

Original Research Article

Study of thermal behaviour of milk protein products using a chemometric approach

ABSTRACT: This study deals with the evaluation of the behavior of viscosity evolution of the Milk Protein Products (MPPs) solutions in different concentrations used in different conditions of thermal processing by means of Rapid Visco Analyzer (RVA). Skimmed milk powder (SMP), whey protein concentrate (WPC), milk protein concentrate (MPC), whey powder (WP) and demineralized whey powder (DWP) were analyzed; the evaluated solid-liquid ratio solutions were $0.1 \text{ g}\cdot\text{g}^{-1}$ to $0.45 \text{ g}\cdot\text{g}^{-1}$ of dry solids in water (DSW) for the MPC, $0.1 \text{ g}\cdot\text{g}^{-1}$ to $0.4 \text{ g}\cdot\text{g}^{-1}$ of DSW for the WPC and $0.5 \text{ g}\cdot\text{g}^{-1}$ to $0.8 \text{ g}\cdot\text{g}^{-1}$ of DSW for SMP, WP and DWP. Brazilian industrial heat treatment set ups were used, with temperatures ranging from 65°C to 95°C and retention time from 5 to 30 minutes. The results were interpreted according to the viscosities during heating and cooling, being possible to optimize the MPPs different parameters conditions with the interpretations of the viscographic profiles based on the thermal behavior of proteins, especially for the SMP, MPC and WPC. The data showed similarity in viscographic characteristics of the solutions when evaluated at 65°C for 30

ABBREVIATIONS

MPPs = Milk protein products; RVA = Rapid Visco Analyzer; SMP = Skimmed milk powder; WPC = whey protein concentrate; MPC = milk protein concentrate; WP = whey powder; DWP = demineralized whey powder; MPC1 = solutions of milk protein concentrate with prior hydration; MPC2 = solutions of milk protein concentrate without prior hydration; DSW= dry solids in water; TCW = Thermocline for Windows; PV = peak viscosity; RV = retention viscosity; FV = final viscosity; $\int/t$ = integration of viscographic profile divided by the total time of analysis; PCA = Principal Component Analysis; PC1 = First Principal Component; PC2 = Second Principal Component; β -LG = β -Lactoglobulin; α -LA = α -Lactalbumin

minutes, at 85°C for 15 minutes and 95°C for 5 minutes, for the MPC and WPC at 95°C for 5 minutes, all of them for different concentrations of proteins in water.

KEYWORDS: milk protein, functionality, viscosity.

1. INTRODUCTION:

Dairy protein products can be defined as concentrated or dried milk products that whenever added to dairy products or other processed food as ingredient contribute to the improvement of the nutritional composition and also to functionality during and after processing (SINGH, 2009).

During the last years the production of dairy protein powder is increasing mainly for baby food production, for special diets, animal feeding and for the food industry applications. Membrane separation processes, vacuum evaporation and spray drying are mandatory steps in the production of dairy proteins powders. The dairy industry has developed new technological processes for extracting and purifying proteins such as milk protein concentrate (MPC), milk protein isolate (MPI), whey protein concentrate (WPC), whey protein isolate (WPI), micellar casein concentrates (MCC) and isolates (MCI), whey concentrates, and selectively demineralized whey concentrates (SCHUCK, 2009).

The process of converting liquid milk or whey into powder alters the nature and behavior of milk components. Factors which affect the ability of water absorption by milk proteins include: composition, protein structure and conformation, surface load and polarity, presence of carbohydrates, lipids and

42 salts, pH, ionic strength, temperature, degree of denaturation and aggregation, and
43 formation of disulfide bridges (DAMODARAN 1996; GARCIA *et al.* 1997).

44 The understanding of the involved interactions and evaluation of the effect
45 of milk proteins as industrial ingredient still needs more studying so as to develop
46 a better suitability of the use of these proteins within each desired technological
47 application in the various processing lines. The understanding of the interaction of
48 milk proteins provides the food industry the formula to obtain a relationship
49 which delivers a better cost / benefit ratio by using each type of protein within the
50 desired characteristics in the final product, thus bringing to market products that
51 use as technological ingredients, proteins with high biological value (FOX, 2003).

52 The Rapid ViscoTM Analyser (RVATM) is a rotational viscometer capable
53 of continuously measure the viscosity of a sample under controlled temperature
54 (BOOTH and BASON, 2007).

55 This study has as goal to investigate the influence of different thermal
56 processing conditions on the evolution of the viscosity of dehydrated dairy protein
57 after adequate rehydration, using the Rapid Viscosity Analyzer (RVA) as
58 simulator.

59

60 2. MATERIALS AND METHODS:

61 Five types of dehydrated MPPs for evaluation were selected, being the
62 selection criteria the industrial use in Brazil, as well the national industrial
63 production, current economic importance, the extent of use in different areas and
64 prospecting for future use (CARVALHO *et al.*, 2010).

Therefore, a sample of industrial production of each MPP was previously selected in the national market or overseas, taking into consideration the quality of products and manufacturing date. The products were acquired and kindly provided by Gemacom Tech Company. The products had their chemical and microbiological characteristics previously informed by their manufacturers through analysis of reports of purchased lots.

Analyses were conducted to determine the results showed by reports at Gemacom Tech Laboratory of Product Analyzes. TABLE 1 shows every analysis carried out.

TABLE 1 Methods of analysis for each parameter of MPP

Analysis	Milk derivate		Whey derivate		
	Skimmed milk powder	Milk protein concentrate	Whey powder	Demineralized whey powder	Whey protein concentrate
Total fat	IN n° 68 de 12/12/2006	AOAC 989.05	FAO, 1976	FAO, 1976	AOAC 989.05
Moisture	FIL 26A: 1993	AOAC 925.45	FIL 26A: 1993	FIL 26A: 1993	AOAC 927.05 17 th
Total protein	IN n° 68 de 12/12/2006	AOAC 991.20	FIL 20B: 1993	FIL 20B: 1993	AOAC 991.20
Protein in dry basis	Calculated	Calculated	Calculated	Calculated	Calculated
Lactose	Calculated	Calculated	Calculated	Calculated	Calculated
Ash	IN n° 68 de 12/12/2006	AOAC 900.02	AOAC 15 th , 1990	AOAC 15 th , 1990	AOAC 900.02
Acidity	FIL 81:1981	FIL 81:1981	FIL 81: 1981	FIL 81: 1981	n.a. ¹
Chloride	n.a. ¹	n.a. ¹	n.a. ¹	LANARA	n.a. ¹
pH	n.a. ¹	AOAC 981.12	Adolfo Lutz	Adolfo Lutz	AOAC 981.12
Specific mass	n.a. ¹	C314 VK Density Tapper	Niro Atomizer	Niro Atomizer	C314 VK Density Tapper
Total count of mesophilic aerobic	FIL 100B:1991	AOAC 989.10	FIL 100B:1991	FIL 100B:1991	AOAC 989.10

Coliforms at 30°C	APHA 1992	AOAC 989.10	APHA 1992	APHA 1992	AOAC 989.10
<i>Escherichia coli</i>	APHA 1992	AOAC 989.10	APHA 1992	APHA 1992	AOAC 989.10
Fungi and yeasts	FIL 94B: 1990	AOAC 989.10	FIL 94B: 1990	FIL 94B: 1990	AOAC 989.10
Coagulase positive staphylococci	FIL 138:1986	AOAC 2003.08	FIL 138:1986	FIL 138:1986	AOAC 2003.08
<i>Salmonella sp</i>	FIL 93B:1995	AOAC 967.25	FIL 93B:1995	FIL 93B:1995	AOAC 967.25

76 ¹ not analysed

77

78 Three processing levels (low, medium and high) were used to simulate
79 different thermal processing for each MPP sample, which were selected based on
80 their usefulness in the processing of cheese, fermented milk, Petit Suisse,
81 pasteurized cream, and flavored milk drinks: 65°C for 30 minutes (low), 85°C for
82 15 minutes (medium) and 95°C for 5 minutes (high). It is important to notice that
83 the three heat treatment processes above used in this investigation are the most
84 usual parameters used by Brazilian industries. As recommended by Bennett *et al.*
85 (2007), all samples were previously hydrated before being subjected to the RVA.
86 Genuine replicates of the solutions were prepared in duplicates using distilled
87 water as solvent and constant stirring for 30 min at 25°C. All these solutions have
88 been prepared with 100 g water and sufficient amount of MPP to obtain the
89 desired concentration of dry basis (dry basis g/water g). The dehydrated products
90 were kept in airtight containers due to the hygroscopic characteristics of the
91 samples, avoiding, thus, absorption of humidity. Before the preparation of each
92 solution, moisture analyzes were performed to obtain the exact calculation of the
93 amount of MPP to be used for its concentration of dry basis. The concentrations
94 of evaluated solutions of each product are shown in TABLE 2.

95 TABLE 2 Solution concentrations of the MPPs evaluated in RVA

Product	Concentration $\text{g}\cdot\text{g}^{-1}$ (dry basis)	
	With prior hydration	Without prior hydration
Skimmed milk powder (SMP)	0.5 / 0.6 / 0.7 / 0.8	X
Milk protein concentrate (MPC)	0.1 / 0.12 / 0.14 / 0.16	0.3 / 0.35 / 0.4 / 0.45
Whey powder (WP)	0.5 / 0.6 / 0.7 / 0.8	X
Demineralized whey powder 40% (DWP)	0.5 / 0.6 / 0.7 / 0.8	X
Whey protein concentrate (WPC)	0.1 / 0.2 / 0.3 / 0.4	X

96 Due to the solubility characteristics of the MPC, thermal processing
97 simulations were also performed without prior hydration, adding this MPP
98 directly to water already in the tank of the equipment. Abbreviations MPC1 and
99 MPC2 refer, respectively, to solutions of MPC with and without prior hydration.

