* (2016) and Morell *et al.* (2015):

$$\sigma = \sigma_0 + K \times \gamma^n \qquad \text{(Eq.1)}$$

where, σ = shear stress (Pa), σ_0 = yield stress (Pa), K = consistency index (Pa*s^n), γ = shear rate (s^{-1}), and n = flow behaviour index.

The thixotropy was evaluated in line with Soukoulis *et al.* (2010) who established Eq. 2 in order to calculate the thixotropic behaviour through the ascending and descending curve of the apparent viscosity (η):

$$\%\ thixotropy = 100 \times \frac{\eta - \eta'}{\eta} \qquad \text{(Eq.2)}$$

where, η and η' = the ascending and descending viscosity, at 50 s^{-1}, respectively.

Small deformations tests (static and dynamic measurements)

The linear viscosity region was previously determined with the weakest structured yogurt mousse through a strain spectrum (0.001 - 0.1%) at 1 Hz of constant frequency (Torres *et al.,* 2010). From this result, frequency sweeps were performed to all samples at a frequency range between 0.1 - 10 Hz, and the constant strain was 0.01%. The G', G'' moduli, and the phase displacement angle (δ = arctan (G''/G')) were obtained from this curve at 10 Hz (Lobato-Calleros *et al.,* 2014; Morell *et al.,* 2015). The creep compliance and the recovery tests were performed during 120 s each one, at a constant σ of 2 Pa. The elastic modulus (E_0), the percentage of recovery, and the liquid viscosity of the linear creep range (η_v) were obtained from the different curves (Lobato-Calleros *et al.,* 2004).

Experimental design and statistical analysis
Experimental design

We employed a response surface methodology (RSM) using a routable central composite design (CCD). The data was analysed with the Statgraphics Centurion XVI 18 (Statistical Graphics Corp., Herndon, VA, USA) software, and 13 runs were obtained from the design, including five replicates of the central point (Table 1). SMP (5.2 - 10.7%) and WPC (2 - 4%) were the independent variables, and rheological parameters (K, n, σ_0, TX, G', G'', δ, η_v, and E_0) were the dependent or response variables. The randomisation helped to reduce the errors of the experiment.

Dairy protein levels optimisation

For the optimisation process, we considered the rheological parameters showing positive and significant interactions ($p < 0.05$), non-significant lack-of-fit, and determination coefficients (R^2) above 90% (Bitaraf *et al.,* 2012). An optimisation was performed in order to meet the standard mousse rheological values (Table 1). Eq. 3 shows the quadratic polynomial model that was fitted to each response.

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \qquad \text{(Eq.3)}$$

where, Y = response, β_0 = constant, β_i = linear coefficient, β_{ii} = quadratic coefficient, and β_{ij} = interaction coefficient, and X_i and X_j = independent variables.

After the optimisation process, a sensory and microstructural characterisation (particle size distributions and differential interference contrast - DIC microscopy) were performed to four samples of interest: optimal sample, standard mousse (non-fermented), high-protein sample (YM6: 10.7% SMP and 4% WPC), and low-protein sample (YM12: 5.35% SMP and 2% WPC). The previous was done to understand the effect of protein level modification in yogurt mousse sensory and physical properties.

Sensory analysis

Sensory profiles by a multidimensional approach, described in ISO 11035:1994, were performed to the aforementioned samples of interest. The yogurt mousses and standard mousse were randomly codified with three digits, and they were evaluated by seven trained and experienced panellists from the Sensory Analysis Laboratory of Universidad de Antioquia, Colombia. The samples were served on 1.5 oz plastic containers at 10°C. The

Table 1. Two-factor central composite design used for the response surface methodology, with dependent variables responses and ANOVA table, for the dependent variables considered for the optimisation process.

Run	**Independent variables**		**Dependent variables responses**			
	SMP (%)	**WPC (%)**	***G'* (Pa)**	**E_0 (Pa)**	**η_v (Pa*s)**	**K (Pa*s^n)**
YM1	5.3	4.0	610.80	387.30	100000.00	1.81
YM2	10.7	2.0	788.90	479.62	100000.00	2.21
YM3	4.2	3.0	293.00	151.45	25000.00	1.13
YM4	8.0	4.4	1069.00	723.59	200000.00	2.68
YM5	8.0	3.0	1123.00	321.34	50000.00	2.15
YM6	10.7	4.0	1688.00	1066.21	250000.00	6.03
YM7	11.8	3.0	1816.00	1125.75	250000.00	4.90
YM8	8.0	1.6	391.00	216.50	20000.00	1.49
YM9	8.0	3.0	459.50	256.21	50000.00	1.42
YM10	8.0	3.0	723.30	440.53	100000.00	2.01
YM11	8.0	3.0	560.50	268.20	50000.00	1.62
YM12	5.3	2.0	267.50	121.51	33333.30	1.24
YM13	8.0	3.0	300.60	346.50	50000.00	1.36
M*	12.4	4.6	777.20	461.04	50000.00	5.01

