

Original Research Article

STUDY OF THE BEHAVIOR OF DAIRY PROTEIN IN DIFFERENT

CONDITIONS OF THERMAL PROCESSING USING CHEMOMETRIC

APPROACH

ABSTRACT: The objective of this study was to evaluate the viscosity evolution behavior of the Milk protein products (MPPs) solutions in different concentrations, when 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 solid-liquid ratio solutions evaluated 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. The 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. Results were interpreted according to the viscosities during heating and cooling. It was possible to optimize the different conditions of parameters of the MPPs with the interpretations of the viscographic profiles obtained in respect to the thermal behavior of proteins, especially for the SMP, MPC and WPC. The data showed similarity in viscographic characteristics of the

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

solutions when evaluated at 65°C for 30 minutes at concentrations 0.29 g·g⁻¹, 0.34 g·g⁻¹ and 0.24 g·g⁻¹ of protein in water respectively, 0.29 g·g⁻¹, 0.26 g·g⁻¹ and 0.16 g·g⁻¹ of protein in water to these MPPs at 85°C for 15 minutes and 95°C for 5 minutes, and also 0.30 g·g⁻¹ and 0.24 g·g⁻¹ of protein in water respectively for the MPC and WPC in the same condition of 95°C for 5 minutes.

KEYWORDS: milk protein, functionality, viscosity.

1. INTRODUCTION:

Dairy protein products can be defined as concentrated or dried milk products which when added to dairy products or other food processed as ingredients contribute to the improvement of the nutritional composition and also functionality during and after processing (SINGH, 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 salts, pH, ionic strength, temperature, degree of denaturation and aggregation, and formation of disulfide bridges (DAMODARAN 1996; GARCIA et al. 1997).

The Rapid Visco TM Analyser (RVATM) or Fast Viscosity Analyzer is a rotational viscometer capable of measuring continuously the viscosity of a sample under controlled temperature (BOOTH; BASON, 2007).

This study had as goal to investigate the influence of different thermal processing conditions on the evolution of the viscosity of dairy protein dehydrated

after adequate rehydration, using the simulator as Rapid Viscosity Analyzer (RVA).

2. MATERIALS AND METHODS:

It was selected five types of dehydrated MPPs for evaluation, being the selection criteria: industrial use in Brazil, the national industrial production, the current economic importance, the extent of use in different areas and prospecting for future use.

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 kindly acquired and provided by the 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 in Laboratory of Products Analyzes, Gemacom Tech Company. Thus, the following analyzes were determined for the products shown in TABLE 1.

TABLE 1 Methods of analysis for each parameter of the 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	Calculated	Calculated	Calculated	Calculated	Calculated

basis					
Lactose	Calculated	Calculated	Calculated	Calculated	Calculated
Ash	IN n° 68 de	AOAC	AOAC	AOAC 15 th ,	AOAC
	12/12/2006	900.02	15 th , 1990	1990	900.02
Acidity	FIL	FIL	FIL 81:	FIL 81: 1981	n.a. ¹
	81:1981	81:1981	1981		
Chloride	n.a. ¹	n.a. ¹	n.a. ¹	LANARA	n.a. ¹
pH	n.a. ¹	AOAC	Adolfo	Adolfo Lutz	AOAC
		981.12	Lutz		981.12
Specific mass	n.a. ¹	C314 VK	Niro	Niro Atomizer	C314 VK
		Density	Atomizer		Density
		Tapper			Tapper
Total count of	FIL	AOAC	FIL	FIL	AOAC
mesophilic	100B:1991	989.10	100B:1991	100B:1991	989.10
aerobic					
Coliforms at	APHA	AOAC	APHA	APHA 1992	AOAC
30°C	1992	989.10	1992		989.10
<i>Escherichia</i>	APHA	AOAC	APHA	APHA 1992	AOAC
<i>coli</i>	1992	989.10	1992		989.10
Fungi and	FIL 94B:	AOAC	FIL 94B:	FIL 94B: 1990	AOAC
yeasts	1990	989.10	1990		989.10
Coagulase	FIL	AOAC	FIL	FIL 138:1986	AOAC
positive	138:1986	2003.08	138:1986		2003.08
staphylococci					
<i>Salmonella sp</i>	FIL	AOAC	FIL	FIL 93B:1995	AOAC
	93B:1995	967.25	93B:1995		967.25

63 ¹ not analysed

64

65 Three processing levels (low, medium and high) were used to simulate different

66 thermal processing in each MPP sample, which were selected for being the most

67 used in the processing of cheese, fermented milk, petitsuisse, pasteurized cream,

68 and flavored milk drinks, pasteurized by dairy industries: 65°C for 30 minutes

69 (low), 85°C for 15 minutes (medium) and 95°C for 5 minutes (high). As

70 recommended by Bennett et. al. (2007), all samples were previously hydrated

71 before being subjected to the RVA. Genuine replicates of the solutions were

72 prepared in duplicates using distilled water as solvent and constant stirring for 30

73 min at 25°C for MPPs. All these solutions have been prepared with 100 g water

74 and sufficient amount of MPP to obtain the desired concentration of dry basis (dry

75 basis g/water g). The dehydrated products were kept in airtight containers due to
 76 the hygroscopic characteristics of the samples, avoiding, thus, absorption of
 77 humidity. Before the preparation of each solution, moisture analyzes were
 78 performed to obtain the exact calculation of the amount of MPP to be used for its
 79 concentration of dry basis. The concentrations of evaluated solutions of each
 80 product are shown in TABLE 2.

81

82 TABLE 2 Solution concentrations of the MPPs evaluated in RVA

83

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

84 Due to the solubility characteristics of the MPC, thermal processing simulations
 85 were also performed without prior hydration, adding this MPP directly to water
 86 already in the tank of the equipment. Abbreviations MPC1 and MPC2 refer,
 87 respectively, to solutions of MPC with and without prior hydration and without
 88 prior hydration. In the simulations, 25 g of each solution were weighed
 89 beforehand in the heating tank of RVA. In the case of MPC, without prior
 90 hydration, calculations were made for the solution prepared directly in the bowl

also presented final mass of 25 g. To ensure full standardization of solutions rotation RVA was set to $104.7 \text{ rad}\cdot\text{s}^{-1}$ during the first 60 seconds at a constant temperature of 30°C . The solutions SMP, WP, DWP and WPC had the pH adjusted to 6.5 using $1 \text{ mol}\cdot\text{L}^{-1}$ solution of sodium hydroxide. After dissolution the pH of MPC solutions ranged from 6.7 to 6.9, and to those, there was no conduction to pH adjustment. The RVA equipment used in this work was the Model Series 4 (RVA-4) from NEWPORT SCIENTIFIC. The software used for data collection was the Thermocline for Windows, TCW. The configurations of the thermal processing simulations are shown in Table 3.