100 In the simulations, 25 g of each solution were weighed beforehand in the heating
101 tank of RVA. In the case of MPC, without prior hydration, calculations were
102 made for the solution prepared directly in the bowl also presented final mass of 25
103 g. To ensure full standardization of solutions, RVA rotation was set to $104.7 \text{ rad}\cdot\text{s}^{-1}$
104 during the first 60 seconds at constant temperature of 30°C . The SMP, WP,
105 DWP and WPC solutions had the pH adjusted to 6.5 using $1 \text{ mol}\cdot\text{L}^{-1}$ of sodium
106 hydroxide solution. After dissolution, the pH of MPC solutions ranged from 6.7 to
107 6.9, and to latter there was no conduction to pH adjustment. The RVA equipment
108 used in this work was the Model Series 4 (RVA-4) from NEWPORT
109 SCIENTIFIC. The software used for data collection was the Thermocline for

110 Windows, TCW. The configurations of the thermal processing simulations are
 111 shown in Table 3.

112 TABLE 3 Settings of thermal processing employed in RVA

Time	Set up	65°C for 30 minutes	85°C for 15 minutes	95°C for 5 minutes
0 min 00 s	Temperature (°C)	30	30	30
0 min 00 s	Rotation (rad.s ⁻¹)	104.7	104.7	104.7
1 min 00 s	Rotation (rad.s ⁻¹)	15.7	15.7	15.7
1 min 00 s	Temperature (°C)	30	30	30
6 min 24 s	Temperature (°C)	65	85	95
36 min 24 s	Temperature (°C)	65	85	95
39 min 06 s	Temperature (°C)	30	30	30
44 min 06 s	Temperature (°C)	30	30	30
44 min 07 s	End	X	X	X

113 For all three treatment levels, the gap between viscosity readings was 4 seconds.

114

115 The gradients of heating and cooling were also standardized between the
 116 levels, being 6.5°C per minute to 13.0°C per minute respectively. Integration
 117 calculations of viscographic profiles were performed in MICROCAL™
 118 ORIGIN® version 6.0 software. All integration calculations were made with the
 119 viscographic profiles as from 67 seconds, discarding, this way, the stirring time at
 120 a speed of 104.7 rad.s⁻¹ as described in step preparation of samples. It was
 121 determined in the experiment that the highest viscosity obtained during thermal
 122 processing before the start of cooling would be represented as peak viscosity
 123 (PV), the viscosity obtained at the end of the retention time of thermal processing
 124 would be represented as retention viscosity (RV), the last viscosity reading
 125 obtained at the end of the analysis would be represented as final viscosity (FV)
 126 and the value of the integration of viscographic profile divided by the total time of
 127 analysis would be represented as area per unit time (J/t). To study the optimization

of the concentration, temperature and time in simulated conditions of thermal processing in RVA, the WPC was selected among the available MPPs for evaluations since it is a high protein product and widely utilized in food, as well as an ingredient which assigns functionality properties.

A 3^3 Box-Behnken factorial design was performed to evaluate the effect of thermal processing on the characteristics of MPPs by monitoring the final viscosity (FV) through the solutions obtained after complete cycle of heating and cooling. The selected factors are shown in table 4.

TABLE 4 Factors and levels used in the 3^3 Box-Behnken factorial design

Factors	Factors levels		
	-1	0	+1
Time (minutes)	5	15	30
Temperature (°C)	65	85	95
Concentration (g·g ⁻¹)	0.2	0.3	0.4

Table 5 presents the matrix of planning, with the levels and factors combinations, where the experiments 13, 14 and 15 are replicates of the center point. The procedures were performed randomly by raffle.

147 TABLE 5 Coded matrix for the WPC 3³ Box-Behnken design

Experimental set randomized	Time (level)	Time (minutes)	Temperature (level)	Temperature (°C)	Concentration (level)	Concentration (g·g ⁻¹)
10	0	15	1	95	-1	0.2
6	1	30	0	85	-1	0.2
3	-1	5	1	30	0	0.3
12	0	15	1	95	1	0.4
4	1	30	1	95	0	0.3
2	1	30	-1	65	0	0.3
14	0	15	0	85	0	0.3
5	-1	5	0	85	-1	0.2
8	1	30	0	85	1	0.4
13	0	15	0	85	0	0.3
15	0	15	0	85	0	0.3
1	-1	5	-1	65	0	0.3
7	-1	5	0	85	1	0.4
9	0	15	-1	65	-1	0.2
11	0	15	-1	65	1	0.4

148

149 **2.1 Data Analysis:**

150 The obtained results were analyzed using the coefficient and regression
151 correlation in order to conclude on the definition of the main factors to be used in
152 interpreting the viscographic profile of MPPs in simulated conditions in RVA.
153 Analyses of variance were also conducted to test differences in the studied
154 variables with MPPs in relation to the concentration and thermal processing. It
155 was applied the system for statistical analyses SAEG 9.1. For the regression
156 analysis, models that had a significance level less than or equal to 5% were
157 accepted as model and as linear and quadratic coefficients, when it was the case.
158 For the analysis of correlation coefficients were accepted with a significance level

159 equals or less than 5%. The response surfaces and calculations of the effects were
 160 obtained by employing the computer program STATISTIC 6.0 (Statsoft, Inc.) and
 161 ANOVA, Microsoft Excel 2007 (Microsoft Co.). For the exploratory analysis by
 162 type of thermal processing among the MPPs, the results of the RVA were
 163 organized into matrices with treatments (MPP and concentration) in the lines and
 164 the viscosity readings averages from all viscographic profile in columns and then
 165 submitted to the Principal Component Analysis (PCA) from the covariance
 166 matrix, using PLS Toolbox v.2.9.2.9 (Eigen Vector Research Inc., Wenatchee,
 167 WA), operating in MATLAB (R2007B, vol. 7.5.0.342, Inc. the Mathworks,
 168 Natick, MA) environment.

169

170 3. RESULTS AND DISCUSSION

171 The samples collected and used in this study presented their compositional
 172 and microbiological characteristics consistent with data from literature (SINGH,
 173 2009). The table 6 presents the MPP's chemical and microbiological composition.

174

175 TABLE 6 Microbiological and chemical composition of MPP

Analysis	Milk derivate			Whey derivate	
	Skimmed milk powder	Milk protein concentrate	Whey powder	Demineralized whey powder	Whey protein concentrate
Total fat ($\text{g}\cdot\text{kg}^{-1}$)	15.9	9.7	15	5	61.5
Moisture ($\text{g}\cdot\text{kg}^{-1}$)	32.5	42.7	18	17	33.9
Total protein ($\text{g}\cdot\text{kg}^{-1}$)	351.4	714.3	123.6	122.8	785.3
Protein in dry basis ($\text{g}\cdot\text{kg}^{-1}$)	363.2	746.2	125.9	124.9	812.8

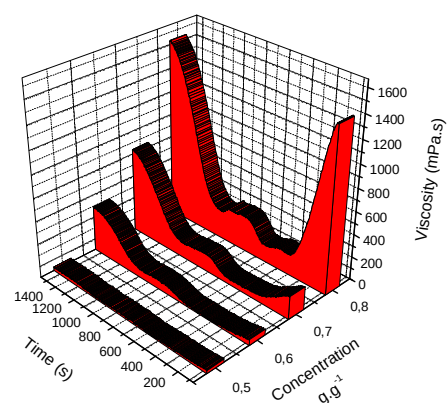
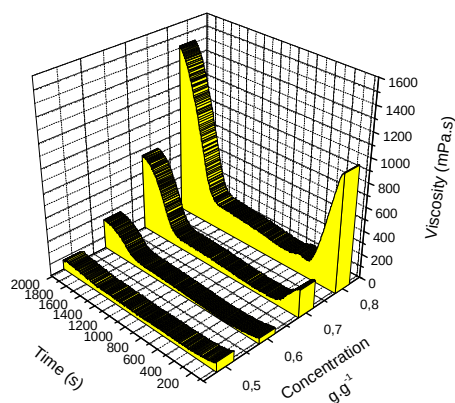
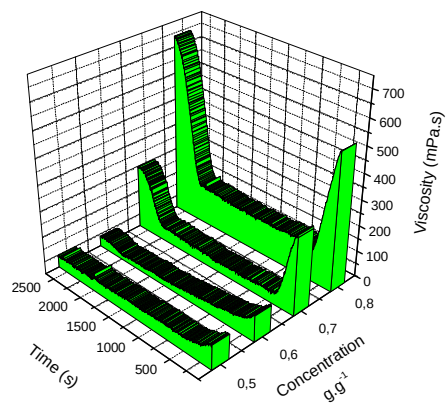
Lactose (g·kg ⁻¹)	511	161.3	768.9	795.9	95.7
Ash (g·kg ⁻¹)	89.2	72.0	74.5	59.3	23.6
Acidity (g ácido lático·L ⁻¹)	1.4	1.5	1.7	1.4	n.a. ¹
Chloride (g·kg ⁻¹)	n.a. ¹	n.a. ¹	n.a. ¹	10.8	n.a. ¹
pH	n.a. ¹	6.95	6.13	6.10	6.33
Specific mass (g·mL ⁻¹)	n.a. ¹	0.52	0.74	0.63	0.43
Total count of mesophilic aerobic	2.6·10 ³ UFC·g ⁻¹	9.6·10 ³ UFC·g ⁻¹	4.2·10 ³ UFC·g ⁻¹	3.9·10 ³ UFC·g ⁻¹	7·10 ² UFC·g ⁻¹
Coliforms at 30°C	< 10 NMP·g ⁻¹	< 10 UFC·g ⁻¹	< 10 NMP·g ⁻¹	< 10 NMP·g ⁻¹	< 10 UFC·g ⁻¹
<i>Escherichia coli</i>	< 1 NMP·g ⁻¹	< 10 UFC·g ⁻¹	< 3 NMP·g ⁻¹	< 1 NMP·g ⁻¹	< 10 UFC·g ⁻¹
Fungi and yeasts	< 10 UFC·g ⁻¹	< 10 UFC·g ⁻¹	40 UFC·g ⁻¹	< 1 UFC·g ⁻¹	< 10 UFC·g ⁻¹
Coagulase positive staphylococci	< 10 UFC·g ⁻¹	< 10 UFC·g ⁻¹	< 1 UFC·g ⁻¹	< 1 UFC·g ⁻¹	< 10 UFC·g ⁻¹
<i>Salmonella sp</i>	absent in 25 g	absent in 25 g	absent in 25 g	absent in 25 g	absent in 25 g

176 ¹ not analyzed

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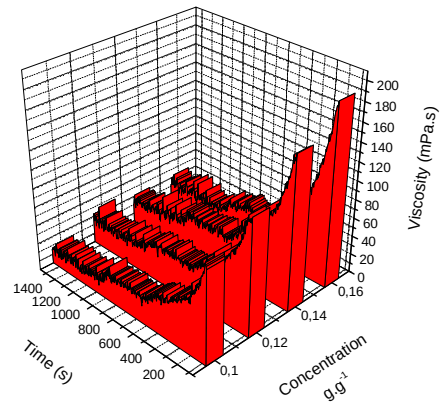
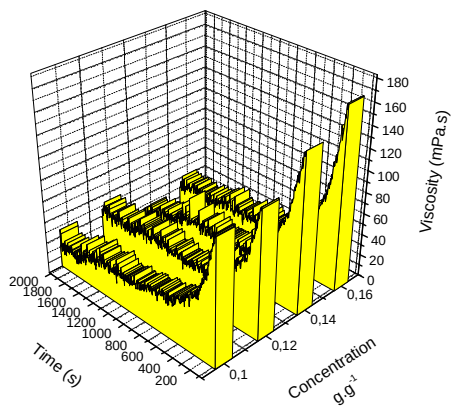
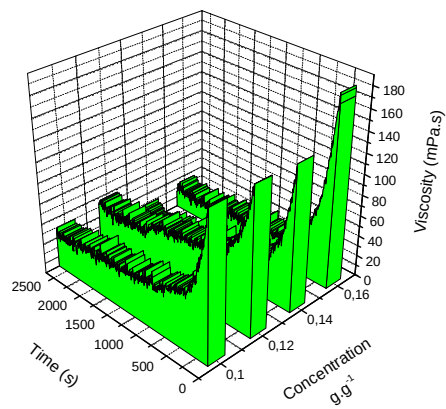
178 The viscographic profiles of simulated thermal processing in RVA for the
179 MPP's are shown in Figure 1 respectively to low, medium and high heat
180 treatment.