Source of variation	***p-value***			
A: SMP	0.0003	0.0004	0.0011	0.0001
B: WPC	0.0020	0.0018	0.0018	0.0009
AB	0.0617	0.1001	0.1359	0.0025
Lack of fit	0.3313	0.4213	0.4824	0.0558
R^2	96.58	96.65	95.72	95.13

*standard dairy mousse (non-fermented), with SMP and WPC levels after 21% water evaporation (SMP and WPC values before evaporation process: 10.7% SMP and 4.0% WPC).

sensory attributes scoring was from 0 to 5, with 0 being the absence of the attribute, 1 being very-low intensity, 2 being low intensity, 3 being medium intensity, 4 being high intensity, and 5 being very-high intensity of the attribute. The attributes were selected from previous studies, as reported by some authors (Menéndez *et al.,* 2006; Meyer *et al.,* 2011) and also from the sensory evaluation itself. The appearance attributes included aerated (aerated A.) and fatty (fatty A.). The flavour and odour attributes included dairy (dairy F. and dairy O.), dairy ferment (dairy ferment F. and dairy ferment O.), fatty (fatty F. and fatty O.), and sweet (sweet F.). The texture attributes included aerated (aerated T.), fatty (fatty T.), creaminess, softness, and mouthfeel.

Microstructural analysis

Differential Interference Contrast (DIC) microscopy

The Differential Interference Contrast (DIC) microscopy analysis was performed to the samples of interest. The micrographs of four production process stages (homogenised mixtures, pasteurised mixtures, yogurts, and aerated yogurts) were taken at 300×, with an inverted optical microscope (Eclipse Ti-E, Nikon, Japan). The undiluted samples were spread onto microscopy glass and immediately they were observed at room temperature (25°C). The images were obtained and stored using an image analysis software (NIS-Elements, Nikon, Japan).

Particle size distribution

A particle size distribution analysis was performed to the samples of interest using a Laser Diffraction-Particle Size Analyzer (MasterSizer 2000, Malvern Ltd., UK). A milk fat refractive index of 1.46 was used as a reference. We analysed the samples in the production steps of "mixture" and "pasteurised mixture" (see the *Samples preparation* section). We did not measure the samples at the yogurt and yogurt mousse steps due to the measurement range of the equipment (< 1000 µm). The samples were placed in a stirring plate for 2 min at 200 rpm for a well-particle-size distribution. About three drops of the sample were added to the Hydro-cell until the obscuration was in the range of 0.0 - 0.3. The data plots were obtained from the equipped software (MasterSizer 2000 software, Malvern Ltd., UK).

Results and discussion

Response surface optimisation analysis

Based on the analysis of variance ANOVA, the modulus (G'), elastic modulus (E_0), creep viscosity (η_v), and consistency index (K) responses showed statistical significance with $p < 0.05$, as well as a goodness of fit, and R^2 above 90% (Table 1). The other rheological responses failed to meet these statistical criteria (Bitaraf *et al.,* 2012). Eqs. 4 to 7 show the quadratic polynomial models for G', E_0, η_v, and K:

$$G' = 2387.03 - 513.269*SMP - 594.058*WPC + 33.1954*SMP2 + 51.9439*SMP*WPC + 75.3821*WPC2$$

(Eq. 4)

$$E_0 = 1480.56 - 312.587*SMP - 436.173*WPC + 20.9027*SMP2 + 29.9813*SMP*WPC + 65.2937*WPC2$$

(Eq. 5)

$$\eta_v = 430842 - 83651.5*SMP - 149222*WPC + 5313.44*SMP2 + 7788.16*SMP*WPC + 24270.8*WPC2$$

(Eq. 6)

$$K = 12.3088 - 2.13676*SMP - 3.48614*WPC + 0.107116*SMP2 + 0.303121*SMP*WPC + 0.302128*WPC2$$

(Eq. 7)

Figures 1A, 1B, and 1C display the surface responses for K, G', and E_0 (η_v surface response not shown). It is apparent that as SMP and WPC concentrations increased, the K, G', and E_0 values also increased. Similar observation occurred to η_v. The latter indicates that both of the dairy proteins content improved the structure (G', E_0), firmness (K) and viscosity (η_v) of the yogurt mousse, as previously mentioned (Sandoval-Castilla *et al.,* 2004; Yu *et al.,* 2016). Based on ANOVA (Table 1), SMP had more influence over the rheological responses than WPC. However, both factors were very significant, and this could be due to the precipitation of caseins from SMP during the fermentation, which influenced the coagulum formation (Parra-Huertas *et al.,* 2015; Marulanda *et al.,* 2016). Whey proteins from WPC also influenced the viscosity and foam formation of the yogurt mousse (Gallardo-Escamilla *et al.,* 2007; Martínez-Padilla *et al.,* 2015).

Based the optimization, the YM2 (10.7% SPM, 2.0% WPC) sample was the most approximated to the standard M (12.4% SMP, 4.6% WPC) in terms of G', E_0, ηv, and K, with a 0.67 of maximum desirability. Therefore, this experimental design allowed us to obtain a formulation rheologically similar to the standard.