100

101 TABLE 3 Settings of thermal processing employed in RVA

Tempo	Set up	65°C for 30 minutes	85°C for 15 minutes	95°C for 5 minutes
0 min 00 s	Temperature ($^{\circ}\text{C}$)	30	30	30
0 min 00 s	Rotation ($\text{rad}\cdot\text{s}^{-1}$)	104.7	104.7	104.7
1 min 00 s	Rotation ($\text{rad}\cdot\text{s}^{-1}$)	15.7	15.7	15.7
1 min 00 s	Temperature ($^{\circ}\text{C}$)	30	30	30
6 min 24 s	Temperature ($^{\circ}\text{C}$)	65	85	95
36 min 24 s	Temperature ($^{\circ}\text{C}$)	65	85	95
39 min 06 s	Temperature ($^{\circ}\text{C}$)	30	30	30
44 min 06 s	Temperature ($^{\circ}\text{C}$)	30	30	30
44 min 07 s	End	X	X	X

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

103 The gradients of heating and cooling were also standardized between the levels,

104 being 6.5°C per minute to 13.0°C per minute respectively. Integration calculations

105 of viscographic profiles were performed in MICROCALTM ORIGIN[®] version

106 6.0 software. All integration calculations were made with the viscographic

107 profiles from the time 67 seconds, discarding, this way, the stirring time at a speed

108 of $104.7 \text{ rad}\cdot\text{s}^{-1}$ as described in step preparation of the samples. It was determined

109 in the experiment that the highest viscosity obtained during thermal processing
110 before the start of cooling would be represented by peak viscosity (PV), the
111 viscosity obtained at the end of the retention time of thermal processing would be
112 represented by retention viscosity (RV), the last viscosity reading obtained at the
113 end of the analysis would be represented by final viscosity (FV) and the value of
114 the integration of viscographic profile divided by the total time of analysis would
115 be represented by area per unit time ($\int/t$). To study the optimization of the
116 concentration, temperature and time in simulated conditions of thermal processing
117 in RVA, the WPC was selected among available MPPs for evaluations, for being
118 a high protein and high utilization product in the food, as an ingredient to assign
119 properties of functionality.

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

124

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

Factors	Factors levels		
	-1	0	+1
Time (minutes)	5	15	30
Temperature (°C)	65	85	95
Concentration ($\text{g}\cdot\text{g}^{-1}$)	0.2	0.3	0.4

126

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.

130

TABLE 5 Planning matrix for the WPC Box-Behnken³

Randomization	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

132

The obtained results were analyzed using the coefficient and regression correlation in order to conclude on the definition of the main factors to be used in interpreting the viscographic profile of MPPs in simulated conditions in RVA. Analyses of variance were also conducted to test differences in the studied variables with MPPs in relation to the concentration and thermal processing. It was applied the system for statistical analyses SAEG 9.1. For the regression

139 analysis, models that had a significance level less than or equal to 5% were
140 accepted as model and as linear and quadratic coefficients, when it was the case.
141 For the analysis of correlation coefficients were accepted with a significance level
142 equals to or less than 5%. The response surfaces and calculations of the effects
143 were obtained by employing the computer program STATISTIC 6.0 (Statsoft,
144 Inc.) and ANOVA, Microsoft Excel 2007 (Microsoft Co.). For the exploratory
145 analysis by type of thermal processing among the MPPs, the results of the RVA
146 were organized into matrices with treatments (MPP and concentration) in the lines
147 and the viscosity readings averages from all viscographic profile in columns and
148 then submitted to the Principal Component Analysis (PCA) from the covariance
149 matrix, using PLS Toolbox v.2.9.2.9 (Eigen Vector Research Inc., Wenatchee,
150 WA), operating in MATLAB (R2007B, vol. 7.5.0.342, Inc . the Mathworks,
151 Natick, MA) environment.

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153

154

155 **3. RESULTS AND DISCUSSION:**

156 The samples collected and used in this study presented their compositional and
157 microbiological characteristics consistent with data from literature (SINGH,
158 2009). The table 6 presents the MPP's chemical and microbiological composition.

159

160

161

162 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 ($\text{g}\cdot\text{kg}^{-1}$)	511	161.3	768.9	795.9	95.7
Ash ($\text{g}\cdot\text{kg}^{-1}$)	89.2	72.0	74.5	59.3	23.6
Acidity ($\text{g}\text{ ácido láctico}\cdot\text{L}^{-1}$)	1.4	1.5	1.7	1.4	n.a. ¹
Chloride ($\text{g}\cdot\text{kg}^{-1}$)	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 ($\text{g}\cdot\text{mL}^{-1}$)	n.a. ¹	0.52	0.74	0.63	0.43
Total count of mesophilic aerobic	$2.6\cdot 10^3$ UFC $\cdot\text{g}^{-1}$	$9.6\cdot 10^3$ UFC $\cdot\text{g}^{-1}$	$4.2\cdot 10^3$ UFC $\cdot\text{g}^{-1}$	$3.9\cdot 10^3$ UFC $\cdot\text{g}^{-1}$	$7\cdot 10^2$ UFC $\cdot\text{g}^{-1}$
Coliforms at 30°C	< 10 NMP $\cdot\text{g}^{-1}$	< 10 UFC $\cdot\text{g}^{-1}$	< 10 NMP $\cdot\text{g}^{-1}$	< 10 NMP $\cdot\text{g}^{-1}$	< 10 UFC $\cdot\text{g}^{-1}$
<i>Escherichia coli</i>	< 1 NMP $\cdot\text{g}^{-1}$	< 10 UFC $\cdot\text{g}^{-1}$	< 3 NMP $\cdot\text{g}^{-1}$	< 1 NMP $\cdot\text{g}^{-1}$	< 10 UFC $\cdot\text{g}^{-1}$
Fungi and yeasts	< 10 UFC $\cdot\text{g}^{-1}$	< 10 UFC $\cdot\text{g}^{-1}$	40 UFC $\cdot\text{g}^{-1}$	< 1 UFC $\cdot\text{g}^{-1}$	< 10 UFC $\cdot\text{g}^{-1}$
Coagulase positive staphylococci	< 10 UFC $\cdot\text{g}^{-1}$	< 10 UFC $\cdot\text{g}^{-1}$	< 1 UFC $\cdot\text{g}^{-1}$	< 1 UFC $\cdot\text{g}^{-1}$	< 10 UFC $\cdot\text{g}^{-1}$
<i>Salmonella sp</i>	absent in 25 g	absent in 25 g	absent in 25 g	absent in 25 g	absent in 25 g