Skimmed milk powder (SMP)



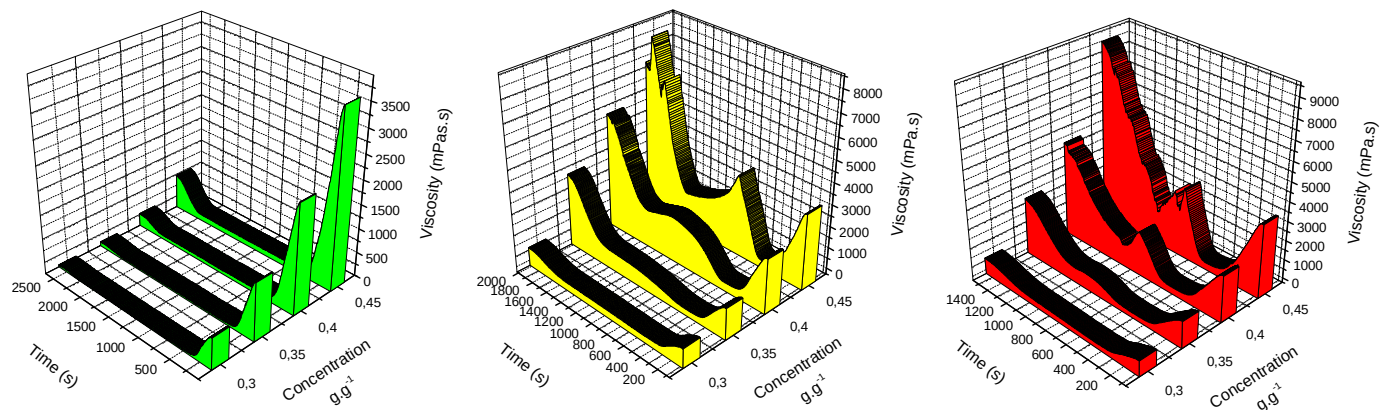
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Milk protein concentrate (MPC₁)



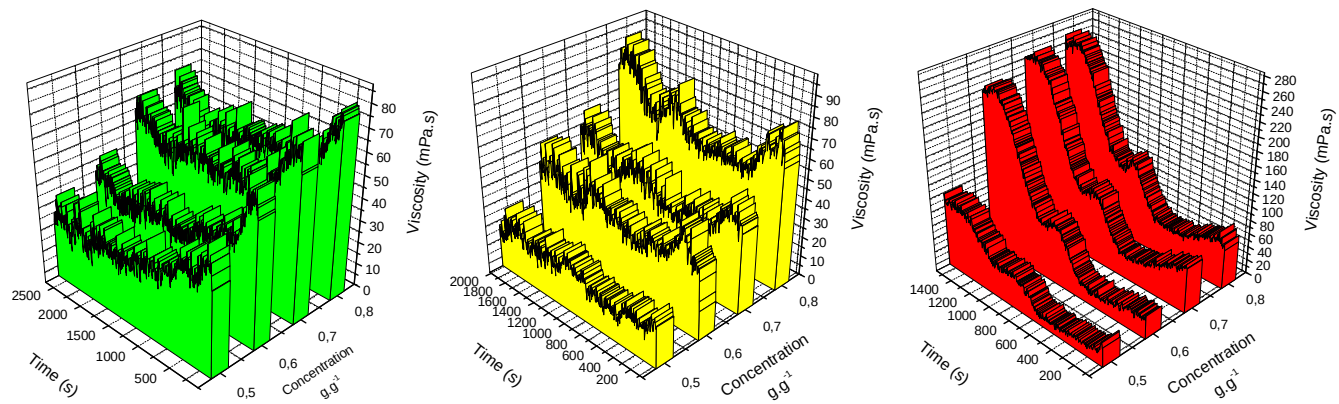
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Milk protein concentrate (MPC₂)



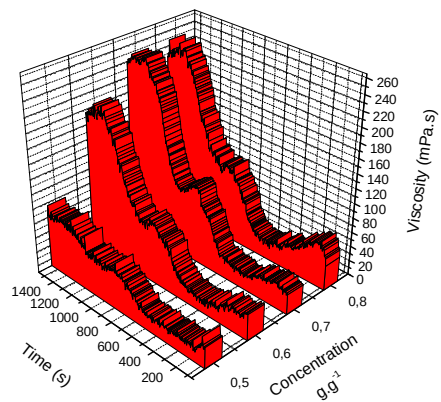
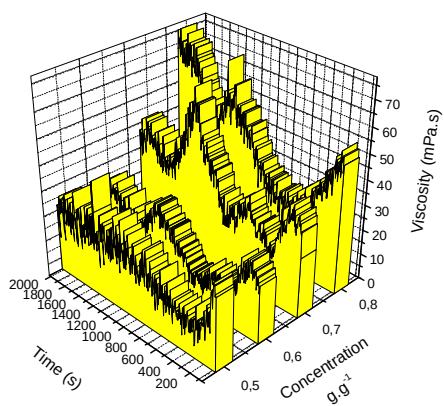
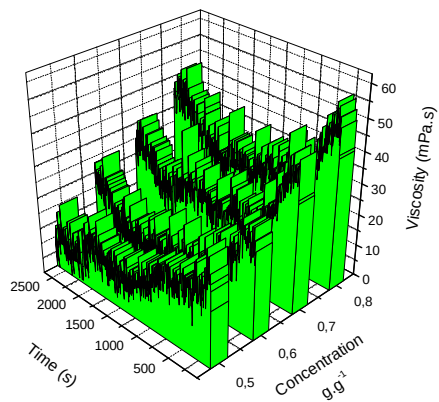
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Whey powder (WP)



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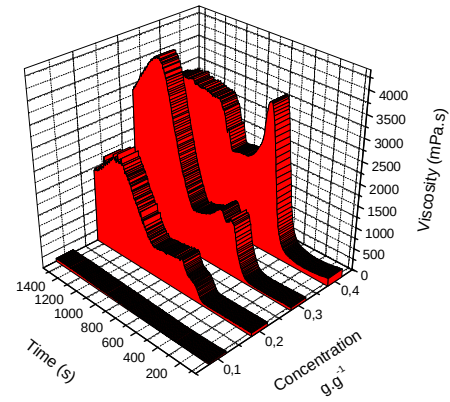
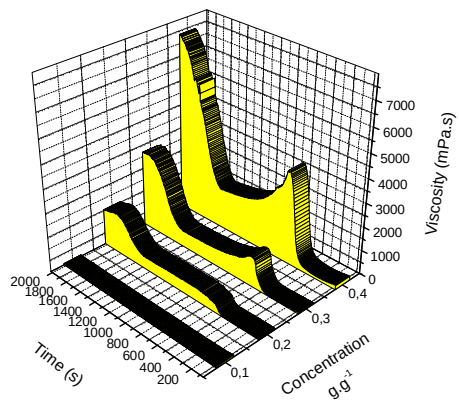
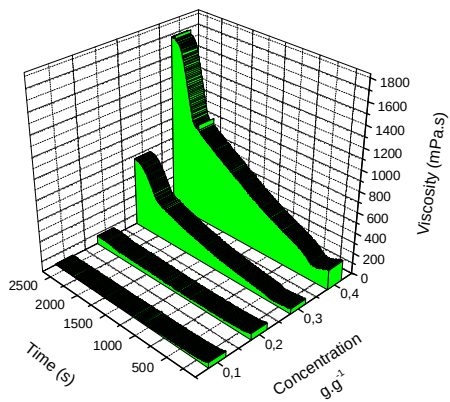
Demineralized whey powder (DWP)



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Whey protein concentrate (WPC)



187

188 FIGURE 1 Viscographic profiles of simulate thermal processing in RVA for the MPPs respectively for low (■), medium (■) and high
189 heat treatment (■)

190 The analysis of variance for PV showed statistically significant effects for
 191 concentration ($P < 0.001$) and thermal processing ($P < 0.003$) in SMP. These
 192 results demonstrate the influence of these two variables in the maximum viscosity
 193 obtained during the process provided by the SMP. The maximum value of PV
 194 obtained for SMP was 318 mPa·s on the condition of $0.8 \text{ g}\cdot\text{g}^{-1}$ at 95°C for 5
 195 minutes and less than 27 mPa·s with $0.6 \text{ g}\cdot\text{g}^{-1}$ subjected to 65°C for 30 minutes.
 196 The maximum value of RV for SMP was 308 mPa·s on the condition of $0.8 \text{ g}\cdot\text{g}^{-1}$
 197 at 95°C for 5 minutes and the minimum 1 mPa·s with $0.6 \text{ g}\cdot\text{g}^{-1}$ subjected to 65°C
 198 during 30 minutes.

199 For the same product the maximum value of FV was 1645 mPa·s on the
 200 condition of $0.8 \text{ g}\cdot\text{g}^{-1}$ at 85°C for 15 minutes and less than 19 mPa·s with $0.5 \text{ g}\cdot\text{g}^{-1}$
 201 subjected to 65°C for 30 minutes. The magnitude of the effects of concentration
 202 and thermal processing on PV, RV and FV clearly manifested ($P < 0.05$), being the
 203 concentration of $0.8 \text{ g}\cdot\text{g}^{-1}$ and binomial 95°C for 5 minutes and 85°C for 15
 204 minutes the most likely to show higher values of viscosity.

205 The analysis of variance for $\int/t$ showed statistically significant effects for
 206 concentration ($P < 0.001$) and thermal processing ($P < 0.001$). These results
 207 demonstrate the influence of these variables in the full viscographic profile of
 208 SMP. The maximum value of $\int/t$ obtained in the study was 563 on the condition of
 209 $0.8 \text{ g}\cdot\text{g}^{-1}$ at 95°C for 5 minutes and 0.6 with at least $16 \text{ g}\cdot\text{g}^{-1}$ subjected to 65°C for
 210 30 minutes. The evaluation of the magnitude of the effects of concentration and
 211 thermal processing on PV, RV and FV can be made to $\int/t$, demonstrating be
 212 statistically significant ($P < 0.05$), with a concentration of $0.8 \text{ g}\cdot\text{g}^{-1}$ and 95°C

binomials for 5 minutes and 85°C for 15 minutes more likely to display higher values of viscosity (see Table 7).