Rheological analysis

Large deformations (flow behaviour)

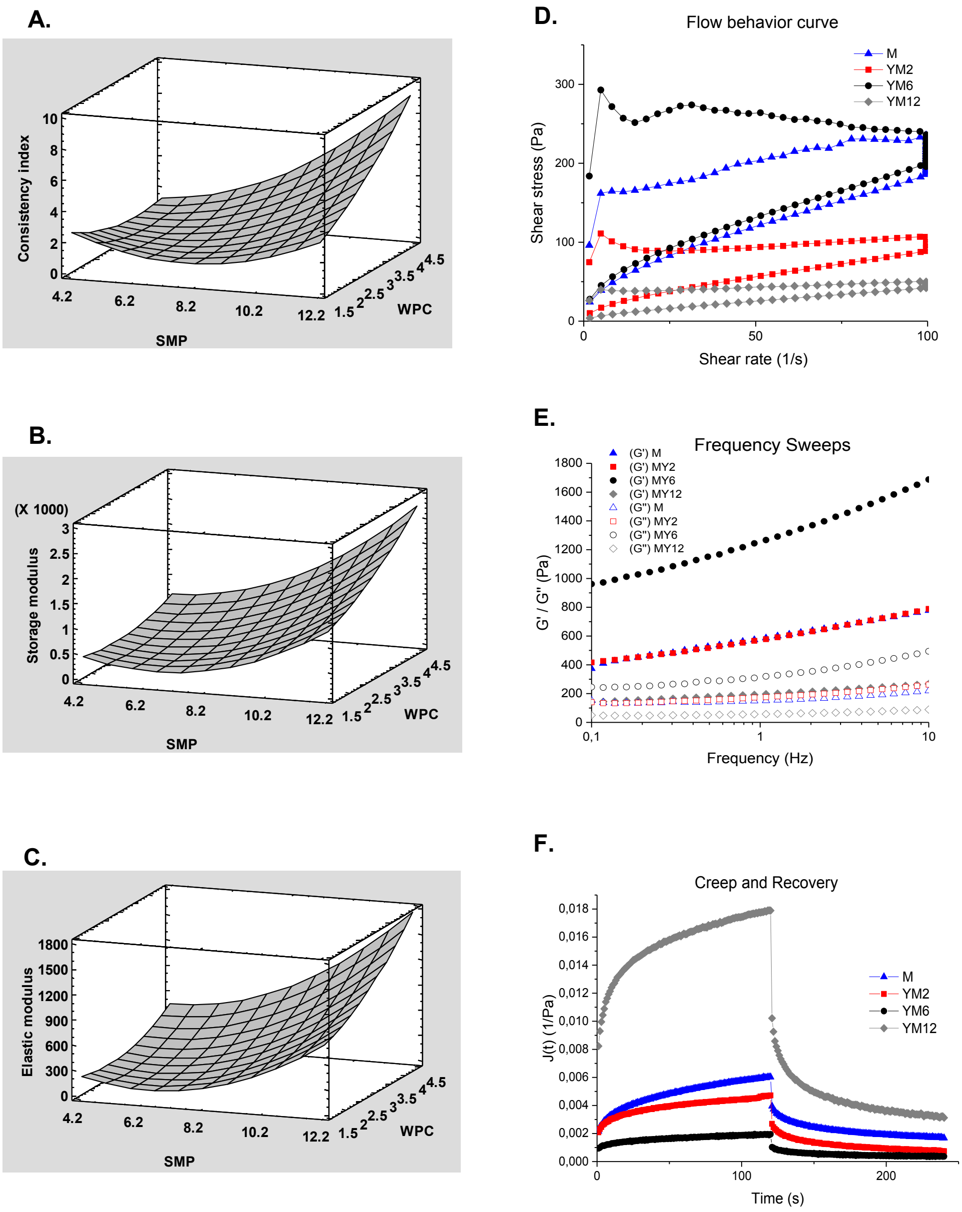
The descending curve from the flow behaviour curve (Figure 1D) shows that all samples presented pseudoplastic (shear thinning) behaviour since the apparent viscosity decreased with the shear rate. Besides, the flow behaviour index values obtained from the Herschel-Bulkley model adjustment were below ($n < 1$) (YM2: 0.78, YM6: 0.73, YM12: 0.84, and M: 0.75) (Yu *et al.,* 2016). The yogurt mousse samples also showed a thixotropic behaviour (Figure 1D) with the formation of a hysteresis area between the ascent and descent curves, indicating that all treatments presented time dependence (Steefe, 1996). Besides, the samples adjusted to the Herschel-Bulkley model had an R^2 above 99%.

The YM6 sample presented higher pseudoplastic behaviour, indicating an increased thixotropy (59.63%). On the contrary, the YM2 sample showed a drop (39.28%), having values that resembled M (40.26%) and YM12 (40.66%) (Figure 1D). For these samples, this behaviour could be related to the WPC content, since YM6 doubled YM2 and YM12 in WPC (Table 1). As mentioned by Torres *et al.* (2018), the application of WPC can have a more significant effect on the firmness of the yogurts, mainly for the reduced-fat ones. Moreover, Lobato-Calleros *et al.* (2004) established that the addition of WPC could improve softness, overall texture, and stability of yogurt.