163 ¹ not analyzed

164

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

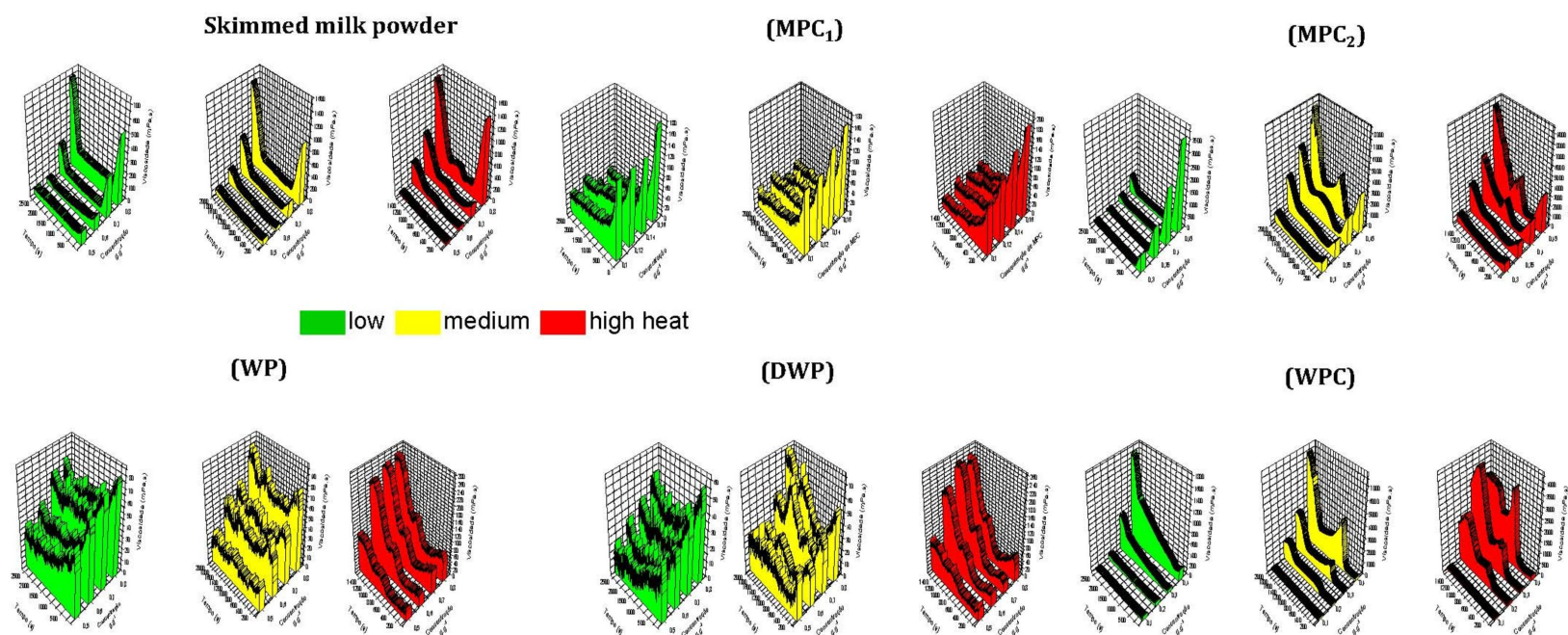


FIGURE 1 Viscographic profiles of simulate thermal processing in RVA for the MPPs respectively for low, medium and high heat treatment

171 The analysis of variance for PV showed statistically significant effects for
172 concentration ($P < 0.001$) and thermal processing ($P < 0.003$) in SMP. These
173 results demonstrate the influence of these two variables in the maximum viscosity
174 obtained during the process provided by the SMP. The maximum value of PV
175 obtained for SMP was 318 mPa·s on the condition of $0.8 \text{ g}\cdot\text{g}^{-1}$ at 95°C for 5
176 minutes and less than 27 mPa·s with $0.6 \text{ g}\cdot\text{g}^{-1}$ subjected to 65°C for 30 minutes.
177 The maximum value of RV for SMP was 308 mPa·s on the condition of $0.8 \text{ g}\cdot\text{g}^{-1}$
178 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
179 during 30 minutes.

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

186 The analysis of variance for $\int/t$ showed statistically significant effects for
187 concentration ($P < 0.001$) and thermal processing ($P < 0.001$). These results
188 demonstrate the influence of these variables in the full viscographic profile of
189 SMP. The maximum value of $\int/t$ obtained in the study was 563 on the condition of
190 $0.8 \text{ g}\cdot\text{g}^{-1}$ at 95°C for 5 minutes and 0.6 with at least $16 \text{ g}\cdot\text{g}^{-1}$ subjected to 65°C for
191 30 minutes. The evaluation of the magnitude of the effects of concentration and
192 thermal processing on PV, RV and FV can be made to $\int/t$, demonstrating be
193 statistically significant ($P < 0.05$), with a concentration of $0.8 \text{ g}\cdot\text{g}^{-1}$ and 95°C

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

196

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

Variables for correlation (J/t)	SMP CC	MPC1 CC	MPC2 CC	WP CC	DWP CC	WPC CC
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 (*)

198 Where: (*) statistically significant ($P < 0.001$); CC = correlation coefficient.

199

200 In the case of MPC1 analysis of variance for PV revealed statistically
201 significant effect for thermal processing ($P < 0.001$). The maximum value of PV
202 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
203 minutes and the minimum 39 mPa·s with $0.1 \text{ g} \cdot \text{g}^{-1}$ subjected to 95°C for 5
204 minutes. The maximum concentration to be prepared from the solution of MPC1
205 with the same procedures performed for other products was $0.16 \text{ g} \cdot \text{g}^{-1}$. This
206 occurred due to the large increase in viscosity at 25°C, making it impossible to
207 maintain agitation of the solution within 30 minutes, which interfered in the
208 warranty of the uniformity of itself. The main factor affecting the solubility of
209 MPC1 seems related to the throughput of water in the powder particle more than
210 the thermal processes during manufacture (SCHUCK et al. 1994). Different
211 methods for producing MPC1 with high solubility in cold water have been

212 proposed, involving the addition of monovalent salts in the retentate prior to
213 drying (CARR, 2002) or partial replacement (approximately 30%) of the calcium
214 content of the retentate sodium ions by ultrafiltration (BHASKAR et al. 2001). To
215 MPC1 analysis of variance for FV showed a statistically significant effect for
216 concentration ($P < 0.001$). The maximum value for MPC1 of FV was 50 mPa·s on
217 the condition of $0.12 \text{ g}\cdot\text{g}^{-1}$ at 65°C for 30 minutes and less than 10 mPa·s with
218 $0.14 \text{ g}\cdot\text{g}^{-1}$ subjected to 85°C for 15 minutes. The viscographic profiles obtained
219 for MPC1 in this concentration range reveal an inherent characteristic of the
220 product which is the high viscosity before processing and significant decrease
221 during the heat treatment, without any increase of viscosity even after cooling.
222 These results are in agreement with the proposal made by Schuck et al. (1994),
223 explaining the low solubility of the MPC1 at low temperatures. The magnitude of
224 the effects of concentration and thermal processing on the FV and PV,
225 respectively, were statistically significant ($P < 0.05$). However, it is possible to
226 correlate any technological factor variables which exhibited the highest values of
227 viscosity. The maximum value of $\int/t$ 59 was obtained in the study of $0.12 \text{ g}\cdot\text{g}^{-1}$ at
228 65°C for 30 minutes and the minimum 0.14 to $29 \text{ g}\cdot\text{g}^{-1}$ subjected to 65°C for 30
229 minutes. The correlation data from $\int/t$ with viscosities PV, RV and FV for the
230 MPC1 are shown in Table 7 and indicate the poor correlation among them.

231 With regard to MPC2, the analysis of variance for PV showed statistically
232 significant effects for concentration ($P < 0.001$) and thermal processing ($P <$
233 0.001). The maximum value of PV for the MPC2 was 3568 mPa·s on the
234 condition of $0.45 \text{ g}\cdot\text{g}^{-1}$ at 85°C for 30 minutes and the minimum was 20 mPa·s