TABLE 7 Correlation coefficients for area per unit of time (J/t)

Variables for correlation (J/t)	SMP	MPC1	MPC2	WP	DWP	WPC
Peak viscosity – PV	0.980 (*)	0.564 (*)	0.984 (*)	0.962 (*)	0.975 (*)	0.967 (*)
Retention viscosity– RV	0.977 (*)	0.778 (*)	0.980 (*)	0.977 (*)	0.978 (*)	0.959 (*)
Final viscosity – FV	0.979 (*)	0.588 (*)	0.991 (*)	0.961 (*)	0.990 (*)	0.938 (*)

Where: (*) statistically significant ($P < 0.001$); **r = Pearson correlation coefficient**

In the case of MPC1 analysis of variance for PV revealed statistically significant effect for thermal processing ($P < 0.001$). The maximum value of PV obtained in the study was 84 mPa·s on the condition of $0.12 \text{ g}\cdot\text{g}^{-1}$ at 65°C for 30 minutes and the minimum 39 mPa·s with $0.1 \text{ g}\cdot\text{g}^{-1}$ subjected to 95°C for 5 minutes. The maximum concentration to be prepared from the solution of MPC1 with the same procedures performed for other products was $0.16 \text{ g}\cdot\text{g}^{-1}$. This occurred due to the large increase in viscosity at 25°C, making it impossible to maintain agitation of the solution within 30 minutes, which interfered in its uniformity. The main factor affecting the solubility of MPC1 seems to be related to the throughput of water in the powder particle more than the thermal processes during manufacture (SCHUCK *et al.* 1994). Different methods for producing MPC1 with high solubility in cold water have been proposed, involving the

231 addition of monovalent salts in the retentate prior to drying (CARR, 2002) or
 232 partial replacement (approximately 30%) of the calcium content of the retentate
 233 sodium ions by ultrafiltration (BHASKAR *et al.* 2001). MPC1 analysis of
 234 variance for FV showed a statistically significant effect for concentration ($P <$
 235 0.001). The maximum value for MPC1 of FV was 50 mPa·s on the condition of
 236 $0.12 \text{ g}\cdot\text{g}^{-1}$ at 65°C for 30 minutes and less than 10 mPa·s with $0.14 \text{ g}\cdot\text{g}^{-1}$ subjected
 237 to 85°C for 15 minutes. The viscographic profiles obtained for MPC1 in this
 238 concentration range reveal an inherent characteristic of the product which is the
 239 high viscosity before processing and significant decrease during the heat
 240 treatment, without any increase of viscosity even after cooling. These results are
 241 in agreement with the proposal made by Schuck *et al.* (1994), explaining the low
 242 solubility of the MPC1 at low temperatures. The magnitude of the effects of
 243 concentration and thermal processing on the FV and PV, respectively, were
 244 statistically significant ($P < 0.05$). However, it is possible to correlate any
 245 technological factor variables which exhibited the highest values of viscosity. The
 246 maximum value of η/t 59 was obtained in the study of $0.12 \text{ g}\cdot\text{g}^{-1}$ at 65°C for 30
 247 minutes and the minimum 0.14 to $29 \text{ g}\cdot\text{g}^{-1}$ subjected to 65°C for 30 minutes. The
 248 correlation data from η/t with viscosities PV, RV and FV for the MPC1 are shown
 249 in Table7 and indicate the poor correlation among them. The low concentration of
 250 protein is the main reason why the results obtained do not have good correlation,
 251 because the viscosity increase in this range is small.

252 With regard to MPC2, the analysis of variance for PV showed statistically
 253 significant effects for concentration ($P < 0.001$) and thermal processing ($P <$

0.001). The maximum value of PV for the MPC2 was 3568 mPa·s on the condition of 0.45 g·g⁻¹ at 85°C for 30 minutes and the minimum was 20 mPa·s with 0.3 g·g⁻¹ subjected to 65°C for 30 minutes. The analysis of variance for RV showed statistically significant effects for concentration ($P < 0.001$) and thermal processing ($P < 0.001$) for MPC2. These results demonstrate the influence of these variables on the viscosity obtained after the retention time, provided by MPC2. The maximum value of RV obtained in the study was 2180 mPa·s on the condition of 0.45 g·g⁻¹ at 95°C for 5 minutes and the minimum was 20 mPa·s with 0.3 g·g⁻¹ subjected to 65°C for 30 minutes. For MPC2 analysis of variance revealed statistically significant effects for FV for concentration ($P < 0.001$) and thermal processing ($P < 0.001$). The maximum FV value obtained in the study was 9050 mPa·s on the condition of 0.45 g·g⁻¹ at 95°C for at least 5 minutes and the minimum was 44 mPa·s with 0.3 g·g⁻¹ subjected to 65°C for 30 minutes. The magnitude of the effects of concentration and thermal processing on PV, RV and FV were statistically significant ($P < 0.05$) for MPC2, being the concentration of 0.45 g·g⁻¹ and the binomial 95°C per 5 minutes and 85°C per 15 minutes the most likely to show higher values of viscosity. The analysis of variance for $\dot{\gamma}/t$ showed statistically significant effects for concentration ($P < 0.001$) and thermal processing ($P < 0.001$). These results demonstrate the influence of these variables on the complete MPC2 viscographic profile. The maximum value of $\dot{\gamma}/t$ obtained in the study was 3113 of 0.45 g·g⁻¹ at 95°C for 5 minutes and the minimum was 58 with 0.3 g·g⁻¹ subjected to 65°C for 30 minutes. The evaluation on the magnitude of the effects of concentration and thermal processing on PV and RV

277 FV can be verified by $\int/t$, demonstrating to be statistically significant ($P < 0.05$),
 278 and the concentration of $0.45 \text{ g}\cdot\text{g}^{-1}$ and the binomial 95°C for 5 minutes and 85°C
 279 for 15 minutes are the most likely to exhibit higher values of viscosity. This
 280 similarity among the results obtained for $\int/t$ with viscosities PV, RV and FV is
 281 deduced when analyzing the correlation coefficients shown in Table 7.

282 For WP, the analysis of variance for PV revealed statistically significant
 283 effects for concentration ($P < 0.001$) and thermal processing ($P < 0.001$). The
 284 maximum value of PV obtained for WP was $126 \text{ mPa}\cdot\text{s}$ on the condition of 0.8
 285 $\text{g}\cdot\text{g}^{-1}$ at 95°C for 5 minutes and the minimum was of $30 \text{ mPa}\cdot\text{s}$ with $0.5 \text{ g}\cdot\text{g}^{-1}$
 286 subjected to 85°C for 15 minutes. The analysis of variance for RV showed
 287 statistically significant effects for concentration ($P < 0.001$) and thermal
 288 processing ($P < 0.001$). These results demonstrate the influence of these variables
 289 on the viscosity obtained after the retention time, provided by WP. The maximum
 290 value of RV was $117 \text{ mPa}\cdot\text{s}$ on the condition of $0.8 \text{ g}\cdot\text{g}^{-1}$ at 95°C for 5 minutes and
 291 the minimum was $10 \text{ mPa}\cdot\text{s}$ with $0.5 \text{ g}\cdot\text{g}^{-1}$ subjected to 65°C for 30 minutes. The
 292 analysis of variance for FV showed statistically significant effects for
 293 concentration ($P < 0.001$) and thermal processing ($P < 0.001$) for the WP. The
 294 maximum value of FV was $256 \text{ mPa}\cdot\text{s}$ on the condition of $0.7 \text{ g}\cdot\text{g}^{-1}$ at 95°C for 5
 295 minutes and the minimum was $7 \text{ mPa}\cdot\text{s}$ with $0.5 \text{ g}\cdot\text{g}^{-1}$ subjected to 65°C for 30
 296 minutes. The analysis of variance for $\int/t$ showed statistically significant effects for
 297 concentration ($P < 0.001$) and thermal processing ($P < 0.001$) for WP. The
 298 maximum value of $\int/t$ obtained was 116 on the condition of $0.8 \text{ g}\cdot\text{g}^{-1}$ at 95°C for
 299 5 minutes and the minimum was 20 with $0.5 \text{ g}\cdot\text{g}^{-1}$ subjected to 65°C for 30

300 minutes. The evaluation on the magnitude of the effects of concentration and
 301 thermal processing on PV, RV and FV can be verified by $\int/t$, demonstrating to be
 302 statistically significant ($P < 0.05$), being the concentrations of $0.8 \text{ g}\cdot\text{g}^{-1}$, $0.7 \text{ g}\cdot\text{g}^{-1}$
 303 and $0.6 \text{ g}\cdot\text{g}^{-1}$, and the binomial 95°C for 5 minutes the ones with the largest
 304 propensity to exhibit higher values of viscosity. This similarity among the results
 305 obtained for $\int/t$ with viscosities PV, RV and FV is deduced when analyzing the
 306 correlation coefficients presented in Table 7.

307 Regarding the DWP, the analysis of variance for PV revealed statistically
 308 significant effects for concentration ($P < 0.001$) and thermal processing ($P <$
 309 0.001). The maximum value of PV obtained in the study was $114 \text{ mPa}\cdot\text{s}$ on the
 310 condition of $0.8 \text{ g}\cdot\text{g}^{-1}$ at 95°C for 5 minutes and the minimum was $27 \text{ mPa}\cdot\text{s}$ with
 311 $0.5 \text{ g}\cdot\text{g}^{-1}$ subjected to 65°C for 30 minutes.

