Evaluation of the synergistic effects of milk proteins in a rapid viscosity analyzer

Rodrigo Stephani,*† Alisson Borges de Souza,† Marccone Augusto Leal de Oliveira,* Ítalo Tuler Perrone,‡
Antônio Fernandes de Carvalho,‡ and Luiz Fernando Cappa de Oliveira*¹

*Núcleo de Espectroscopia e Estrutura Molecular, Departamento de Química, Universidade Federal de Juiz de Fora, 36036-330, Juiz de Fora, MG, Brazil

†Tate & Lyle Gemacon Tech, Bruno Simili, 36092-050, Juiz de Fora, MG, Brazil

‡Department of Food Technology, Federal University of Viçosa, 36571-000, Viçosa, MG, Brazil

ABSTRACT

Protein systems (PS) are routinely used by companies from Brazil and around the globe to improve the texture, yield, and palatability of processed foods. Understanding the synergistic behavior among the different protein structures of these systems during thermal treatment under the influence of pH can help to better define optimum conditions for products and processes. The interpretation of the reactions and interactions that occur simultaneously among the protein constituents of these systems as dispersions during thermal processing is still a major challenge. Here, using a rapid viscosity analyzer, we observed the rheological changes in the startup viscosities of 5 PS obtained by combining varying proportions of milk protein concentrate and whey protein concentrate under different conditions of pH (5.0, 6.5, and 7.0) and heat processing (85°C/15 min and 95°C/5 min). The solutions were standardized to 25% of total solids and 17% of protein. Ten analytical parameters were used to characterize each of the startup-viscosity ramps for 35 experiments conducted in a $2 \times 3 \times 5$ mixed planning matrix, using principal component analysis to interpret behavioral similarities. The study showed the clear influence of pH 5.5 in the elevation of the initial temperature of the PS startup viscosity by at least 5°C, as well as the effect of different milk protein concentrate:whey protein concentrate ratios above 15:85 at pH 7.0 on the viscographic profile curves. These results suggested that the primary agent driving the changes was the synergism among the reactions and interactions of casein with whey proteins during processing. This study reinforces the importance of the rapid viscosity analyzer as an analytical tool for

the simulation of industrial processes involving PS, and the use of the startup viscosity ramp as a means of interpreting the interactions of system components with respect to changes related to the treatment temperature.

Key words: whey protein concentrate, milk protein concentrate, rapid viscosity analyzer, principal component analysis

INTRODUCTION

Milk proteins are important for human nutrition. They are responsible for a wide variety of functional dynamic properties that are widely exploited by the food industry with the objective of improving the texture, yield, and palatability of processed products. Different methods for the industrial-scale production of milk proteins have been developed in recent years. As a result, a wide variety of specifically designed products has been manufactured, with highly concentrated protein products as the most important from the industrial point of view. These include CN, caseinate, whey protein concentrate (**WPC**), whey protein isolate, milk protein concentrate (**MPC**), and milk protein isolate. The processes used in the manufacture of these products tend to modify the native structure of the milk proteins, which may lead to new protein-protein interactions and therefore have an effect on their technological functionality (Singh, 2009).

Milk proteins are commonly added as ingredients in the manufacture of formulated milk products such as processed cheeses to increase the concentration of protein, the yield of the final product, or both. Milk proteins have also been added to improve the textural characteristics of different types of fermented milk. The use of milk proteins in nutritional drinks is growing; for example, in such applications, MPC contributes to the protein fraction of the milk (i.e., CN and whey proteins) in the same proportion as the original milk, but at a much lower lactose concentration (Baldwin

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¹Corresponding author: luiz.oliveira@ufjf.edu.br

and Pearce, 2005). Whey protein concentrate has applications in meat products, beverages, baked goods, and children's products (Kinsella, 1984; De Wit, 1989). The main functional characteristics of these products are related to the whey proteins and include their strong interactions with water, as well as emulsification, aeration, and gelling capabilities. These features depend not only on the protein composition but also on the various processes to which they are subjected during manufacturing (De Wit et al., 1986). The process of converting milk or liquid whey into powder alters the nature and behavior of the milk components. Factors affecting the water absorption ability of milk proteins include composition; protein conformation and structure; surface charge and polarity; presence of carbohydrates, lipids, and salts; pH; ionic strength; temperature; degree of denaturation and aggregation; and disulfide bond formation (Damodaran, 1996; Garcia et al., 1997; Fox and McSweeney, 2003).