235 with $0.3 \text{ g}\cdot\text{g}^{-1}$ subjected to 65°C for 30 minutes. The analysis of variance for RV
236 showed statistically significant effects for concentration ($P < 0.001$) and thermal
237 processing ($P < 0.001$) for MPC2. These results demonstrate the influence of these
238 variables on the viscosity obtained after the retention time, provided by MPC2.
239 The maximum value of RV obtained in the study was $2180 \text{ mPa}\cdot\text{s}$ on the
240 condition of $0.45 \text{ g}\cdot\text{g}^{-1}$ at 95°C for 5 minutes and the minimum was $20 \text{ mPa}\cdot\text{s}$ with
241 $0.3 \text{ g}\cdot\text{g}^{-1}$ subjected to 65°C for 30 minutes. For MPC2 analysis of variance
242 revealed statistically significant effects for FV for concentration ($P < 0.001$) and
243 thermal processing ($P < 0.001$). The maximum FV value obtained in the study
244 was $9050 \text{ mPa}\cdot\text{s}$ on the condition of $0.45 \text{ g}\cdot\text{g}^{-1}$ at 95°C for at least 5 minutes and
245 the minimum was $44 \text{ mPa}\cdot\text{s}$ with $0.3 \text{ g}\cdot\text{g}^{-1}$ subjected to 65°C for 30 minutes. The
246 magnitude of the effects of concentration and thermal processing on PV, RV and
247 FV were statistically significant ($P < 0.05$) for MPC2, being the concentration of
248 $0.45 \text{ g}\cdot\text{g}^{-1}$ and the binomial 95°C per 5 minutes and 85°C per 15 minutes the most
249 likely to show higher values of viscosity. The analysis of variance for $\int/t$ showed
250 statistically significant effects for concentration ($P < 0.001$) and thermal
251 processing ($P < 0.001$). These results demonstrate the influence of these variables
252 on the complete MPC2 viscographic profile. The maximum value of $\int/t$ obtained
253 in the study was 3113 of $0.45 \text{ g}\cdot\text{g}^{-1}$ at 95°C for 5 minutes and the minimum was
254 58 with $0.3 \text{ g}\cdot\text{g}^{-1}$ subjected to 65°C for 30 minutes. The evaluation on the
255 magnitude of the effects of concentration and thermal processing on PV and RV
256 FV can be made to $\int/t$, demonstrating to be statistically significant ($P < 0.05$), and
257 the concentration of $0.45 \text{ g}\cdot\text{g}^{-1}$ and the binomial 95°C for 5 minutes and 85°C for

15 minutes are the most likely to exhibit higher values of viscosity. This similarity among the results obtained for $\int/t$ with viscosities PV, RV and FV is deduced when analyzing the correlation coefficients shown in Table 7.

For WP, the analysis of variance for PV revealed statistically significant effects for concentration ($P < 0.001$) and thermal processing ($P < 0.001$). The maximum value of PV obtained for WP was 126 mPa·s on the condition of 0.8 g·g⁻¹ at 95°C for 5 minutes and the minimum was of 30 mPa·s with 0.5 g·g⁻¹ subjected to 85°C for 15 minutes. The analysis of variance for RV 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 viscosity obtained after the retention time, provided by WP. The maximum value of RV was 117 mPa·s on the condition of 0.8 g·g⁻¹ at 95°C for 5 minutes and the minimum was 10 mPa·s with 0.5 g·g⁻¹ subjected to 65°C for 30 minutes. The analysis of variance for FV showed statistically significant effects for concentration ($P < 0.001$) and thermal processing ($P < 0.001$) for the WP. The maximum value of FV was 256 mPa·s on the condition of 0.7 g·g⁻¹ at 95°C for 5 minutes and the minimum was 7 mPa·s with 0.5 g·g⁻¹ subjected to 65°C for 30 minutes. The analysis of variance for $\int/t$ showed statistically significant effects for concentration ($P < 0.001$) and thermal processing ($P < 0.001$) for WP. The maximum value of $\int/t$ obtained was 116 on the condition of 0.8 g·g⁻¹ at 95°C for 5 minutes and the minimum was 20 with 0.5 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, RV and FV can be made to $\int/t$, demonstrating to be

281 statistically significant ($P < 0.05$), being the concentrations of $0.8 \text{ g}\cdot\text{g}^{-1}$, $0.7 \text{ g}\cdot\text{g}^{-1}$
282 and $0.6 \text{ g}\cdot\text{g}^{-1}$, and the binomial 95°C for 5 minutes the ones with the largest
283 propensity to exhibit higher values of viscosity. This similarity among the results
284 obtained for $\int/t$ with viscosities PV, RV and FV is deduced when analyzing the
285 correlation coefficients presented in Table 7.

286 Regarding the DWP, the analysis of variance for PV revealed statistically
287 significant effects for concentration ($P < 0.001$) and thermal processing ($P <$
288 0.001). The maximum value of PV obtained in the study was $114 \text{ mPa}\cdot\text{s}$ on the
289 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
290 $0.5 \text{ g}\cdot\text{g}^{-1}$ subjected to 65°C for 30 minutes.

291 The analysis of variance for RV showed statistically significant effects for
292 concentration ($P < 0.001$) and thermal processing ($P < 0.001$) for DWP. The
293 maximum value of RV obtained in the study was $110 \text{ mPa}\cdot\text{s}$ on the condition of
294 $0.8 \text{ g}\cdot\text{g}^{-1}$ at 95°C for 5 minutes and the minimum was $10 \text{ mPa}\cdot\text{s}$ with $0.6 \text{ g}\cdot\text{g}^{-1}$
295 subjected to 65°C for 30 minutes. The analysis of variance for FV showed
296 statistically significant effects for concentration ($P < 0.002$) and thermal
297 processing ($P < 0.001$) for the DWP. The maximum FV value obtained in the
298 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
299 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
300 magnitude of the effects of concentration and thermal processing on PV, RV and
301 FV were statistically significant ($P < 0.05$) for the DWP, being the concentrations
302 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 was the most likely
303 to show higher values of viscosity. The analysis of variance for $\int/t$ showed

304 statistically significant effects for concentration ($P < 0.001$) and thermal
305 processing ($P < 0.001$). The maximum value of η/t obtained in the study was 99 on
306 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
307 $\text{g}\cdot\text{g}^{-1}$ subjected to 65°C for 30 minutes. The evaluation on the magnitude of the
308 effects of concentration and thermal processing on PV, RV and FV can be made to
309 η/t , demonstrating to be statistically significant ($P < 0.05$), being the
310 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
311 one with the highest propensity to exhibit higher values of viscosity. This
312 similarity among the results obtained for η/t with viscosities PV, RV and FV is
313 deduced when analyzing the correlation coefficients shown in Table 7.

314 For WPC analysis of variance for PV revealed statistically significant
315 effects for concentration ($P < 0.001$) and thermal processing ($P < 0.001$). The
316 maximum value of PV obtained in the study was $3892 \text{ mPa}\cdot\text{s}$ on the condition of
317 $0.4 \text{ g}\cdot\text{g}^{-1}$ at 85°C for 30 minutes and the minimum was $26 \text{ mPa}\cdot\text{s}$ with $0.1 \text{ g}\cdot\text{g}^{-1}$
318 subjected to 65°C for 30 minutes. The variance analysis for RV showed
319 statistically significant effects for concentration ($P < 0.001$) and thermal
320 processing ($P < 0.001$) for the WPC. The maximum value of RV obtained in the
321 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
322 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
323 analysis of variance for FV showed statistically significant effects for
324 concentration ($P < 0.001$) and thermal processing ($P < 0.005$) for WPC. The
325 maximum value obtained in the study was $10105 \text{ mPa}\cdot\text{s}$ under the condition of 0.4
326 $\text{g}\cdot\text{g}^{-1}$ at 85°C for 15 minutes and the minimum was $6 \text{ mPa}\cdot\text{s}$ with $0.1 \text{ g}\cdot\text{g}^{-1}$

327 subjected to 65°C for 30 minutes. The magnitude of the effects of concentration
328 and thermal processing on PV, RV and FV were statistically significant ($P <$
329 0.05), being the concentration of 0.4 g·g⁻¹ and binomial 95°C for 5 minutes and
330 85°C for 15 minutes the most likely to show higher values of viscosity. The
331 analysis of variance for η/t showed statistically significant effects for concentration
332 ($P < 0.001$) and thermal processing ($P < 0.001$). These results demonstrate the
333 influence of these variables on viscosity obtained after retention. The maximum
334 value of η/t obtained in the study was 3062 on condition of 0.4 g·g⁻¹ at 85°C for
335 15 minutes and the minimum was 11 with 0.1 g·g⁻¹ subjected to 65°C for 30
336 minutes. The magnitude of the effects of concentration and thermal processing on
337 PV, RV and FV can be made to η/t , demonstrating to be statistically significant (P
338 < 0.05), with a concentration of 0.4 g·g⁻¹ and the binomial 95°C for 5 minutes and
339 85°C for 15 minutes the most likely to exhibit higher values of viscosity. This
340 similarity among the results obtained for η/t with viscosities PV, RV and FV is
341 deduced when analyzing the correlation coefficients shown in Table 7.