312 The analysis of variance for RV showed statistically significant effects for
 313 concentration ($P < 0.001$) and thermal processing ($P < 0.001$) for DWP. The
 314 maximum value of RV obtained in the study was $110 \text{ mPa}\cdot\text{s}$ on the condition of
 315 $0.8 \text{ g}\cdot\text{g}^{-1}$ at 95°C for 5 minutes and the minimum was $10 \text{ mPa}\cdot\text{s}$ with $0.6 \text{ g}\cdot\text{g}^{-1}$
 316 subjected to 65°C for 30 minutes. The analysis of variance for FV showed
 317 statistically significant effects for concentration ($P < 0.002$) and thermal
 318 processing ($P < 0.001$) for the DWP. The maximum FV value obtained in the
 319 study was $255 \text{ mPa}\cdot\text{s}$ on the condition of $0.8 \text{ g}\cdot\text{g}^{-1}$ at 95°C for 5 minutes and the
 320 minimum was $9 \text{ mPa}\cdot\text{s}$ with $0.5 \text{ g}\cdot\text{g}^{-1}$ subjected to 65°C for 30 minutes. The
 321 magnitude of the effects of concentration and thermal processing on PV, RV and
 322 FV were statistically significant ($P < 0.05$) for the DWP, being the concentrations

323 of $0.8 \text{ g}\cdot\text{g}^{-1}$ and $0.7 \text{ g}\cdot\text{g}^{-1}$, and the binomial 95°C for 5 minutes the most likely to
 324 show higher values of viscosity. The analysis of variance for η/t showed
 325 statistically significant effects for concentration ($P < 0.001$) and thermal
 326 processing ($P < 0.001$). The maximum value of η/t obtained in the study was 99 on
 327 the condition of $0.8 \text{ g}\cdot\text{g}^{-1}$ at 95°C for 5 minutes and the minimum was 15 with 0.5
 328 $\text{g}\cdot\text{g}^{-1}$ subjected to 65°C for 30 minutes. The evaluation on the magnitude of the
 329 effects of concentration and thermal processing on PV, RV and FV can be verified
 330 by η/t , demonstrating to be statistically significant ($P < 0.05$), being the
 331 concentrations of $0.8 \text{ g}\cdot\text{g}^{-1}$ and $0.7 \text{ g}\cdot\text{g}^{-1}$, and the binomial 95°C for 5 minutes the
 332 one with the highest propensity to exhibit higher values of viscosity. This
 333 similarity among the results obtained for η/t with viscosities PV, RV and FV is
 334 deduced when analyzing the correlation coefficients shown in Table 7.

335 For WPC analysis of variance for PV revealed statistically significant
 336 effects for concentration ($P < 0.001$) and thermal processing ($P < 0.001$). The
 337 maximum value of PV obtained in the study was $3892 \text{ mPa}\cdot\text{s}$ on the condition of
 338 $0.4 \text{ g}\cdot\text{g}^{-1}$ at 85°C for 30 minutes and the minimum was $26 \text{ mPa}\cdot\text{s}$ with $0.1 \text{ g}\cdot\text{g}^{-1}$
 339 subjected to 65°C for 30 minutes. The variance analysis for RV showed
 340 statistically significant effects for concentration ($P < 0.001$) and thermal
 341 processing ($P < 0.001$) for the WPC. The maximum value of RV obtained in the
 342 study was $2086 \text{ mPa}\cdot\text{s}$ on the condition of $0.4 \text{ g}\cdot\text{g}^{-1}$ at 95°C for 5 minutes and at
 343 the minimum was $2 \text{ mPa}\cdot\text{s}$ with $0.1 \text{ g}\cdot\text{g}^{-1}$ subjected to 65°C for 30 minutes. The
 344 analysis of variance for FV showed statistically significant effects for
 345 concentration ($P < 0.001$) and thermal processing ($P < 0.005$) for WPC. The

346 maximum value obtained in the study was 10105 mPa.s under the condition of 0.4
 347 $\text{g}\cdot\text{g}^{-1}$ at 85°C for 15 minutes and the minimum was 6 mPa.s with 0.1 $\text{g}\cdot\text{g}^{-1}$
 348 subjected to 65°C for 30 minutes. The magnitude of the effects of concentration
 349 and thermal processing on PV, RV and FV were statistically significant ($P <$
 350 0.05), being the concentration of 0.4 $\text{g}\cdot\text{g}^{-1}$ and binomial 95°C for 5 minutes and
 351 85°C for 15 minutes the most likely to show higher values of viscosity. The
 352 analysis of variance for η/t showed statistically significant effects for concentration
 353 ($P < 0.001$) and thermal processing ($P < 0.001$). These results demonstrate the
 354 influence of these variables on viscosity obtained after retention. The maximum
 355 value of η/t obtained in the study was 3062 on condition of 0.4 $\text{g}\cdot\text{g}^{-1}$ at 85°C for
 356 15 minutes and the minimum was 11 with 0.1 $\text{g}\cdot\text{g}^{-1}$ subjected to 65°C for 30
 357 minutes. The magnitude of the effects of concentration and thermal processing on
 358 PV, RV and FV can be verified by η/t , demonstrating to be statistically significant
 359 ($P < 0.05$), with a concentration of 0.4 $\text{g}\cdot\text{g}^{-1}$ and the binomial 95°C for 5 minutes
 360 and 85°C for 15 minutes the most likely to exhibit higher values of viscosity. This
 361 similarity among the results obtained for η/t with viscosities PV, RV and FV is
 362 deduced when analyzing the correlation coefficients shown in Table 7.

363 In this study, we can infer that increases in viscosity observed in different
 364 simulations of thermal processing had no direct contribution on denaturation of
 365 casein molecules, since they have almost no secondary or tertiary structures. As
 366 described by Walstra *et al.* (2001), caseins do not suffer **denaturation** by heating at
 367 temperatures below 100°C, which occurs only at higher temperatures. This way,
 368 one can assign the increased viscosity of the solutions of MPPs during processing

369 to thermal denaturation of whey protein and their association with the casein
370 micelles. A common behavior in many assessed simulations was $PV > RV$, ie, the
371 mean peak viscosities were higher than the average of the viscosities of retention.
372 We can attribute this loss of viscosity to two factors. The first is the physical
373 stress to which the solutions are subjected during the whole time of analysis (15.7
374 $\text{rad}\cdot\text{s}^{-1}$), causing the protein structure to lose part of its ability to interact with
375 water due to the mechanical energy applied and the flow of product within the tub
376 for heating. The second is related to the aggregation of β -LG and also aggregate
377 formation of β -LG / α -LA that will occur at a rate associated with duration of
378 exposure to high temperature. During the retention time, the whey proteins will
379 unfold in a short period of time, thus presenting opportunity for more monomers
380 of unfolded β -LG self-aggregate (OLDFIELD *et al.* 1998), therefore decreasing
381 the interaction with water, thereby lowering the viscosity of the solution.

382 During the cooling step, another common effect to various tests was
383 substantial increase in viscosity until the final temperature of 30°C . The increased
384 viscosity of the protein solutions during cooling is an attributed behavior not only
385 to the decrease in molecular mobility due to decreased energy system, but also to
386 the whey protein combinations to the surfaces of casein micelles during thermal
387 processing, modifying their characteristics, and forming larger structures more
388 hydrophilic, being capable of influencing the functionality of some dairy protein
389 products (SINGH, 2009).

390 The 3^3 Box-Behnken factorial design was conducted in simulated
391 conditions with the WPC in RVA and Table 8 presents a coded matrix and results.

392 Analyzing the results in Table 8, it appears that the highest value obtained for FV
 393 (8578 mPa·s) was provided on the condition of 0.4 g·g⁻¹ at 95°C for 15 minutes
 394 and the lowest FV (29 mPa·s) on the condition of 0.2 g·g⁻¹ at 65°C for 15 minutes.
 395 These results demonstrate the influence of these three variables in the thermal
 396 denaturation of whey protein of WPC. One FV of 8293 mPa·s close to the
 397 maximum achieved in prior condition was on the condition of 0.4 g·g⁻¹ at 85°C for
 398 5 minutes, showing that it is possible to optimize the application of energy and
 399 keep a final equivalent result in aspect of the desired viscosity. Regression
 400 analysis by response surface for final viscosity was performed using full quadratic
 401 model. The estimators of population parameters of the model were calculated by
 402 the minimum squares method X and Y matrices used to determine the estimators
 403 are shown in Figure 2.

404
 405 TABLE 8 Coded matrix for 3³ Box-Behnken design and results of final viscosity -
 406 FV (mPa·s)

Experiment	Time	Temperature	Concentration	Final viscosity (mPa.s)
1	-1	-1	0	150
2	1	-1	0	669
3	-1	1	0	3119
4	1	1	0	4380
5	-1	0	-1	1827
6	1	0	-1	2198
7	-1	0	1	8293
8	1	0	1	2752
9	0	-1	-1	29
10	0	1	-1	2139
11	0	-1	1	932
12	0	1	1	8578
13	0	0	0	3180
14	0	0	0	3188
15	0	0	0	2595

407 Time (minutes): (-1) 5; (0) 15; (+1) 30; Temperature (°C): (-1) 65; (0) 85; (+1) 95;
 408 Concentration (g·g⁻¹): (-1) 0.2; (0) 0.3; (+1) 0.4
 409

$$X = \begin{bmatrix} M & X_1 & X_2 & X_3 & X_1^2 & X_2^2 & X_3^2 & X_{12} & X_{13} & X_{23} \\ +1 & -1 & -1 & 0 & +1 & +1 & 0 & +1 & 0 & 0 \\ +1 & +1 & -1 & 0 & +1 & +1 & 0 & -1 & 0 & 0 \\ +1 & -1 & +1 & 0 & +1 & +1 & 0 & -1 & 0 & 0 \\ +1 & +1 & +1 & 0 & +1 & +1 & 0 & +1 & 0 & 0 \\ +1 & -1 & 0 & -1 & +1 & 0 & +1 & 0 & +1 & 0 \\ +1 & +1 & 0 & -1 & +1 & 0 & +1 & 0 & -1 & 0 \\ +1 & -1 & 0 & +1 & +1 & 0 & +1 & 0 & -1 & 0 \\ +1 & +1 & 0 & +1 & +1 & 0 & +1 & 0 & +1 & 0 \\ +1 & 0 & -1 & -1 & 0 & +1 & +1 & 0 & 0 & +1 \\ +1 & 0 & +1 & -1 & 0 & +1 & +1 & 0 & 0 & -1 \\ +1 & 0 & -1 & +1 & 0 & +1 & +1 & 0 & 0 & -1 \\ +1 & 0 & +1 & +1 & 0 & +1 & +1 & 0 & 0 & +1 \\ +1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ +1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ +1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix} \quad Y = \begin{bmatrix} V_F \\ 150 \\ 669 \\ 3119 \\ 4380 \\ 1827 \\ 2198 \\ 8293 \\ 2752 \\ 29 \\ 2139 \\ 932 \\ 8578 \\ 3180 \\ 3188 \\ 2595 \end{bmatrix}$$

Where:

- X_1 is the time factor;
- X_2 is the temperature factor;
- X_3 is the concentration factor;
- X_1^2 is quadratic parameter for time;
- X_2^2 is quadratic parameter for temperature;
- X_3^2 is quadratic parameter for concentration;
- X_{12} is the interaction between time and temperature;
- X_{13} is the interaction between time and concentration;
- X_{23} is the interaction between temperature and concentration.

410

411 *FIGURE 2 Matrices X and Y used to determine the estimators*

412

413 Table 9 shows calculated values for the coefficients and p-values for the
 414 adjusted model. Using a significance level of 4%, it is considered that a factor
 415 affects the response if the coefficients differ from zero or the p-value is less than
 416 0.04 (RAGONESE *et al.* 2002).