Understanding the synergistic interactions and evaluating the effects of milk proteins as industrial ingredients are still required to better develop suitable uses and achieve each desired technological profile in different lines of processing (e.g., processed cheese). Understanding the interactions of milk proteins will enable the food industry to obtain better cost:benefit relationships by using the most suitable protein for the desired characteristics in the final product. In this way, products can be brought to market using different protein powder systems (**PS**) as technological ingredients that better use the synergistic capacities of their components. In view of these interactions, the pH of the medium is presented as a major factor influencing the intensity and type of reactions that occur. Denaturation and protein interactions occur in different forms and intensities when the pH of the medium varies (Anema, 2008). The unfolding of the milk protein fractions can affect the rheological properties of the food system and have a positive or negative effect on consumer acceptance of a dairy product.

The rapid viscosity analyzer (**RVA**) is a rotational viscometer with a computer interface that is capable of continuously measuring the viscosity of a sample under controlled temperature conditions (Booth and Bason, 2007). The instrument is commonly used to evaluate the intrinsic viscosity properties of starches, cereals, pastas, and other foods. The ability to suspend the RVA sample in a solvent, maintain the suspension throughout the test, and apply appropriate agitation can be configured as an industrial-like process, which lends it great value in many research processes. It is particularly useful for materials that require complex tests that use variable temperature and stirring condi-

tions, allowing the material to demonstrate its properties with respect to viscosity. This instrument has already been used in product development, as well as studies of the effects of heat treatment on milk proteins and the way proteins interact with other ingredients (Metzger et al., 2002; Kapoor et al., 2004; Prow, 2004; Kapoor and Metzger, 2005; Prow and Metzger, 2005; Onwulata et al., 2013).

We sought to characterize the viscosity changes that occur during the heating of dispersions containing various ratios of WPC and MPC under different pH and temperature conditions. The synergistic reactions and interactions from the thermal denaturation of the proteins were evaluated by interpreting 10 direct and derived parameters related to the variation in the startup viscosity during the beginning of processing. Thus, we aimed to characterize the influence of varying CN and whey protein proportions in PS powder mixtures containing WPC and MPC, especially the effects on the physical and rheological properties of these mixtures for application as dairy ingredients under different pH conditions when processing is simulated in a RVA.

MATERIALS AND METHODS

Samples and Preparation of Experiments

Commercial samples of WPC and MPC containing 70% of protein on a dry basis were selected for the RVA experiments, due to our interest in their use as dairy ingredients. The products, which are not domestically produced, were imported, acquired, and kindly provided by Tate & Lyle Gemacom Tech S/A. The MPC was chosen because of its protein composition, which is equivalent to the original milk and maintains a ratio of caseins and whey proteins of approximately 80:20 (Supplemental Figures S1 and S2; <http://dx.doi.org/10.3168/jds.2015-9300>). The WPC was selected because it uniquely contributes whey proteins (Supplemental Figure S3; <http://dx.doi.org/10.3168/jds.2015-9300>).

The total fat, moisture, total protein, ash, and pH were analyzed according to the components of most interest to the study, employing methods described by AOAC International (2000).

To evaluate the effects of different MPC:WPC ratios on the rheological behavior during thermal processing, 5 PS powder samples were prepared by dry mixing according to the proportions shown in Table 1. The MPC:WPC ratios ranged from 0:100 to 30:70 to evaluate the influence of the gradual change in the ratio of caseins and whey proteins (~80:20) to an approximate reversal of the original proportion in milk in sample

Table 1. Proportions of milk protein concentrate (MPC) and whey protein concentrate (WPC) in dry mixed protein system powder samples

Protein system	MPC:WPC (%)
A	0:100
B	7.5:92.5
C	15:85
D	22.5:77.5
E	30:70

E (~24:76). The calculated protein compositions of this PS are shown in Supplemental Figures S4 and S5 (<http://dx.doi.org/10.3168/jds.2015-9300>).