342 In this study, we can infer that increases in viscosity observed in different
343 simulations of thermal processing had no direct contribution on denaturation of
344 casein molecules, since they have almost no secondary or tertiary structures. As
345 described by Walstra et al. (2001), caseins do not insolubilized by heating at
346 temperatures below 100°C, which occurs only at higher temperatures. This way,
347 one can assign the increased viscosity of the solutions of MPPs during processing
348 to thermal denaturation of whey protein and their association with the casein
349 micelles. A common behavior in many assessed simulations was $PV > RV$, ie, the

mean peak viscosities were higher than the average of the viscosities of retention. We can attribute this loss of viscosity to two factors. The first is the physical stress to which the solutions are subjected during the whole time of analysis ($15.7 \text{ rad}\cdot\text{s}^{-1}$), causing the protein structure to lose part of its ability to interact with water due to the mechanical energy applied and the flow of product within the tub for heating. The second is related to the aggregation of β -LG and also aggregate formation of β -LG / α -LA that will occur at a rate associated with duration of exposure to high temperature. During the retention time, the whey proteins will unfold in a short period of time, thus presenting opportunity for more monomers of unfolded β -LG self-aggregate (OLDFIELD et al. 1998b), therefore decreasing the interaction with water, thereby lowering the viscosity of the solution.

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

The Box-Behnken factorial design 3^3 was conducted in simulated conditions with the WPC in RVA and Table 8 presents a planning matrix and results. Analyzing the results in 8, it appears that the highest value obtained for FV ($8578 \text{ mPa}\cdot\text{s}$) was provided on the condition of $0.4 \text{ g}\cdot\text{g}^{-1}$ at 95°C for 15

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

TABLE 8 Planning matrix for Box-Behnken 3³ and results of final viscosity - 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

Time (minutes): (-1) 5; (0) 15; (+1) 30; Temperature (°C): (-1) 65; (0) 85; (+1) 95;

Concentration (g·g⁻¹): (-1) 0.2; (0) 0.3; (+1) 0.4

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

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.

389

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

391

392 The TABLE 9 shows the calculated values for the coefficients and p-
 393 values for the adjusted model. Using a significance level of 4%, it is considered
 394 that a factor affects the response if the coefficients differ from zero or the p-value
 395 is less than 0.04 (RAGONESE et al. 2002).

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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	$1.38 \cdot 10^3$	$1.70 \cdot 10^2$	$1.48 \cdot 10^{-2}$

The results presented in TABLE 9 show that the terms: temperature, concentration, temperature versus temperature, time versus concentration and temperature versus concentration are significant for FV at a level of 96% of confidence. This way, all the variables affect the results of final viscosity. The model adjustment was assessed by analysis of variance (ANOVA). The adjusted regression model has not the lack of adjustment in the confidence level of 96%, since the *p*-value was of 0.046 value for the ratio between the mean square due to the lack of adjustment and the quadratic mean and due to the pure error with three and two degrees of freedom, respectively. Thus it was possible to obtain nine response surfaces for the final viscosity model as represented in FIGURES 3, 4 and 5. It is possible to observe that the highest final viscosity 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 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 vary the temperature levels of the lowest (65°C) to 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 $0.4 \text{ g}\cdot\text{g}^{-1}$ of dry basis in water in a condition

438 of 95°C, has a great effect of whey protein thermal denaturation due to high
439 temperature and high concentration of proteins. Dannenberg and Kessler (1988)
440 showed how the kinetics of thermal denaturation of whey proteins has a
441 significant effect at temperatures above 90°C, especially in β -LG and α -LA. The
442 change in protein conformation increases the available surface area and exhibits
443 peptides and amino acid side chains, before hidden and which, in this way, can
444 interact with water (KINSELLA, 1982) thereby increasing the viscosity.
445 However, due to absence of other proteins such as caseins, self-aggregation of β -
446 LG and aggregation of β -LG/ α -LA occur at a rate associated with the time of
447 exposure to high temperature. Thus, it is observed that this type of interaction has
448 a negative effect on the increase of viscosity. At higher temperatures and faster
449 heating rates, all whey proteins of milk start to unfold in a short period of time,
450 thereby presenting more opportunity for the monomers β -LG unfolded self-
451 aggregating (OLDFIELD et al. 1998b), and thus a decrease in interaction with
452 water is expected.

453

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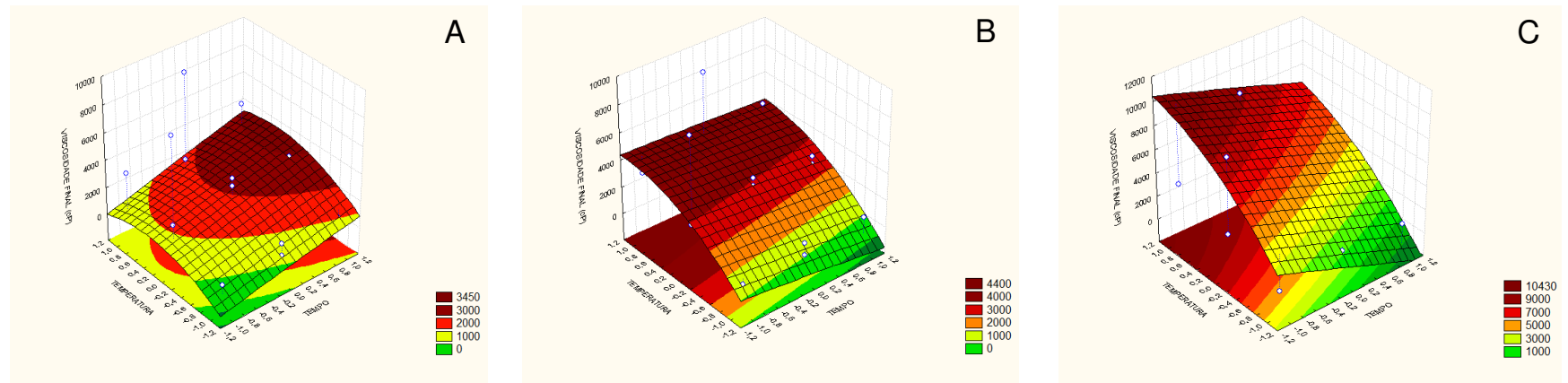
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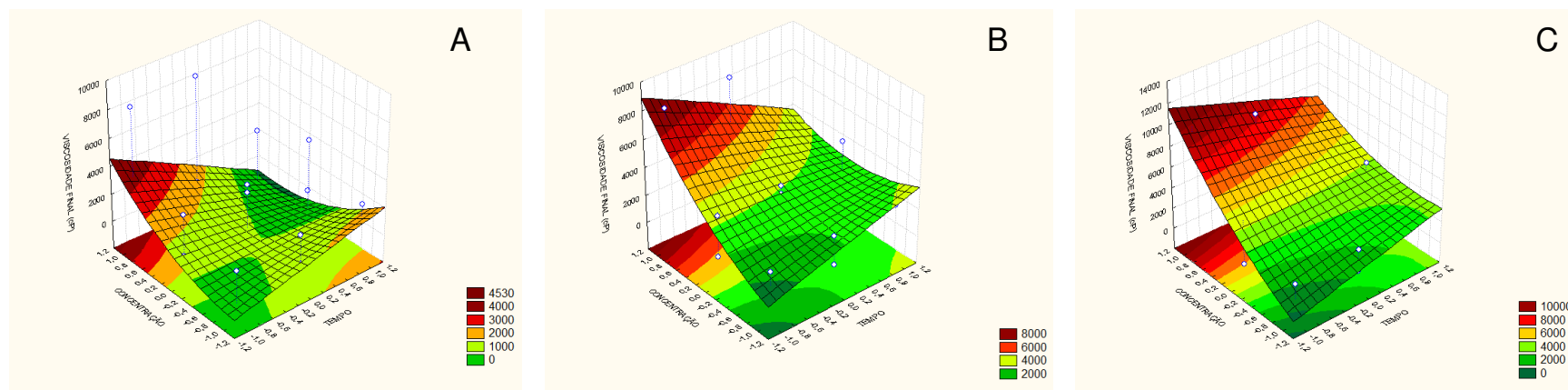