YM6 showed the highest values of K (6.03 Pa*sn), σ_0 (27.81 Pa), thixotropy (49.63%), and the lowest n (0.73), thus demonstrating the effect of fermentation in the reinforcement of gel. On the contrary, M had more dairy solid content, but it did not go through a fermentation process (K: 5.01 Pa*sn, σ_0: 24.17 Pa, *%Tx*: 40.26%, and n: 0.75). This might be due to the complex interactions between the κ-casein and serum proteins that are favoured by the heat treatment and acidic medium (Andoyo *et al.,* 2014; Gösta, 2019a). Other authors have found correlations between the firmness of the gel and the yield stress, and it might be due to the lactic acid bacteria metabolism and the exopolysaccharide production, which improves water retention (Morell *et al.,* 2015).

Small deformations

Frequency sweeps



[1]Figure 1. Response surfaces for (A) consistency index (*K*); (B) storage modulus (*G'*); and (C) elastic modulus (E_0) with SMP levels (5.3 - 10.7%) as *x-axis*, WPC levels (2.0 - 4.0%) as *y-axis*, and *K, G',* or E_0 as the *z-axis*. Curves for M (standard sample), YM2 (optimal sample), YM6 (high-protein sample), and YM12 (low-protein sample): (D) flow behaviour; (E) frequency sweeps [filled markers as storage modulus (*G'*) and empty ones as loss modulus (*G''*)]; and (F) creep compliance and recovery.

Figure 1E shows that all samples of mousse presented a gel structure since the *G'* curve was always above the *G''* curve (Steefe, 1996; Yu *et al.*, 2016). Menéndez et al. (2006) studied the viscoelastic behaviour of yogurt mousse with ovalbumin addition, and they identified a gel-like behaviour, confirming the present findings. The results indicate that the YM2 (*G'*: 788.90 Pa and *G''*: 262.20 Pa) frequency sweeps resembled the standard M (*G'*: 777.20 Pa and *G''*: 219.70 Pa).

Even though δ did not adjust to the model for optimisation, yogurt mousse gels were strong with values below 0.35 rad (YM2: 0.32 rad, YM6: 0.28 rad, YM12: 0.32 rad, and M: 0.28 rad) (Steefe, 1996), being YM6 and M the strongest ones. While M had a higher concentration on dairy proteins (Table 1), yogurt mousse proteins functionalised through fermentation also generated strong gels, similar to the evidence provided by Morell *et al.* (2015), where the increase in viscosity due to fermentation protein interactions intensified the gel strength.

Creep compliance and recovery test

Figure 1F shows that mousses had a viscoelastic behaviour, which means a partial recovery when the applied stress is removed (Menéndez *et al.,* 2006). However, based on the percentage of recovery, all samples had considerable recovery values (YM2: 84.26%, YM6: 81.03%, YM12: 82.53%, and M: 72.09%), thus demonstrating that the elastic proportion was higher in all cases.

The η_v and E_0 variables explained the structure of the mousses with R^2 values of 95.72 and 96.65%, respectively. E_0 was directly related to the elastic ratio, indicating similar values for M (461.04 Pa) and YM2 (479.62 Pa). This confirms the tendency observed in the frequency sweeps, where G' was also similar in these two formulations (Menéndez *et al.,* 2006). On the other hand, the viscose proportion (η_v) seems to be improved by fermentation and not by solid concentration (YM2: 100,000.00 Pa*s, YM6: 250,000.00 Pa*s, YM12: 33,333.33 Pa*s, and M: 50,000.00 Pa*s). These results follow the evidence obtained from the dynamic tests.

Sensory profiles by a multidimensional approach

Figure 2 shows the sensory profiles by a multidimensional approach obtained for YM2, YM6, YM12, and M. The qualification of the fermentation-related attributes of taste and odour varied since M was a non-fermented sample. This is consistent with the results obtained by Aragon-Alegro *et al.* (2007) and Cardarelli *et al.* (2008), where chocolate mousses with the addition of probiotic cultures generated significant changes in the sensory perception. Besides, the changes in the aromas and flavours of the yogurt samples are a result of the fermentation and the variations in solid content (Soukoulis *et al.,*2007). In terms of texture, YM6 obtained higher aerated texture and creaminess scores. As the creaminess is directly related to the viscosity in dairy products (Kokini, 1987), we inferred that the consistency index (K) could be correlated with this sensory attribute since K is the "global" viscosity in non-Newtonian fluids (Steefe, 1996).

It is also important to highlight that WPC could be related to the airy and light sensations in yogurt mousse due to its ability to form a foam (Martínez-Padilla *et al.,* 2015). Osorio *et al.* (2014) found that the foaming ability is associated with the partial deployment of the protein during heat treatment (pasteurisation), which favours the flexibility of the chain. The texture attributes were similarly rated for YM2 and the standard M, thus indicating that the surface response optimisation provided a formulation with comparable rheological and sensory characteristics. This confirms that the adjustment model was successful. It is worth noting that the evaluation had an emphasis on the texture attributes of the product.