According to the recommendation by Bennett et al. (2007), all samples were rehydrated for 12 h before being subjected to RVA. Assays were performed on duplicate samples using water as solvent, with sodium hydroxide solution (0.1 mol·L⁻¹) or lactic acid (10 mol·L⁻¹) for pH adjustment. Each sample was weighed directly in the RVA test cup, calculating the dosage to obtain a concentration of 25% dry basis per 100 g of water (dry basis g/100 g of water) and final approximate amount of 25.0 g per test. Due to their hygroscopicity, the dried products were kept in airtight containers to prevent moisture absorption. Before the tests, moisture analysis was performed to calculate the exact amount of sample used on a dry basis. To completely standardize the analyses, the RVA rotation was set to 104.7 rad·s⁻¹ (1,000 rpm) for the first 60 s at a constant temperature of 30°C, and 15.7 rad·s⁻¹ (150 rpm) thereafter. The solutions were adjusted to pH 5.5, 6.5, and 7.0, with a maximum acceptable variation of ± 0.1 pH units.

RVA Parameters

To simulate the conditions that are most often used for the processing of fermented milk, petit suisse, pasteurized cream, and pasteurized flavored milk drinks by the dairy industry, our experiments were conducted under medium (85°C for 15 min) and high (95°C for

Table 3. Thermal processing, central point (90°C/10 min)

Time	Command	Value
0 min 00 s	Temperature (°C)	30
0 min 00 s	Rotation (rad·s ⁻¹)	104.7
1 min 00 s	Rotation (rad·s ⁻¹)	15.7
1 min 00 s	Temperature (°C)	30
17 min 40 s	Temperature (°C)	90
27 min 40 s	Temperature (°C)	90
44 min 20 s	Temperature (°C)	30
48 min 20 s	Temperature (°C)	30
48 min 21 s	End	X

5 min) thermal processing conditions. To calculate the average error of the model, experiments were also conducted at 90°C for 10 min; these conditions were considered a central point, X₁, for the model and held no technological interest, but were merely a statistical tool.

The instrument used in this work was the model RVA-4 from Newport Scientific Pty Ltd. (Warriewood, Australia). The data collection software was Thermocline for Windows (Warriewood, Australia). The configurations of the thermal processing simulations are described in Tables 2, 3, and 4. For all 3 heat treatment levels, the interval between viscosity readings was 1 s. The heating and cooling gradients were standardized at 3.6°C·min⁻¹. Figure 1 plots the given parameters.

The viscographic profiles were interpreted with respect to 6 collected analytical datapoints and 4 that were derived, as described in Table 5. Figure 2 graphically presents the collected and calculated data in a viscographic profile.

2 × 3 × 5 Planning Matrix

To study the relationships between the MPC:WPC ratio, thermal processing, and pH under the simulated processing conditions in the RVA, a 2 × 3 × 5 mixed planning matrix was constructed. The selected factors are presented in Table 6.

Table 2. Thermal processing settings, middle level (85°C/15 min)

Time	Command	Value
0 min 00 s	Temperature (°C)	30
0 min 00 s	Rotation (rad·s ⁻¹)	104.7
1 min 00 s	Rotation (rad·s ⁻¹)	15.7
1 min 00 s	Temperature (°C)	30
16 min 00 s	Temperature (°C)	85
31 min 00 s	Temperature (°C)	85
46 min 00 s	Temperature (°C)	30
50 min 00 s	Temperature (°C)	30
50 min 01 s	End	X

Table 4. Thermal processing, high level (95°C/5 min)

Time	Command	Value
0 min 00 s	Temperature (°C)	30
0 min 00 s	Rotation (rad·s ⁻¹)	104.7
1 min 00 s	Rotation (rad·s ⁻¹)	15.7
1 min 00 s	Temperature (°C)	30
19 min 00 s	Temperature (°C)	95
24 min 00 s	Temperature (°C)	95
42 min 00 s	Temperature (°C)	30
46 min 00 s	Temperature (°C)	30
46 min 01 s	End	X