(A) $z=2987.7-423.75*x-30.1*x^2+2054.5*y-878.1*y^2+185.5*x*y-1478.*(-1)*x+1384.*(-1)*y-985.3$

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

FIGURA 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)

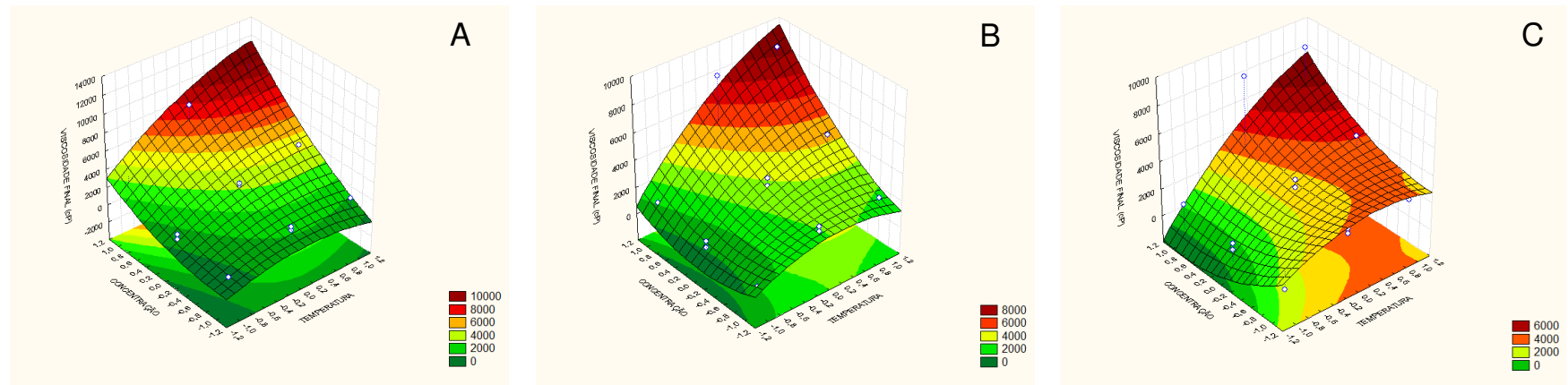


(A) $z=2987.7-423.75*x-30.1*x^2+1795.25*y+809.9*y^2+185.5*(-1.)*x-1478.*x*y+1384.*(-1.)*y-2932.6$

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

FIGURA 4 Response surfaces obtained for final viscosity (FV) under the simulated conditions for WPC on RVA: (A) constant temperature on leve -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) $z=2987.7+2054.5*x-878.1*x^2+1795.25*y+809.9*y^2+185.5*1.*x-1478.*1.*y+1384.*x*y-453.8$

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

493 With the aim of obtaining an exploratory analysis by looking for patterns
 494 with all MPPs, the results of the three types of heat processing were organized in
 495 three different matrices, with treatments (MPP type and concentration) lines and
 496 the average readings of viscosity of the entire viscographic profile in the columns.
 497 Therefore, we obtained the matrix 1(24 x 375), matrix 2 (24 x 490) and matrix 3
 498 (24 x 646) for the high, medium and low heat treatments respectively, being
 499 'number of treatments x number of mean viscosities read '. "Assays were
 500 identified in order that they are presented in TABLE 10.

501

502 TABLE 10 Identification of the experiments with MPPs on RVA

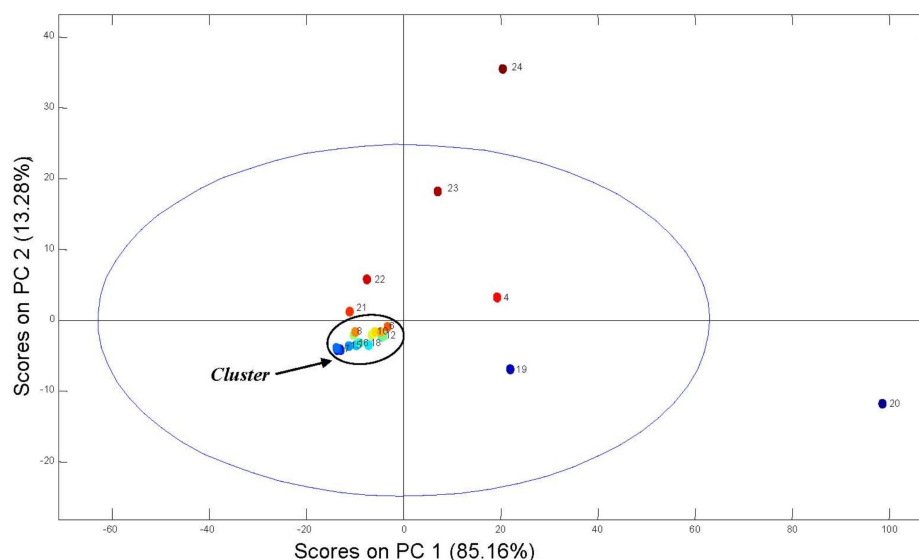
MPP type	Dry matter concentration (g·g ⁻¹)	Identification
Skimmed milk powder – SMP	0.5	1
	0.6	2
	0.7	3
	0.8	4
Milk protein concentrate – MPC ₁	0.1	5
	0.12	6
	0.14	7
	0.16	8
Whey powder – WP	0.5	9
	0.6	10
	0.7	11
	0.8	12
Demineralized whey powder – DWP	0.5	13
	0.6	14
	0.7	15