Differential interference contrast microscopy and particle size distribution

Homogenised mixtures

The particle size distribution measurements showed that homogenised mixtures (prior to pasteurisation process) in Figure 3A present a trimodal distribution explained it follows:

i. Zone I contains caseins from the SMP (0.01 - 0.1 µm) (Gösta, 2019b) that are well-distributed in the food matrix. It is important to have in mind that M and YM6 had the same SMP and WPC

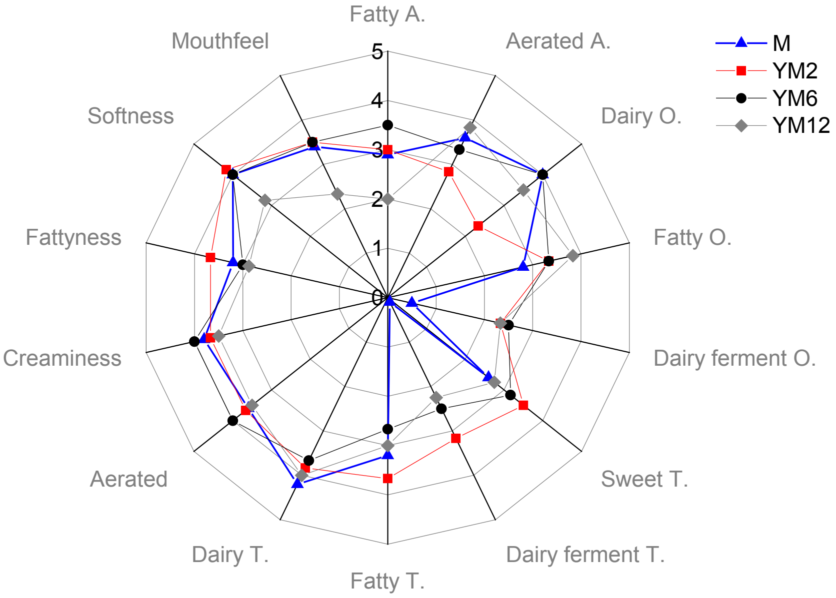


Figure 2. Sensory profiles by a multidimensional approach for M (standard sample), YM2 (optimal sample), YM6 (high-protein sample), and YM12 (low-protein sample).

concentrations before the pasteurisation process (Table 1). Therefore, we expected to obtain a similar particle size volume percentage in this zone for both of these samples (Abdalla *et al.,* 2017). The volume percentage decreased with the protein levels reduction (YM2 with 10.7% SMP and 2% WPC, YM12 with 5.3% SMP and 2% WPC).

ii. Zone II corresponds to greater size particles, which are fat globes (1 - 10 µm) (Torres *et al.,* 2010; Gösta, 2019b) coming from the cream. YM2, YM6, and YM12 showed similar particle size distribution volume percentage, which is congruent because all samples have the same fat composition (See *Sample preparation* section). However, since M and YM6 (with similar composition before the evaporation process) (Table 1) showed a different particle size distribution volume percentage in this zone, it can be suggested that the performed homogenisation process did not homogenise fat globes as was expected in M sample.

Other components like lactose, sugar, and salts that were present in the solution were not detected by the equipment (Gösta, 2019b), as it was observed in the micrographs (Figure 4). In summary,homogenised mixtures showed similar particle size distributions, and looked alike in this step of the process.

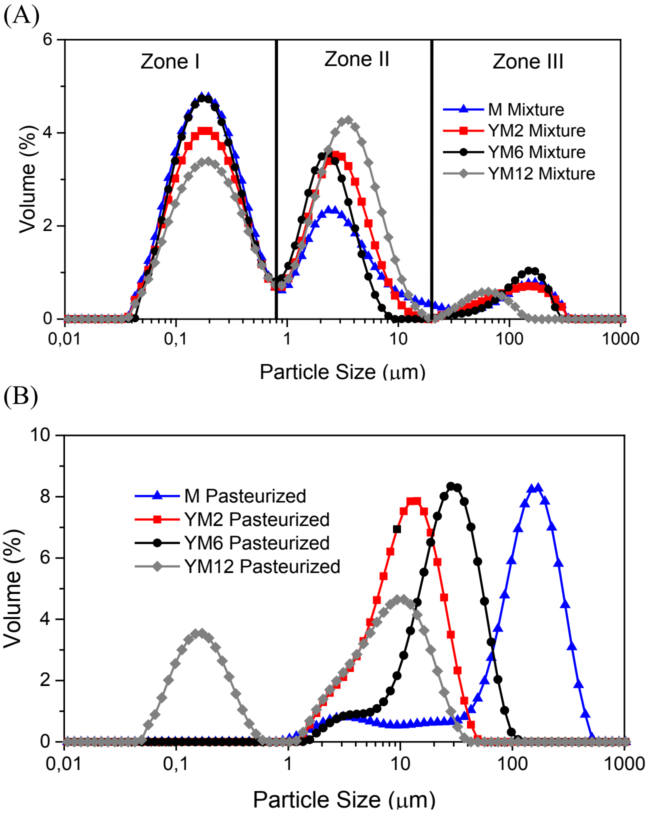


Figure 3. Particle size distribution plots for M (standard sample), YM2 (optimal sample), YM6 (high-protein sample), and YM12 (low-protein sample): (A) mixtures and (B) pasteurised mixtures