Table 5. Description of the collected and calculated data in the viscographic profile

Data	Identification	Unit
1	Initial viscosity (V_i)	mPa·s
2	Peak viscosity (V_p)	mPa·s
3	Startup viscosity (ΔV)	mPa·s
4	Initial peak temperature (T_i)	°C
5	Final peak temperature (T_p)	°C
6	Temperature difference (ΔT)	°C
7	Initial peak time (t_i)	min
8	Final peak time (t_f)	min
9	Peak time interval (Δt)	min
10	Startup viscosity $\times$ peak time interval relationship ($\Delta V/\Delta t$)	mPa·s min ⁻¹

Table 7 shows the planning matrix with its levels and factors combinations, where experiments 31 to 35 are replications of the center point. The procedures were performed at random by drawing lots.

Analysis of RVA Results

For exploratory analysis of the RVA, the results were arranged in a 35×10 matrix with the experiments in the rows and the 6 collected plus 4 calculated data points from the viscographic profiles in the columns. The data were subjected to principal component analysis (PCA) from the covariance matrix using PLS Toolbox (Eigenvector Research Inc., Wenatchee, WA), operating in Matlab (R2007B v.7.5.0.342, The MathWorks Inc., Natick, MA). Then, the results were preprocessed using unit variance scaling (autoscale) to correct for differences in scales among the results.

RESULTS AND DISCUSSION

Physical and Chemical Characteristics of the Samples

Milk protein composition is an important factor in a rheological study of thermal processing. By determining the moisture content of each product, we could use a fixed amount of total solids in each test and eliminate the possibility of variation caused by different moisture contents. Hence, the concentrations of the prepared solutions were calculated on the basis of the solids contents of each protein concentrate, and are given as DM in water.

The chemical characterization of the samples also provides important information about the protein and fat contents. The amount of protein on a dry basis is a common descriptor for protein concentrates; their hygroscopic natures can contribute to the possibility

Figure 1. Rapid viscosity analyzer configuration parameters. MPC = milk protein concentrate; WPC = whey protein concentrate. Color version available online.

Figure 2. Collected analytical parameters: initial viscosity (V_i), peak viscosity (V_p), initial peak temperature (T_i), final peak temperature (T_p), initial peak time (t_i), and final peak time (t_f). Calculated analytical parameters: startup viscosity (ΔV), temperature difference (ΔT), peak time interval (Δt), and startup viscosity $\times$ peak time interval ($\Delta V/\Delta t$). Color version available online.

of analytical error due to moisture gain, and therefore, the mass increase of the product and a decrease in the total protein content.

Table 8 shows the protein, total fat, moisture, lactose, and fixed mineral residue contents, as well as the pH of the protein concentrates used in the RVA thermal processing simulations. These values were consistent with data from the literature (Singh, 2009).

Table 9 demonstrates the standardization of the protein content in all evaluations based on the protein concentrations shown in Table 8. Using the concentration to be used in the assay, 25% on a dry basis in water solution, we obtained a constant level of 17% protein in 100 g of water used in preparing the samples.

Analytical Characterization of Startup Viscosity Ramps

The $2 \times 3 \times 5$ mixed planning design was carried out under the simulated conditions with the samples; Table 10 shows the design matrix and the results.

Sample E afforded the highest obtained value of ΔV (7,841 mPa·s) at pH 7.0 and 85°C/15 min (experiment 15), as well as the lowest ΔV (1,581 mPa·s) at pH 5.5 and 95°C/5 min (experiment 20). Among the $\Delta V/\Delta t$ results, the highest and lowest obtained values were again for sample E: 2,066 mPa·s min⁻¹ at pH 7.0 and 95°C/5 min (experiment 30), and 323 mPa·s min⁻¹ at pH 5.5 and 85°C/15 min (experiment 5), respectively. Thus, the startup viscosity and the relationship between the startup viscosity and the peak time interval are shown to influence the evolution of viscosity.