	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

503

504 The use of Principal Component Analysis made possible the joint
505 evaluation of 9000, 11760 and 15504 experimental data for the high, medium and
506 low heat treatment respectively. This collection of data makes the information
507 provided by PCA significantly under the relevant analytical point of view.
508 FIGURE 6 shows the PCA scores in the first two components to the low heat
509 treatment (matrix 3), representing 98.44% of the total variance of the data. With
510 the projection of two main components, it is possible to observe the formation of
511 different groups among the MPPs samples. In the positive quadrant for PC1 and
512 PC2, it is observed that tests 4, 23 and 24 are in the same region determined by
513 the first main component, which explains the greater variety of samples (85.16%).
514 Respectively we have the rehearsals $0.8 \text{ g} \cdot \text{g}^{-1}$ of dry matter of the SMP, $0.4 \text{ g} \cdot \text{g}^{-1}$
515 and $0.45 \text{ g} \cdot \text{g}^{-1}$ of dry matter from MPC2. In the positive quadrant for PC1 and
516 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
517 matter of WPC) in the same region for PC2. The positive quadrant for PC2 and
518 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

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



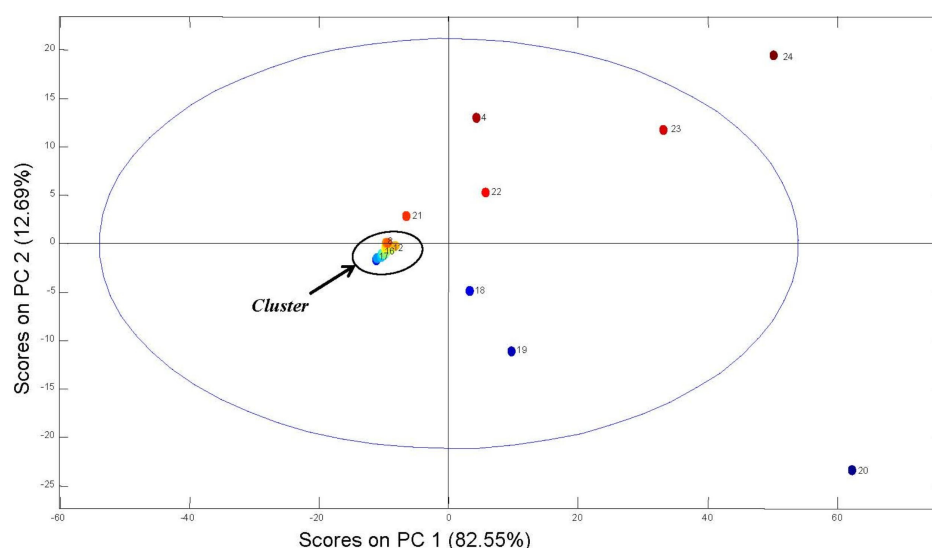
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522 *FIGURE 6 Graphic of score for PC1 vs. PC2 under the low treatment conditions*
523 *(65°C for 30 minutes). Where: 4 is equal to SMP (0.29 g.g⁻¹ of protein), 19 is*
524 *equal to WPC (0.24 g.g⁻¹ of protein), 20 is equal to WPC (0.33 g.g⁻¹ of protein),*
525 *21 is equal to MPC (0.22 g.g⁻¹ of protein), 22 is equal to SMP (0.26 g.g⁻¹ of*
526 *protein), 23 is equal to SMP (0.30 g.g⁻¹ of protein), 24 is equal to SMP (0.34 g.g⁻¹*
527 *of protein).*

528

529 It is possible to see in Figure 6 that the PC1 identified the influence of low heat
530 treatment on product solutions with the highest protein content and PC2
531 differentiated the influence of heat treatment on the origin of MPPs (milk or
532 whey), except for those involved in cluster due to the low protein concentration.
533 The protein content of separation in PC1 for MPPs originated form of milk (upper
534 right quadrant of the graph) was 0.29 g.g⁻¹ of protein for SMP and 0.30 g.g⁻¹
535 protein to MPC2, close values considering the difference in concentration of dry

536 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
537 MPC2). For the whey originated, the content of separation in PC1 was $0.24 \text{ g}\cdot\text{g}^{-1}$
538 protein from WPC, indicating a higher influence of the low heat treatment on the
539 components of WPC over the SMP and MPC2. Tests 4, 19 and 24 are in the same
540 region of PC1, indicating their similarity in viscographic characteristics, these
541 being SMP, WPC and MPC2, respectively. FIGURE 7 shows the PCA scores in
542 the first two components to medium thermal treatment (matrix 2), representing
543 95.24% of the total variance of the data. With the projection of two main
544 components, it is also possible to observe the formation of different groups among
545 the MPPs samples.



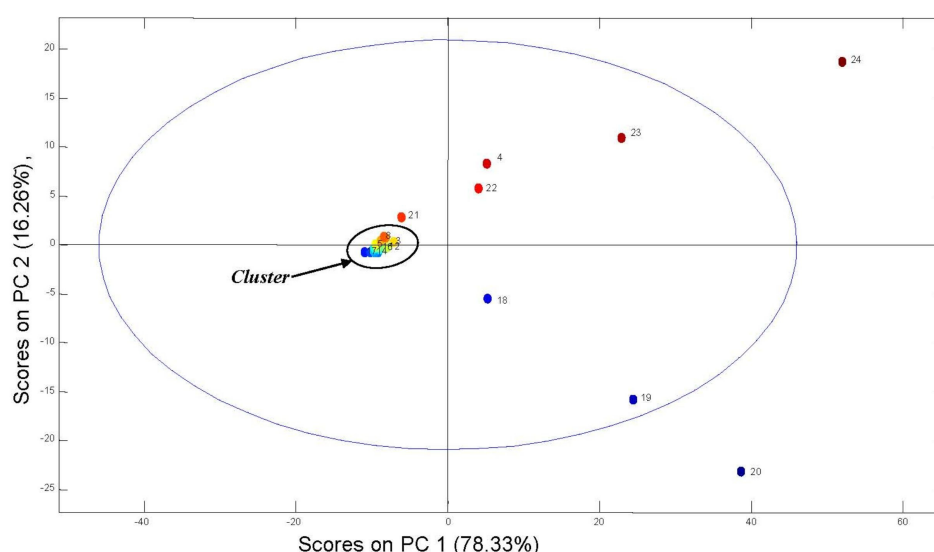
546

547 *FIGURE 7 Graphic of score for PC1 vs. PC2 under the medium treatment*
548 *conditions (85°C for 15 minutes). Where: 4 is equal to SMP ($0.29 \text{ g}\cdot\text{g}^{-1}$ of*
549 *protein), 19 is equal to WPC ($0.24 \text{ g}\cdot\text{g}^{-1}$ of protein), 20 is equal to WPC ($0.33 \text{ g}\cdot\text{g}^{-1}$*
550 *of protein), 21 is equal to MPC ($0.22 \text{ g}\cdot\text{g}^{-1}$ of protein), 22 is equal to SMP (0.26*
551 *$\text{g}\cdot\text{g}^{-1}$ of protein), 23 is equal to SMP ($0.30 \text{ g}\cdot\text{g}^{-1}$ of protein), 24 is equal to SMP*
552 *($0.34 \text{ g}\cdot\text{g}^{-1}$ of protein).*