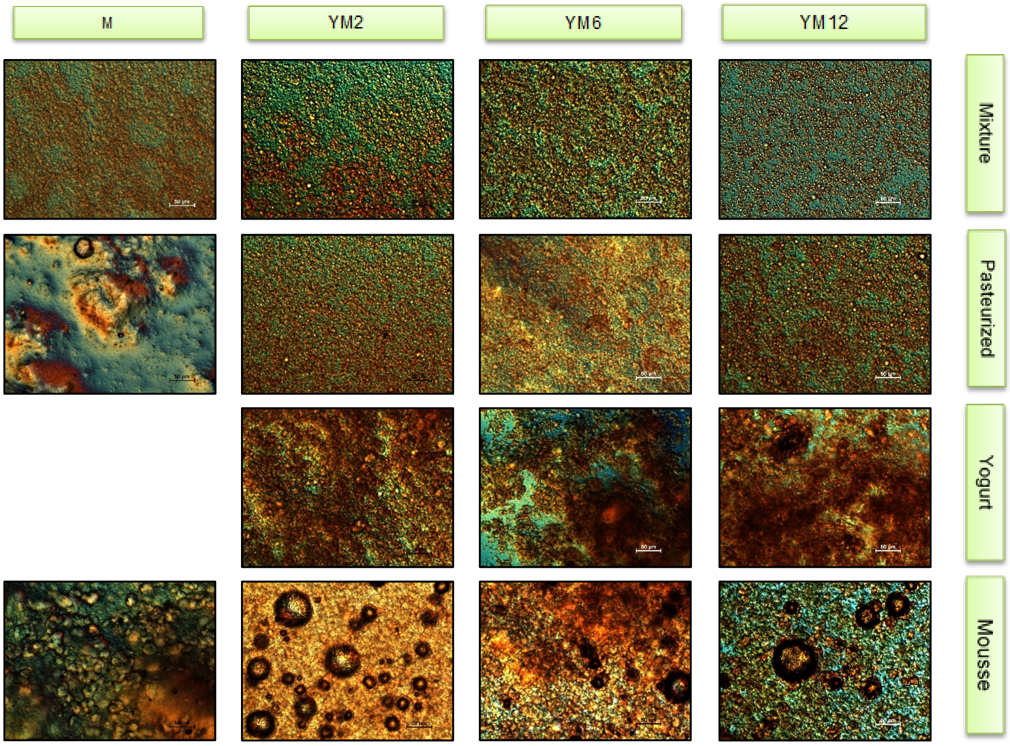


Figure 4. DIC micrographs (300×) for M (standard sample), YM2 (optimal sample), YM6 (high-protein sample), and YM12 (low-protein sample) at process stages of homogenised mixtures, pasteurised mixtures, yogurt (fermentation), and aerated yogurt (mousse).

Pasteurised mixtures

Regarding the pasteurised mixtures (Figure 3B), the systems M, YM2, and YM6 seemed to be mono-modal, and increased in particle size. The latter might be caused by the coagulation (denaturation) process that globular proteins undergo during heat treatment above 80°C (Gösta, 2019a). As the M sample concentrated after the evaporation process, it had the most significant particle size distribution values, followed by YM6 and YM2, which resembled the evidenced behaviour in Figure 4 (pasteurised samples). Figure 4 also suggests that the systems might have larger particle sizes out of the equipment measurement range (> 1 mm).

On the other hand, YM12 seemed to be the least affected by the pasteurisation process (Figure 3B), as it kept the same peak of zone I (Figure 3A), while zone II peak slightly increased. It means that the casein proportion did not vary. Figure 4 shows these outcomes when comparing homogenised and pasteurised samples for YM12.

The particle agglomeration in pasteurised samples also affected the gel formation in the fermentation process (yogurt) of YM2, YM6, and YM12, and subsequently, the air incorporation and air stabilisation in yogurt mousse (Figure 4). Following this, WPC enhanced the gel formation in YM6 (Sandoval-Castilla *et al.,* 2004), but the air incorporation process seemed to be more effective in YM2 and YM12. This might be due to very high viscosity in YM6 and M, and in contrast to other authors, the WPC did not favour air retention in this case (Martínez-Padilla *et al.,* 2015). Additionally, it is essential to mention that air bubbles had different particle sizes, which is consistent with results from Duquenne *et al.* (2016). Compared to another study (Attalla and El-Hussieny, 2017), our yogurt mousses had more noticeable air bubble incorporation.