417

418

419 TABLE 9 Estimation of the final viscosity model - FV

Term	Final viscosity - FV		
	Coefficient	Standard error ($\pm$)	<i>p</i> -value
Constant	$2.99 \cdot 10^3$	$1.96 \cdot 10^2$	$4.29 \cdot 10^{-3}$
Time	$-4.24 \cdot 10^2$	$1.20 \cdot 10^2$	$7.19 \cdot 10^{-2}$
Temperature	$2.05 \cdot 10^3$	$1.20 \cdot 10^2$	$3.41 \cdot 10^{-3}$
Concentration	$1.80 \cdot 10^3$	$1.20 \cdot 10^2$	$4.46 \cdot 10^{-3}$
Time x Time	$-3.01 \cdot 10^1$	$1.77 \cdot 10^2$	$8.81 \cdot 10^{-1}$
Temperature x Temperature	$-8.78 \cdot 10^2$	$1.77 \cdot 10^2$	$3.83 \cdot 10^{-2}$
Concentration x Concentration	$8.10 \cdot 10^2$	$1.77 \cdot 10^2$	$4.46 \cdot 10^{-2}$
Time x Temperature	$1.86 \cdot 10^2$	$1.70 \cdot 10^2$	$3.89 \cdot 10^{-1}$
Time x Concentration	$-1.48 \cdot 10^3$	$1.70 \cdot 10^2$	$1.30 \cdot 10^{-2}$
Temperature x Concentration	$1.38 \cdot 10^3$	$1.70 \cdot 10^2$	$1.48 \cdot 10^{-2}$

420

421 The results presented in Table 9 show that the terms: temperature,
422 concentration, temperature versus temperature, time versus concentration and
423 temperature versus concentration are significant for FV at a level of 96% of
424 confidence. In this way, all the variables affect the results of final viscosity. The
425 model adjustment was assessed by analysis of variance (ANOVA). The adjusted
426 regression model does not have the lack of adjustment in the confidence level of
427 96%, since the *p*-value was of 0.046 value for the ratio between the mean square
428 due to the lack of adjustment and the quadratic mean and due to the pure error
429 with three and two degrees of freedom, respectively. Thus it was possible to
430 obtain nine response surfaces for the final viscosity model as represented in
431 FIGURES 3, 4 and 5. It is possible to observe that the highest final viscosity
432 values come in different regions, depending on the variable that is used as a

constant. Analyzing the models with constant concentrations of WPC (FIGURES 3, 4 and 5) at the lowest concentration ($0.2 \text{ g}\cdot\text{g}^{-1}$), the highest values of FV are obtained in the high level region for the time and for medium and high temperature (+1, 0, +1) (FIGURE 3). On the average level of concentration ($0.3 \text{ g}\cdot\text{g}^{-1}$), it is possible to obtain higher values of FV (Figure 4) in the three time levels and in the medium and high levels of temperature (-1, 0, +1; 0, +1). Considering the high level of concentration ($0.4 \text{ g}\cdot\text{g}^{-1}$), one obtains the highest values of FV (FIGURE 4) in the region of negative and positive levels respectively for time and temperature (-1, +1). Similar analyzes can be made for models with constant processing temperatures (Figures 3, 4 and 5). In the three temperature levels (65°C , 85°C and 95°C), the highest values FV (FIGURES 3, 4 and 5 respectively) are obtained in the regions of negative levels for the time and positive levels for the concentration (-1, +1). As it can be seen in the models for the constant concentrations, in these models the FVs also have the tendency to increase in absolute values as the temperature levels vary from the lowest (65°C) to the highest (95°C). In all three areas where time was kept constant (FIGURES. 3, 4 and 5), the highest values of FV are obtained in the regions of positive levels for the temperature and concentration (+1, +1). In these cases, there is an inverse tendency of the behavior of FV in relation to the models of constant temperature. A decrease in the absolute values of the final viscosities occurs according to the variation of the levels of time from the lowest (5 min) to the highest (30 minutes). This behavior can be attributed to the denaturation kinetics of whey protein. In the positive levels of temperature and concentration, the WPC solution with $0.4 \text{ g}\cdot\text{g}^{-1}$

456 of dry basis in water in a condition of 95°C has a great effect of thermal
457 denaturation of whey protein due to high temperature and high concentration of
458 proteins.

459 The interactions of various reactive groups such as thiol, or the
460 hydrophobic moieties exposed on the surface of protein molecules, leading to the
461 formation of covalent or non-covalent intermolecular bonds, which result in
462 protein and / or polymerization aggregation. Such molecular changes alter the
463 functional characteristics of proteins such as hydration, solubility, solution
464 viscosity, film formation, and gelation adsorption at the interface between
465 aqueous and lipid phases (DAMODARAN 1996).

466 The rapid and significant increase in the viscosity of solutions of the MPPs
467 in RVA during heating, resulting often in tilting viscosity can be explained with
468 respect to denaturation of whey proteins to be considered as cooperation changes:
469 the rupture of some of the bonds within molecule causes the remaining bonds to
470 become less stable and many are broken simultaneously. This explains why the
471 denaturation occurs in a very narrow range of temperature and concentration
472 (WALSTRA et al. 2001).

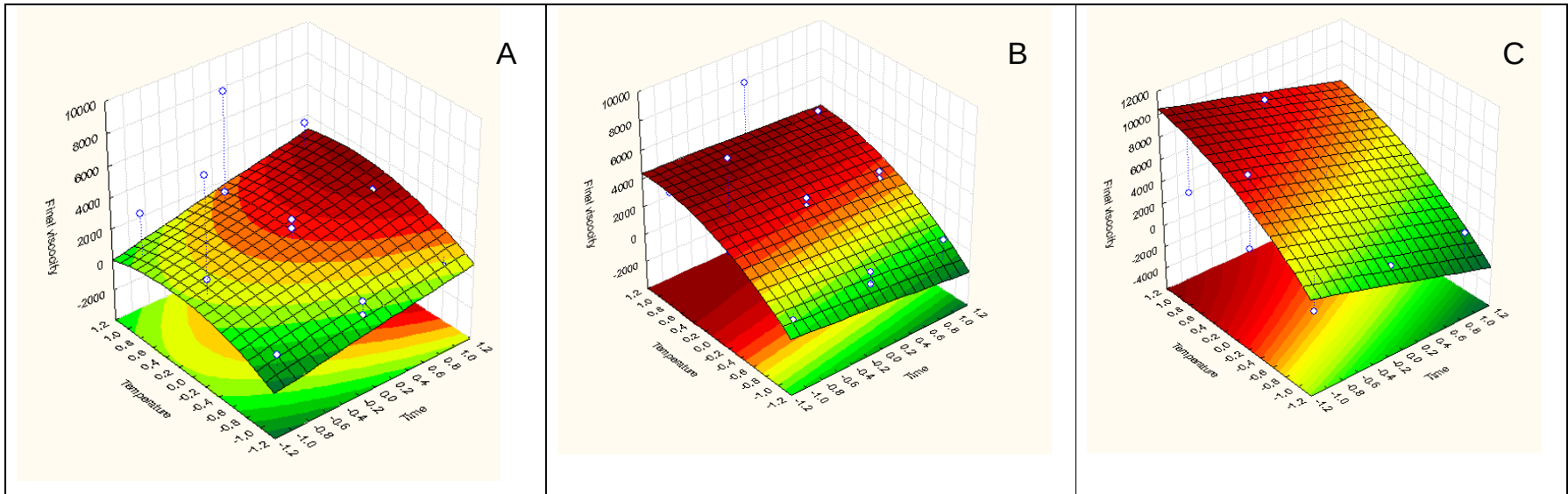
473 Dannenberg and Kessler (1988) showed how the kinetics of thermal
474 denaturation of whey proteins has a significant effect at temperatures above 90°C,
475 especially in β -LG and α -LA. The change in protein conformation increases the
476 available surface area and exhibits peptides and amino acid side chains, before
477 hidden that in turn can interact with water (KINSELLA, 1982) thereby
478 increasing the viscosity. However, due to absence of other proteins such as

479 caseins, self-aggregation of β -LG and aggregation of β -LG/ α -LA occur at a rate
480 associated with the time of exposure to high temperature. Thus, it is observed that
481 this type of interaction has a negative effect on the increase of viscosity. At higher
482 temperatures and faster heating rates, all whey proteins of milk start to unfold in a
483 short period of time, thereby presenting more opportunity for the unfolded β -LG
484 monomers self-aggregate (OLDFIELD *et al.* 1998), and thus a decrease in
485 interaction with water is expected.

486

487

488

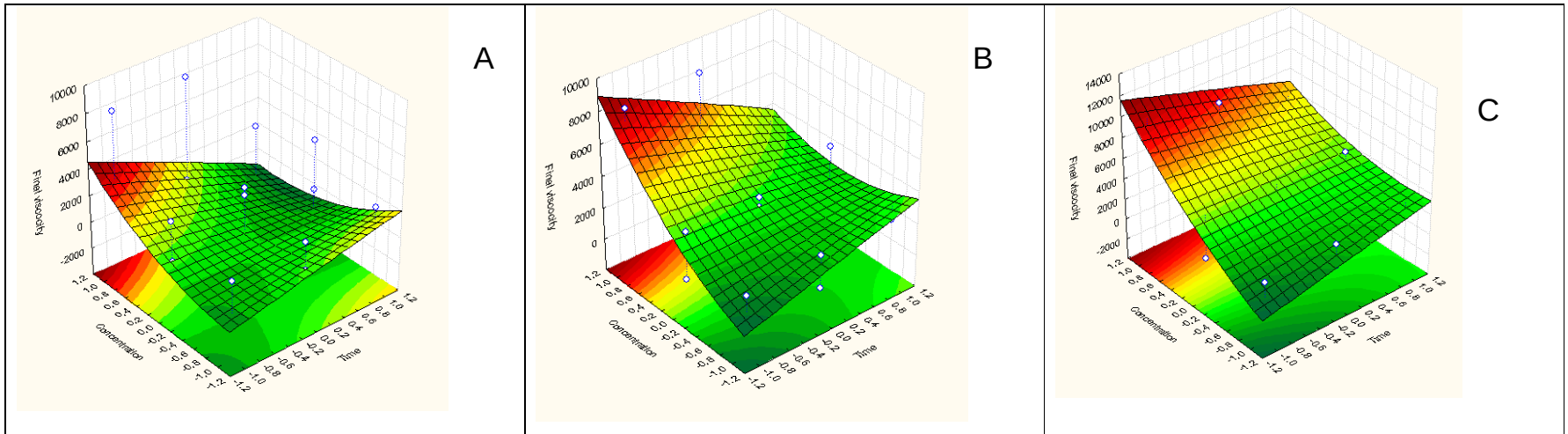


(A) $z=2987.7-423.75*x-30.1*x^2+2054.5*y-878.1*y^2+185.5*x*y-1478.*(-1.)*x+1384.*(-1.)*y-985.3$