553

554 For the medium heat treatment, in the positive quadrant for PC1 and PC2, it is
555 observed that besides the trials 4, 23 and 24 which were already in the low heat
556 treatment, the test 22 is also presents itself in the same region determined by the
557 first main component for the SMP to $0.8 \text{ g}\cdot\text{g}^{-1}$ of dry matter. It is also observed,
558 displacement of the experiments 23 and 24 for the right side of the quadrant,
559 leaving clear the influence of heat treatment on average MPC2 to the
560 concentration of $0.4 \text{ g}\cdot\text{g}^{-1}$ and $0.45 \text{ g}\cdot\text{g}^{-1}$ of dry matter. In the positive quadrant for
561 PC1 and negative for PC2, it is also seen the insertion of a further test (number 18,
562 WPC $0.16 \text{ g}\cdot\text{g}^{-1}$ of dry weight) besides the 19 and 20 ($0.3 \text{ g}\cdot\text{g}^{-1}$ and $0.4 \text{ g}\cdot\text{g}^{-1}$ dry
563 matter from WPC) in different regions for PC2. The positive quadrant for PC2
564 and the negative for PC1 shows only the test 21 ($0.3 \text{ g}\cdot\text{g}^{-1}$ of dry matter MPC2)
565 and in the negative for PC1 and PC2 all other tests in a cluster. It is possible to see
566 in Figure 7 that the PC1 continued identifying the influence of heat treatment on
567 product solutions with the highest protein content and PC2 differentiating the
568 influence of heat treatment on the origin of MPPs (milk or whey), except for those
569 involved in cluster due to low protein concentration. The protein content of the
570 separation in PC1 for the MPPs originated from milk (upper right quadrant of the
571 graph) was $0.29 \text{ g}\cdot\text{g}^{-1}$ protein from SMP and $0.26 \text{ g}\cdot\text{g}^{-1}$ of protein for MPC2,
572 indicating that the MPC2 presents greater influence of heat treatment compared to
573 the average SMP for the concentration ranges studied. For those originated of
574 whey, the content of separation in PC1 was $0.16 \text{ g}\cdot\text{g}^{-1}$ protein WPC, indicating a
575 higher influence of the medium heat treatment on the components of the product

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



582

583 *FIGURE 8 Graphic of score for PC1 vs. PC2 under the high treatment conditions*
584 *(95°C for 5 minutes). Where: 4 is equal to SMP (0.29 g.g⁻¹ of protein), 19 is equal*
585 *to WPC (0.24 g.g⁻¹ of protein), 20 is equal to WPC (0.33 g.g⁻¹ of protein), 21 is*
586 *equal to MPC (0.22 g.g⁻¹ of protein), 22 is equal to SMP (0.26 g.g⁻¹ of protein), 23*
587 *is equal to SMP (0.30 g.g⁻¹ of protein), 24 is equal to SMP (0.34 g.g⁻¹ of protein).*
588

589 The provision of test scores in the graph to the high heat treatment does not differ
590 much compared to medium heat treatment. However, it is possible to note
591 similarities among the trials 4, 22 and 18 (respectively 0.8 g.g⁻¹ of dry matter from
592 SMP, 0.35 g.g⁻¹ dry matter from MPC2, and 0.2 g.g⁻¹ of dry matter from WPC),

593 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
594 matter from WPC) in PC1. It is possible to see in FIGURE 8 that the PC1
595 continued identifying the influence of the heat treatment in the solutions of
596 products with higher protein content, determining, inclusive, similarity of the
597 evolution of the viscosity among the SMP, MPC WPC at concentrations of 0.29
598 $\text{g}\cdot\text{g}^{-1}$, $0.26 \text{ g}\cdot\text{g}^{-1}$ and $0.16 \text{ g}\cdot\text{g}^{-1}$ of protein respectively, besides the MPC and WPC
599 $0.30 \text{ g}\cdot\text{g}^{-1}$ and $0.24 \text{ g}\cdot\text{g}^{-1}$ protein also for PC1. The products which were arranged
600 in the cluster, did not have their influence characteristics of the heat treatment
601 well defined due to the low protein concentration, indicating that this method of
602 analysis explains better the products of high protein in the solution, refer to the
603 solutions of MPC1 are also attached to the cluster. For the three thermal
604 processing, it was possible to use the PCA graphs to identify if the origin of the
605 MPP was of milk or whey in solutions of high protein content. It was also possible
606 to note the similarities of the influences of heat treatment on the development of
607 viscosity in solutions of the SMP, MPC2 and WPC and, making it possible to
608 determine the protein concentration required to obtain the same evolution of
609 viscosity among the solutions of these products.

610 **4. CONCLUSION**

611 Based in this work and the results obtained, it can be concluded that:

- 612 - The contents of dry matter and protein contributed to the increased viscosity of
- 613 the solutions of MPPs, when subjected to thermal processing;
- 614 - The variables of time and temperature of heat treatment influenced the
- 615 viscographic profiles, the viscosities peak, retention and final solutions of MPPs;

- 616 - The MPPs viscographic profiles were adequate to reveal the characteristics of
- 617 functionality during and after the heat treatment;
- 618 - The principal components analysis of the viscographic profiles during thermal
- 619 processing solutions have demonstrated that the MPP was originated from milk or
- 620 whey, and
- 621 - The RVA was convenient for the simulation of thermal processing of solutions
- 622 for MPPs.

623 **5. References**

- 624 Bennett L, Pollard A, Ross AS (2007) Characterizing the properties of protein-
- 625 rich ingredients and foods. In: Crosbie, G. B.; Ross, A. S., (1st ed.) The RVA
- 626 Handbook. (pp.9-175) New York: AACC International.
- 627 Bhaskar GV, Singh H, Blazey ND (2001) Milk protein concentrate products and
- 628 process. International Patent Specification WO01/41578, Palmerston North, New
- 629 Zealand, New Zealand Dairy Research Institute
- 630 Booth R, Bason M Principles of operation and experimental techniques. In:
- 631 Crosbie G B, Ross AS, The RVA Handbook. (2007). AACC International: United
- 632 States of America
- 633 Carr AJ (2002) Milk protein concentrate products and uses thereof. International
- 634 Patent Specification WO02/196208A2, Wellington, New Zealand, New Zealand
- 635 Dairy Research Institute
- 636 Damodaran S (1996) In: Nakai, S.; Modler, H. W. (2nd ed.) Food proteins:
- 637 properties and characterization (pp.1-50). New York: VCH

- 638 Garcia MC, Torre M, Marina ML, Laborda F, Rodriquez AR. (1997) Composition
639 and characterization of soybean and related products. Critical Reviews in Food
640 Science and Nutrition, Boca Raton, 37, 361-391
- 641 Oldfield DJ, Singh H, Taylor MW (1998) Association of β -lactoglobulin and α -
642 lactalbumin with the casein micelles in skim milk heated in an ultra-high
643 temperature plant, International Dairy Journal, 8, 765-770
- 644 Ragonese R, Macka M, Hughes J, Petoez P (2002) The use of the Box– Behnken
645 experimental design in the optimisation and robustness testing of a capillary
646 electrophoresis method for the analysis of ethambutol hydrochloride in a
647 pharmaceutical formulation. Journal of Pharmaceutical and Biomedical Analysis
648 27, 995-1007
- 649 Schuck P, Piot M, Mejean S, Le Graet Y, Fauquant J, Bule G, Mabouis J (1994)
650 Spray drying of native phosphocaseinate obtained by membrane microfiltration.
651 Le Lait, 74, 375-388
- 652 Singh H (2009) Protein interactions and functionality of milk protein products. In:
653 Corredig, M. (1st ed.). Dairy-derived ingredients: food and nutraceutical uses (pp.
654 644-669). United Kingdom:Woodhead Publishing
- 655 Walstra P, Geurts T J, Noomen A, Jellema A, Van Boekel M A J S (2001) Dairy
656 Technology Principles of milk properties and processes. (1st ed.). Zaragoza:
657 Acibia.