Figure 3 plots the results of 35 experiments in relation to the startup viscosity ΔV , demonstrating the synergistic effects among the components of samples C, D, and E at pH 7.0 under medium and high temperature processing conditions. The increase in this rheological parameter (ΔV) was well captured by the RVA.

It is possible to conclude that the viscosity increase is related to the reduced mobility of water molecules due to 3 major factors: the increase in electrostatic interactions, the increased exposure of hydrophobic

Table 6. Factors and levels for $2 \times 3 \times 5$ mixed factorial design matrix

Factor	Level				
	(-1)	(-0.5)	(0)	(0.5)	(1)
X ₁ : thermal processing	85°C/15 min		90°C/10 min		95°C/5 min
X ₂ : pH	5.5		6.5		7.0
X ₃ : MPC:WPC ¹ (%)	0:100	7.5:92.5	15:85	22.5:77.5	30:70

¹MPC = milk protein concentrate; WPC = whey protein concentrate.

Table 7. Encoded matrix for $2 \times 3 \times 5$ mixed factorial design with quintuplicate in the central point

Experiment	X ₁	X ₂	X ₃
1	-1	-1	-1
2	-1	-1	-0.5
3	-1	-1	0
4	-1	-1	0.5
5	-1	-1	1
6	-1	0	-1
7	-1	0	-0.5
8	-1	0	0
9	-1	0	0.5
10	-1	0	1
11	-1	1	-1
12	-1	1	-0.5
13	-1	1	0
14	-1	1	0.5
15	-1	1	1
16	1	-1	-1
17	1	-1	-0.5
18	1	-1	0
19	1	-1	0.5
20	1	-1	1
21	1	0	-1
22	1	0	-0.5
23	1	0	0
24	1	0	0.5
25	1	0	1
26	1	1	-1
27	1	1	-0.5
28	1	1	0
29	1	1	0.5
30	1	1	1
31	0	0	0
32	0	0	0
33	0	0	0
34	0	0	0
35	0	0	0

groups of the proteins, and networking due to protein interactions. It can be inferred that such effects are closely related to the thermal denaturation of the whey proteins in the PS during heating and the interactions that occur simultaneously between the system constituents in the dispersions during heat processing. The differences in viscosity may also be interpreted as due to changes in the concentrations of the whey proteins and CN in accordance with the MPC:WPC ratio in the prepared samples, noting that higher concentrations of MPC produce higher startup viscosity values.

Changes in protein conformation can affect the thermodynamics of binding with water by changing the availability of polar or hydration sites. The transition

Table 8. Physicochemical data for the protein concentrates¹

Analysis	WPC	MPC
Total fat (g/100 g)	5.02	0.8
Moisture (g/100 g)	5.0	5.8
Total protein (g/100 g)	65.39	66.29
Protein on dry basis ² (g/100 g)	68.83	70.37
Lactose ² (g/100 g)	20.89	19.91
Ash (g/100 g)	3.7	7.2
pH	6.4	6.9

¹WPC = whey protein concentrate; MPC = milk protein concentrate.

²Calculated values.

from the compact globular conformation of the native protein to a random conformation results in an increase of the available surface area as well as the exposure of the peptides and side-chain AA (which had been previously protected), which can then interact with water (Kinsella, 1982).

Figure 4 shows the results of experiments with respect to initial peak temperatures. In the pH 5.5 trials, the minimum increase was 5°C compared with the runs at pH 6.5 and 7.0; the discussion is presented in the Interpretation and Discussion of Observed Behaviors section.

Figure 5 shows the viscographic profiles of samples A through E at pH 5.5, 6.5, and 7.0 under the processing conditions of technological interest: 85°C/15 min and 95°C/5 min.