(B) $z=2987.7-423.75*x-30.1*x^2+2054.5*y-878.1*y^2+185.5*x*y-1478.*0.*x+1384.*0.*y+0.$

(C) $z=2987.7-423.75*x-30.1*x^2+2054.5*y-878.1*y^2+185.5*x*y-1478.*1.*x+1384.*1.*y+2605.2$

FIGURE 3 Response surfaces obtained for final viscosity (FV) under the simulated conditions for WPC on RVA: (A) constant concentration on level -1 (0,2 g.g⁻¹), (B) constant concentration on level 0 (0,3 g.g⁻¹) and (C) constant concentration on level +1 (0,4 g.g⁻¹)

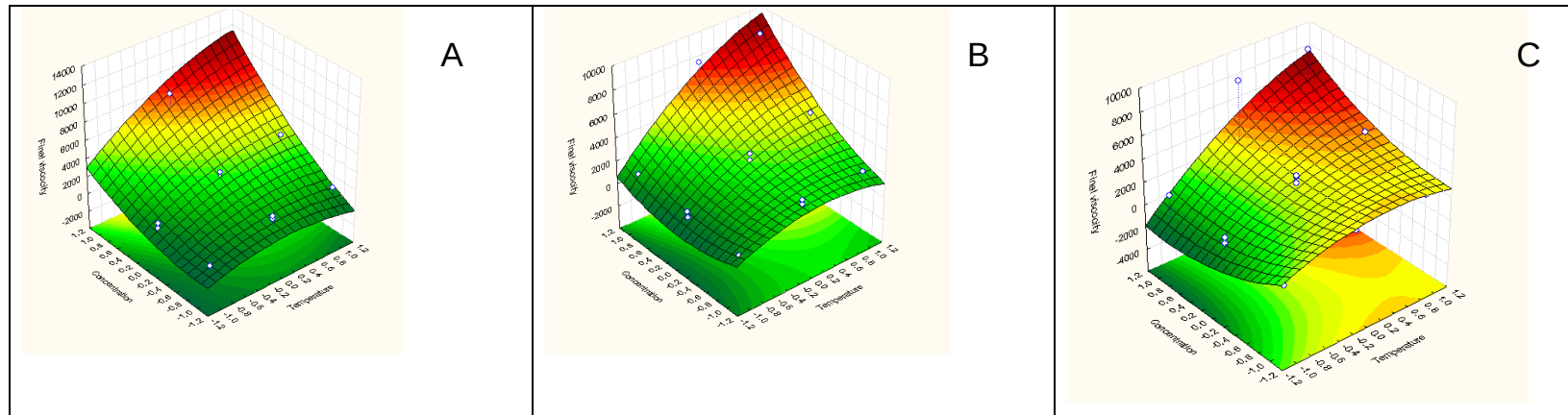


(A) $z=2987.7-423.75*x-30.1*x^2+1795.25*y+809.9*y^2+185.5*(-1)*x-1478.*x*y+1384.*(-1)*y-2932.6$

(B) $z=2987.7-423.75*x-30.1*x^2+1795.25*y+809.9*y^2+185.5*0.*x-1478.*x*y+1384.*0.*y+0.$

(C) $z=2987.7-423.75*x-30.1*x^2+1795.25*y+809.9*y^2+185.5*1.*x-1478.*x*y+1384.*1.*y+1176.4$

FIGURE 4 Response surfaces obtained for final viscosity (FV) under the simulated conditions for WPC on RVA: (A) constant temperature on level -1 (65°C), (B) constant temperature on level 0 (85°C) and (C) constant temperature on level +1 (95°C)



- (A) $z=2987.7+2054.5*x-878.1*x^2+1795.25*y+809.9*y^2+185.5*(-1.)*x-1478.*(-1.)*y+1384.*x*y+393.7$
 (B) $z=2987.7+2054.5*x-878.1*x^2+1795.25*y+809.9*y^2+185.5*0.*x-1478.*0.*y+1384.*x*y+0.$
 (C) $z=2987.7+2054.5*x-878.1*x^2+1795.25*y+809.9*y^2+185.5*1.*x-1478.*1.*y+1384.*x*y-453.8$

FIGURE 5 Response surfaces obtained for final viscosity (FV) under the simulated conditions for WPC on RVA: (A) constant time under level -1 (5 minutes), (B) constant time under level 0 (15 minutes) and (C) constant time under level +1 (30 minutes)

518 With the aim of obtaining an exploratory analysis by looking for patterns
 519 with all MPPs, the results of the three types of heat processing were organized in
 520 three different matrices, with treatments (MPP type and concentration) lines and
 521 the average readings of viscosity of the entire viscographic profile in the columns.
 522 Therefore, we obtained the matrix 1(24 x 375), matrix 2 (24 x 490) and matrix 3
 523 (24 x 646) for the high, medium and low heat treatments respectively, 'number of
 524 treatments x number of mean viscosities read'. Assays were identified in the order
 525 that they are presented in TABLE 10.

526

527 TABLE 10 Identification of the experiments with MPPs on RVA

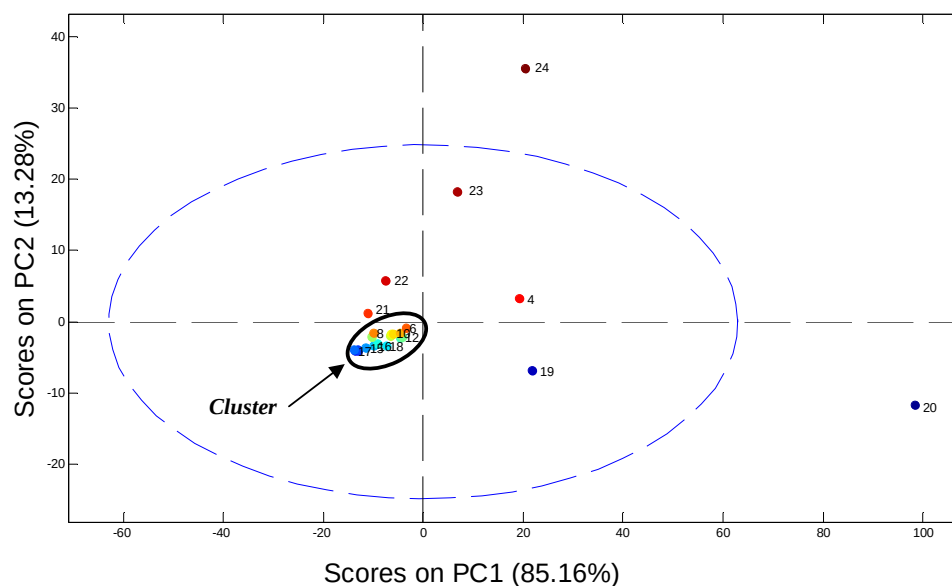
MPP type	Dry matter concentration (g·g ⁻¹)	Identification
Skimmed milk powder – SMP	0.5	1
	0.6	2
	0.7	3
	0.8	4
Milk protein concentrate – MPC ₁	0.1	5
	0.12	6
	0.14	7
	0.16	8
Whey powder – WP	0.5	9
	0.6	10
	0.7	11
	0.8	12
Demineralized whey powder – DWP	0.5	13
	0.6	14
	0.7	15

	0.8	16
Whey protein concentrate - WPC	0.1	17
	0.2	18
	0.3	19
	0.4	20
Milk protein concentrate – MPC ₂ (without prior hydration)	0.3	21
	0.35	22
	0.40	23
	0.45	24

528

529 The use of Principal Component Analysis made it possible for the joint
530 evaluation of 9000, 11760 and 15504 experimental data and for the high, medium
531 and low heat treatment respectively. This collection of data makes the information
532 provided by PCA significant under a relevant analytical point of view. FIGURE 6
533 shows the PCA scores in the first two components to the low heat treatment
534 (matrix 3), representing 98.44% of the total variance of the data. With the
535 projection of two main components, it is possible to observe the formation of
536 different groups among the MPPs samples. In the positive quadrant for PC1 and
537 PC2, it is observed that tests 4, 23 and 24 are in the same region determined by
538 the first main component, which explains the greater variety of samples (85.16%).
539 Respectively, we have the rehearsals $0.8 \text{ g} \cdot \text{g}^{-1}$ of dry matter of the SMP, $0.4 \text{ g} \cdot \text{g}^{-1}$
540 and $0.45 \text{ g} \cdot \text{g}^{-1}$ of dry matter from MPC₂. In the positive quadrant for PC1 and
541 negative for PC2, it is seen the samples 19 and 20 ($0.3 \text{ g} \cdot \text{g}^{-1}$ and $0.4 \text{ g} \cdot \text{g}^{-1}$ of dry
542 matter of WPC) in the same region for PC2. The positive quadrant for PC2 and
543 negative for PC1 presents tests 21 and 22 ($0.3 \text{ g} \cdot \text{g}^{-1}$ and $0.35 \text{ g} \cdot \text{g}^{-1}$ of dry basis

544 from MPC2) and in the negative for PC1 and PC2 all other experiments in a
 545 cluster.