Conclusion

The results of the present work show that the WPC and SMP levels variation had a significant influence over the rheological properties of yogurt mousse, especially in the rheological variables from the small deformations tests. These discoveries resulted in an improved elastic behaviour for samples with higher WPC and SMP concentrations, as evidenced in the statistical analysis. The flow behaviour and thixotropy of yogurt mousses also improved with the increase of WPC and SMP levels, even though these variables were not statistically significant. Moreover, the response surface methodology optimisation allowed us to obtain a yogurt mousse (10.7% SMP and 2% WPC) with similar rheological characteristics to a standard non-fermented mousse (12.4% SMP and 4.6% WPC) that also resembled in sensory terms (texture). Therefore, we could confirm that the rheology of yogurt mousse could be correlated to its sensory texture, as observed in other dairy products. However, the microstructural analysis showed that there were noticeable differences between the yogurt mousses and the non-fermented sample. Besides, it needs to be mentioned that the fermentation process could have improved the structuring and air incorporation of yogurt mousses, resulting in lower protein levels for the optimal mousse in contrast to the non-fermented standard, as observed in the DIC micrographs. Finally, we suggest more studies related to the variation of other nutrients and process variables to understand the rheological, sensory, and microstructural behaviour of this product.

Acknowledgement

The present work was financially supported by Facultad de Ciencias Farmacéuticas y Alimentarias and CODI of Universidad de Antioquia, Colombia (Grant CIQF-275). Grupo de Investigación en Análisis Sensorial and Grupo de Coloides of Universidad de Antioquia, Colombia, also provided funds for the completion of the present work.

References

Abdalla, A. K., Smith, K. and Lucey, J. 2017. Physical properties of nonfat dry milk and skim milk powder. International Journal of Dairy Science 12(2): 149-154.

Andoyo, R., Guyomarc'h, F., Cauty, C. and Famelart, M.-H. 2014. Model mixtures evidence the respective roles of whey protein particles and casein micelles during acid gelation. Food Hydrocolloids 37: 203-212.

Aragon-Alegro, L. C., Alarcon Alegro, J. H., Roberta Cardarelli, H., Chih Chiu, M. and Isay Saad, S. M. 2007. Potentially probiotic and synbiotic chocolate mousse. LWT - Food Science and Technology 40(4): 669-675.

Attalla, N. R. and El-Hussieny, E. A. 2017. Characteristics of nutraceutical yoghurt mousse fortified with chia seeds. International Journal of Environment, Agriculture and Biotechnology 2(4): 2033-2046.

Bitaraf, M. S., Khodaiyan, F., Mohammadifar, M. A. and Mousavi, S. M. 2012. Application of response surface methodology to improve fermentation time and rheological properties of probiotic yogurt containing *Lactobacillus reuteri*. Food and Bioprocess Technology 5(4): 1394-1401.

Buriti, F. C. A., Castro, I. A. and Saad, S. M. I. 2010. Effects of refrigeration, freezing and replacement of milk fat by inulin and whey protein concentrate on texture profile and sensory acceptance of synbiotic guava mousses. Food Chemistry 123(4): 1190-1197.

Cardarelli, H. R., Aragon-Alegro, L. C., Alegro, J. H. A., de Castro, I. A. and Saad, S. M. I. 2008. Effect of inulin and *Lactobacillus paracasei* on sensory and instrumental texture properties of functional chocolate mousse. Journal of the Science of Food and Agriculture 88(8): 1318-1324.

Chandan, R. C. and O'Rell, K. R. 2006. Manufacture of various types of yogurts. In Chandan, R. C. (ed). Manufacturing Yogurt and Fermented Milks. United States: Blackwell Publishing.

Duquenne, B., Vergauwen, B., Capdepon, C., Boone, M. A., De Schryver, T., Van Hoorebeke, L., ... and De Block, J. 2016. Stabilising frozen dairy mousses by low molecular weight gelatin peptides. Food Hydrocolloids 60: 317-323.

Gallardo-Escamilla, F. J., Kelly, A. L. and Delahunty, C. M. 2007. Mouthfeel and flavour of fermented whey with added hydrocolloids. International Dairy Journal 17(4): 308-315.

Gösta, B. 2019a. Cultured milk products. In Gösta, B. (ed). Dairy Processing Handbook, p. 255-276. Sweden: Tetra Pak International.

Gösta, B. 2019b. The chemistry of milk. In Gösta, B. (ed). Dairy Processing Handbook, p. 17-42. Sweden: Tetra Pak International.

Kokini, J. L. 1987. The physical basis of liquid food texture and texture-taste interactions. Journal of Food Engineering 6(1): 51-81.

Komatsu, T. R., Buriti, F. C. A., da Silva, R. C., Lobo, A. R., Colli, C., Gioielli, L. A. and Saad, S. M. I. 2013. Nutrition claims for functional guava mousses produced with milk fat substitution by inulin and/or whey protein concentrate based on heterogeneous food legislations. LWT - Food Science and Technology 50(2): 755-765.