Exploratory Analysis in the Simulated Conditions in the RVA

A $2 \times 3 \times 5$ mixed factorial design with a quintuplicate in the central point was used to evaluate the synergistic effects of milk proteins in a RVA. A traditional calculation was used to evaluate the effects and interactions of thermal treatment (X₁), pH (X₂), and ratio MPC:WPC (X₃) as shown in Table 11. In the present study, different coefficients were significant within a 95% confidence interval (*P*-values lower than 0.05). However, individual information to each factor using traditional calculation was not interesting in the present case. For a better understanding of the RVA simulations in the present study, an exploratory analysis was carried out by PCA. In the model built by PCA, 4 major components were chosen that captured 96.22%

Table 9. Protein content in the solutions used in the rapid viscosity analyzer tests

Analysis	A (0:100)	B (7.5:92.5)	C (15:85)	D (22.5:77.5)	E (30:70)
Protein on dry basis (g/100 g)	68.83	68.95	69.06	69.18	69.29
Protein in solution, 25% (g/100 g) ¹	17.0	17.0	17.0	17.0	17.0

¹Calculated value.

Table 10. Statistical results for $2 \times 3 \times 5$ mixed factorial design matrix with the analytical data collected by rapid viscosity analyzer (RVA)¹

Analytical data collected from the heating ramp of viscometric profiles													
	1	2	3	4	5	6	7	8	9	10			
	Initial vis. (V _i), mPa·s	Peak vis. (V _p), mPa·s	Startup vis. (ΔV), mPa·s	Initial peak temp. (T _i), °C	Final peak temp. (T _p), °C	Temp. difference (ΔT), °C	Initial peak time (t _i), min	Final peak time (t _f), min	Peak time interval (Δt), min	Startup vis. × peak time interval (ΔV/Δt)			
X ₁ (thermal treatment)	X ₂ (pH)	X ₃ (MPC;WPC)											
1 85°C/15 min	5.5	A	184	3,164	2,980	85.00	76.85	13.82	18.05	4.23	704		
2 85°C/15 min	5.5	B	184	2,986	2,802	85.00	78.80	14.33	18.70	4.37	642		
3 85°C/15 min	5.5	C	7	2,949	2,942	85.05	78.80	14.33	19.67	5.34	551		
4 85°C/15 min	5.5	D	122	2,154	2,032	85.00	78.75	14.33	19.22	4.88	416		
5 85°C/15 min	5.5	E	63	1,803	1,740	85.00	77.40	13.95	19.33	5.38	323		
6 85°C/15 min	6.5	A	97	2,742	2,645	85.05	72.65	12.67	16.25	3.58	738		
7 85°C/15 min	6.5	B	123	2,489	2,366	84.90	72.60	12.30	16.40	3.75	631		
8 85°C/15 min	6.5	C	69	2,446	2,377	85.05	71.15	12.28	16.10	3.82	623		
9 85°C/15 min	6.5	D	96	2,477	2,381	85.10	72.70	12.40	16.10	3.42	697		
10 85°C/15 min	6.5	E	41	2,938	2,897	84.95	71.65	12.42	16.35	3.93	737		
11 85°C/15 min	7.0	A	149	4,229	4,080	85.00	70.60	12.10	16.23	4.13	987		
12 85°C/15 min	7.0	B	118	3,167	3,049	82.85	71.40	12.35	15.42	3.07	993		
13 85°C/15 min	7.0	C	43	5,320	5,277	84.00	70.90	11.90	15.48	3.58	1,473		
14 85°C/15 min	7.0	D	78	6,183	6,105	84.95	71.25	12.30	16.83	4.53	1,347		
15 85°C/15 min	7.0	E	105	7,841	7,736	84.90	71.65	12.42	16.37	3.95	1,958		
16 95°C/5 min	5.5	A	185	3,110	2,925	88.45	78.30	10.15	17.18	2.78	1,051		
17 95°C/5 min	5.5	B	182	2,701	2,519	88.05	79.10	8.95	17.05	2.42	1,042		
18 95°C/5 min	5.5	C	146	2,399	2,253	88.15	78.55	9.60	17.08	2.62	861		
19 95°C/5 min	5.5	D	73	1,983	1,910	89.85	77.65	12.20	17.58	3.36	568		
20 95°C/5 min	5.5	E	127	1,708	1,581	89.15	79.15	10.00	17.40	2.77	571		
21 95°C/5 min	6.5	A	69	2,861	2,792	85.70	71.70	14.00	16.40	3.82	732		
22 95°C/5 min	6.5	B	131	2,420	2,289	85.85	72.90	12.95	16.47	3.57	642		
23 95°C/5 min	6.5	C	107	2,150	2,043	84.70	73.50	11.20	16.13	3.06	667		
24 95°C/5 min	6.5	D	49	2,711	2,662	85.50	72.00	13.50	16.37	3.72	716		
25 95°C/5 min	6.5	E	52	3,467	3,415	87.50	71.95	15.55	16.90	4.25	804		
26 95°C/5 min	7.0	A	110	3,396	3,286	81.45	70.10	11.35	15.27	3.15	1,043		
27 95°C/5 min	7.0	B	106	3,283	3,177	82.65	71.25	12.12	15.58	3.13	1,014		
28 95°C/5 min	7.0	C	54	6,218	6,164	86.40	70.40	16.00	16.62	4.42	1,396		
29 95°C/5 min	7.0	D	62	6,413	6,351	87.55	71.15	12.43	16.93	4.50	1,411		
30 95°C/5 min	7.0	E	102	6,851	6,749	83.20	71.35	12.48	15.75	3.27	2,066		
31 90°C/10 min	6.5	C	113	2,143	2,030	84.85	72.75	11.85	16.23	3.31	613		
32 90°C/10 min	6.5	C	91	2,553	2,462	83.65	71.25	12.40	15.92	3.43	717		
33 90°C/10 min	6.5	C	132	2,668	2,536	83.65	72.35	11.30	15.92	3.15	804		
34 90°C/10 min	6.5	C	82	2,773	2,691	84.10	71.00	13.10	16.03	3.60	748		
35 90°C/10 min	6.5	C	136	2,702	2,566	83.65	72.50	11.15	15.93	3.10	829		