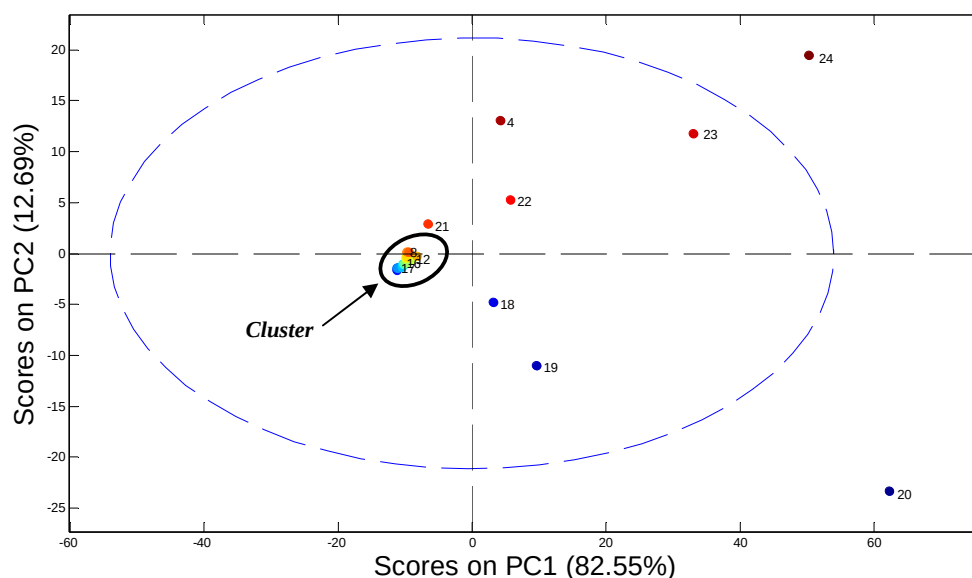
546

547 *FIGURE 6 Graphic of score for PC1 vs. PC2 under the low treatment conditions*
 548 *(65°C for 30 minutes). Where: 4 is equal to SMP (0.29 g.g⁻¹ of protein), 19 is*
 549 *equal to WPC (0.24 g.g⁻¹ of protein), 20 is equal to WPC (0.33 g.g⁻¹ of protein),*
 550 *21 is equal to MPC (0.22 g.g⁻¹ of protein), 22 is equal to SMP (0.26 g.g⁻¹ of*
 551 *protein), 23 is equal to SMP (0.30 g.g⁻¹ of protein), 24 is equal to SMP (0.34 g.g⁻¹*
 552 *of protein).*

553

554 It is possible to see in Figure 6 that the PC1 identified the influence of low
 555 heat treatment on product solutions with the highest protein content and PC2
 556 differentiated the influence of heat treatment on the origin of MPPs (milk or
 557 whey), except for those involved in cluster due to the low protein concentration.
 558 The protein content of separation in PC1 for MPPs originated form of milk (upper
 559 right quadrant of the graph) was 0.29 g.g⁻¹ of protein for SMP and 0.30 g.g⁻¹

560 protein to MPC2, close values considering the difference in concentration of dry
 561 matter of trial 4 ($0.8 \text{ g}\cdot\text{g}^{-1}$ dry matter from SMP) and 23 ($0.3 \text{ g}\cdot\text{g}^{-1}$ dry matter from
 562 MPC2). For the whey originated, the content of separation in PC1 was $0.24 \text{ g}\cdot\text{g}^{-1}$
 563 protein from WPC, indicating a higher influence of the low heat treatment on the
 564 components of WPC over the SMP and MPC2. Tests 4, 19 and 24 are in the same
 565 region of PC1, indicating their similarity in viscographic characteristics, these
 566 being SMP, WPC and MPC2, respectively. FIGURE 7 shows the PCA scores in
 567 the first two components to medium thermal treatment (matrix 2), representing
 568 95.24% of the total variance of the data. With the projection of two main
 569 components, it is also possible to observe the formation of different groups among
 570 the MPPs samples.



571
 572 *FIGURE 7 Graphic of score for PC1 vs. PC2 under the medium treatment*
 573 *conditions (85°C for 15 minutes). Where: 4 is equal to SMP ($0.29 \text{ g}\cdot\text{g}^{-1}$ of*
 574 *protein), 19 is equal to WPC ($0.24 \text{ g}\cdot\text{g}^{-1}$ of protein), 20 is equal to WPC ($0.33 \text{ g}\cdot\text{g}^{-1}$*
 575 *of protein), 21 is equal to MPC ($0.22 \text{ g}\cdot\text{g}^{-1}$ of protein), 22 is equal to SMP (0.26*

576 $g \cdot g^{-1}$ of protein), 23 is equal to SMP ($0.30 g \cdot g^{-1}$ of protein), 24 is equal to SMP
 577 ($0.34 g \cdot g^{-1}$ of protein).

578

579 For the medium heat treatment, in the positive quadrant for PC1 and PC2,
 580 it is observed that besides the trials 4, 23 and 24 which were already in the low
 581 heat treatment, the test 22 is also presented in the same region determined by the
 582 first main component for the SMP at $0.8 g \cdot g^{-1}$ of dry matter. It is also observed,
 583 displacement of the experiments 23 and 24 for the right side of the quadrant, thus
 584 clarifying the influence of heat treatment on average MPC2 for the concentration
 585 of $0.4 g \cdot g^{-1}$ and $0.45 g \cdot g^{-1}$ of dry matter. In the positive quadrant for PC1 and
 586 negative for PC2, it is also seen the insertion of a further test (number 18, WPC
 587 $0.16 g \cdot g^{-1}$ of dry weight) besides the 19 and 20 ($0.3 g \cdot g^{-1}$ and $0.4 g \cdot g^{-1}$ dry matter
 588 from WPC) in different regions for PC2. The positive quadrant for PC2 and the
 589 negative for PC1 shows only the test 21 ($0.3 g \cdot g^{-1}$ of dry matter MPC2) and in the
 590 negative for PC1 and PC2 all other tests in a cluster. It is possible to see in Figure
 591 7 that the PC1 continued identifying the influence of heat treatment on product
 592 solutions with the highest protein content and PC2 differentiating the influence of
 593 heat treatment on the origin of MPPs (milk or whey), except for those involved in
 594 cluster due to low protein concentration. The protein content of the separation in
 595 PC1 for the MPPs originated from milk (upper right quadrant of the graph) was
 596 $0.29 g \cdot g^{-1}$ protein from SMP and $0.26 g \cdot g^{-1}$ of protein for MPC2, indicating that
 597 the MPC2 presents greater influence of heat treatment compared to the average
 598 SMP for the concentration ranges studied. For those originated of whey, the
 599 content of separation in PC1 was $0.16 g \cdot g^{-1}$ protein WPC, indicating a higher

influence of the medium heat treatment on the components of the product compared to the low thermal processing. These three tests are in the same region of PC1, indicating similarity in the viscographic characteristics of them. The FIGURE 8 shows the PCA scores in the first two components to the medium thermal treatment (matrix 1), representing 94.59% of total variation of data. With the projection of two main components, one can observe the formation of different groups among the MPPs samples.

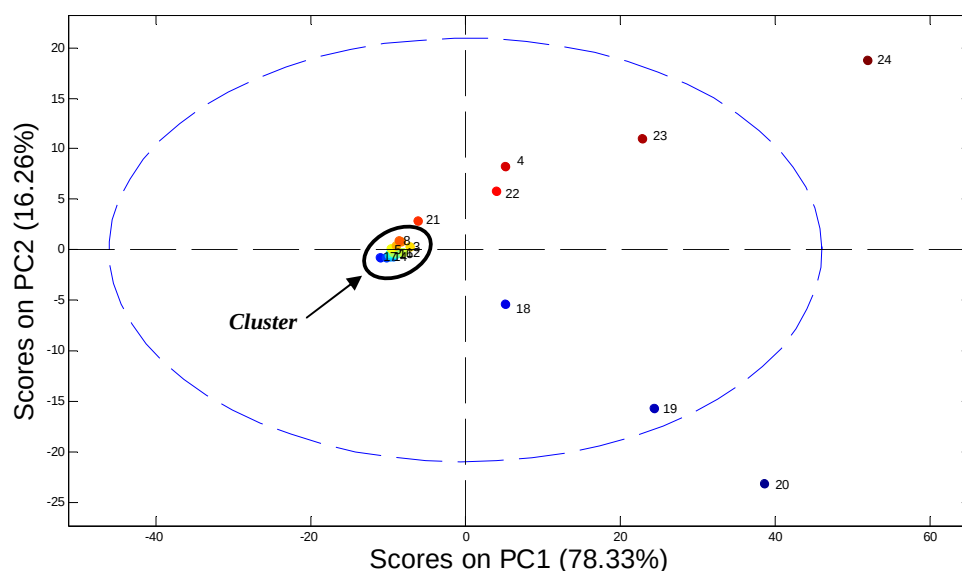


FIGURE 8 Graphic of score for PC1 vs. PC2 under the high treatment conditions (95°C for 5 minutes). Where: 4 is equal to SMP (0.29 g.g⁻¹ of protein), 19 is equal to WPC (0.24 g.g⁻¹ of protein), 20 is equal to WPC (0.33 g.g⁻¹ of protein), 21 is equal to MPC (0.22 g.g⁻¹ of protein), 22 is equal to SMP (0.26 g.g⁻¹ of protein), 23 is equal to SMP (0.30 g.g⁻¹ of protein), 24 is equal to SMP (0.34 g.g⁻¹ of protein).

The provision of test scores in the graph to the high heat treatment does not differ much compared to medium heat treatment. However, it is possible to

notice similarities among the trials 4, 22 and 18 (respectively $0.8 \text{ g}\cdot\text{g}^{-1}$ of dry matter from SMP, $0.35 \text{ g}\cdot\text{g}^{-1}$ dry matter from MPC2, and $0.2 \text{ g}\cdot\text{g}^{-1}$ of dry matter from WPC), and also tests 23 and 19 ($0.4 \text{ g}\cdot\text{g}^{-1}$ of dry matter from MPC2 and $0.3 \text{ g}\cdot\text{g}^{-1}$ of dry matter from WPC) in PC1. It is possible to see in FIGURE 8 that the PC1 continued identifying the influence of the heat treatment on the solutions of products with higher protein content, determining, inclusive, similarity of the evolution of the viscosity among the SMP, MPC WPC at concentrations of $0.29 \text{ g}\cdot\text{g}^{-1}$, $0.26 \text{ g}\cdot\text{g}^{-1}$ and $0.16 \text{ g}\cdot\text{g}^{-1}$ of protein respectively, besides the MPC and WPC $0.30 \text{ g}\cdot\text{g}^{-1}$ and $0.24 \text{ g}\cdot\text{g}^{-1}$ protein also for PC1. The products, which were arranged in the cluster, did not have their influence characteristics of the heat treatment well defined due to the low protein concentration, indicating that this method of analysis explains better for the products of high protein in the solution, refer to the solutions of MPC1 are also attached to the cluster. For the three thermal processing, it was possible to use the PCA graphs to identify if the origin of the MPP was of milk or whey in solutions of high protein content. It was also possible to notice the similarities of the influences of heat treatment on the development of viscosity in solutions of the SMP, MPC2 and WPC and, making it possible to determine the protein concentration required to obtain the same evolution of viscosity among the solutions of these products.

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639 4. CONCLUSION

640 Based on this work and the results obtained, it can be concluded that:

- 641 - The contents of dry matter and protein contributed to the increased viscosity of
- 642 the solutions of MPPs, when subjected to thermal processing;
- 643 - The variables of time and temperature of heat treatment influenced the
- 644 viscographic profiles, the viscosities peak, retention and final solutions of MPPs;
- 645 - The MPPs viscographic profiles were adequate to reveal the characteristics of
- 646 functionality during and after the heat treatment;
- 647 - The principal components analysis of the viscographic profiles during thermal
- 648 processing solutions have demonstrated that the MPP was originated from milk or
- 649 whey;
- 650 - The RVA was convenient for the simulation of thermal processing of solutions
- 651 for MPPs.

652

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