Lobato-Calleros, C., Martınez-Torrijos, O., Sandoval-Castilla, O., Pérez-Orozco, J. P. and Vernon-Carter, E. J. 2004. Flow and creep compliance properties of reduced-fat yoghurts containing protein-based fat replacers. International Dairy Journal 14(9): 777-782.

Lobato-Calleros, C., Ramírez-Santiago, C., Vernon-Carter, E. J. and Alvarez-Ramirez, J. 2014. Impact of native and chemically modified starches addition as fat replacers in the viscoelasticity of reduced-fat stirred yogurt. Journal of Food Engineering 131: 110-115.

Martínez-Padilla, L. P., García-Rivera, J. L., Romero-Arreola, V. and Casas-Alencáster, N. B. 2015. Effects of xanthan gum rheology on the foaming properties of whey protein concentrate. Journal of Food Engineering 156: 22-30.

Marulanda, M., Granados, C. and García-Zapateiro, L. A. 2016. Sensory analysis and physical-chemical estimation of the useful life of a yogurt beverage based on sweet whey fermented with *Estreptococcus Salivarius* ssp., *Thermophilus* and *Lactobacillus casei* ssp. *casei*. Producción + Limpia 11(1): 94-102.

Menéndez, M., Paredes, B., Iglesias, O., Rendueles, M. and Díaz, M. 2006. Rheological behavior and organoleptic effects of ovalbumin addition in yogurt mousse production. Journal of Dairy Science 89(3): 951-962.

Meyer, D., Bayarri, S., Tárrega, A. and Costell, E. 2011. Inulin as texture modifier in dairy products. Food Hydrocolloids 25(8): 1881-1890.

Miquelim, J. N., Lannes, S. C. S. and Mezzenga, R. 2010. pH Influence on the stability of foams with protein-polysaccharide complexes at their interfaces. Food Hydrocolloids 24(4): 398-405.

Morell, P., Hernando, I., Llorca, E. and Fiszman, S. 2015. Yogurts with an increased protein content and physically modified starch: rheological, structural, oral digestion and sensory properties related to enhanced satiating capacity. Food Research International 70: 64-73.

Osorio, J., Monjes, J., Pinto, M., Ramírez, C., Simpson, R. and Vega, O. 2014. Effects of spray drying conditions and the addition of surfactants on the foaming properties of a whey protein concentrate. LWT - Food Science and Technology 58(1): 109-115.

Parra-Huertas, R. A., Barrera-Rojas, L. J. and Rojas-Parada, D. C. 2015. Evaluation of the addition of oats, mango and stevia in yogurt made from mixtures of goat's and cow's semi-skimmed milk. Corpoica Ciencia y Tecnología Agropecuaria 16(2): 167-179.

Patel, P., Parekh, T. and Subhash, R. 2008. Development of probiotic and synbiotic chocolate mousse: a functional food. Biotechnology 7(4): 769-774.

Sah, B. N. P., Vasiljevic, T., McKechnie, S. and Donkor, O. N. 2016. Physicochemical, textural and rheological properties of probiotic yogurt fortified with fiber-rich pineapple peel powder during refrigerated storage. LWT - Food Science and Technology 65: 978-986.

Sandoval-Castilla, O., Lobato-Calleros, C., Aguirre-Mandujano, E. and Vernon-Carter, E. J. 2004. Microstructure and texture of yogurt as influenced by fat replacers. International Dairy Journal 14(2): 151-159.

Soukoulis, C., Panagiotidis, P., Koureli, R. and Tzia, C. 2007. Industrial yogurt manufacture: monitoring of fermentation process and improvement of final product quality. Journal of Dairy Science 90(6): 2641-2654.

Soukoulis, C., Rontogianni, E. and Tzia, C. 2010. Contribution of thermal, rheological and physical measurements to the determination of sensorially perceived quality of ice cream containing bulk sweeteners. Journal of Food Engineering 100(4): 634-641.

Steefe, J. F. 1996. Rheological methods in food process engineering. 2nd ed. United States: Freeman Press.

Torres, I. C., Amigo, J. M., Knudsen, J. C., Tolkach, A., Mikkelsen, B. Ø. and Ipsen, R. 2018. Rheology and microstructure of low-fat yoghurt produced with whey protein microparticles as fat replacer. International Dairy Journal 81: 62-71.

Torres, J. D., Tárrega, A. and Costell, E. 2010. Storage stability of starch-based dairy desserts containing long-chain inulin: rheology and particle size distribution. International Dairy Journal 20(1): 46-52.

Xavier-Santos, D., Bedani, R., Perego, P., Converti, A. and Saad, S. M. I. 2019. *L. acidophilus* La-5, fructo-oligosaccharides and inulin may improve sensory acceptance and texture profile of a synbiotic diet mousse. LWT 105: 329-335.

Yu, H.-Y., Wang, L. and McCarthy, K. L. 2016. Characterization of yogurts made with milk solids nonfat by rheological behavior and nuclear magnetic resonance spectroscopy. Journal of Food and Drug Analysis 24(4): 804-812.