¹Temp. = temperature; vis. = viscosity; MPC = milk protein concentrate; WPC = whey protein concentrate; A: 0:100; B: 7.5:92.5; C: 15:85; D: 22.5:77.5; E: 30:70.

Figure 3. Graph of 35 trials versus startup viscosity.

of the total variance. The choice of the principal components was based on a plot of the eigenvalues versus the number of principal components. The 10 collected and calculated analytical parameters in the heating ramps of the 35 viscographic profiles from the RVA were used to compose the 35×10 matrix. Figure 6 shows the score plots of the first 2 components, which

clearly reveal the formation of 3 major regions, I, II, and III. Also in Figure 6, the plot of the samples versus PC1 scores readily identifies the PC1 with respect to the characteristics of the grouped experiments.

Region I consists of experiments 13, 14, 15, 28, 29, and 30, which were performed using samples C, D, and E in heat treatments at pH 7.0 and 85°C/15 min or

Figure 4. Graph of 35 trials versus initial peak temperature (°C).

Figure 5. Viscographic profiles of samples A to E (variable X_3) during heat treatment (variable X_1 ; dashed line) at 85°C/15 min (A_{85} to E_{85}) and 95°C/5 min (A_{95} to E_{95}) at different pH (variable X_2): 5.5 (black), 6.5 (dark gray), and 7.0 (light gray).

Table 11. Statistical results for 2 × 3 × 5 mixed factorial design with quintuplicate in the central point

Factor	Initial viscosity			Peak viscosity			Startup viscosity			Initial peak temperature			Final peak temperature		
	Coeff ¹ (1)	Error (1)	P (1)	Coeff (2)	Error (2)	P (2)	Coeff (3)	Error (3)	P (3)	Coeff (4)	Error (4)	P (4)	Coeff (5)	Error (5)	P (5)
Constant	74.067	7.005	4.53 × 10 ^{-4*}	2.520	0.073	4.19 × 10 ^{-6*}	2.446	0.074	4.89 × 10 ^{-6*}	72.505	0.230	6.08 × 10 ^{-10*}	85.507	0.153	6.10 × 10 ^{-11*}
X ₁	2.533	4.388	5.95 × 10 ⁻¹	-0.041	0.046	4.25 × 10 ⁻¹	-0.043	0.046	4.03 × 10 ⁻¹	0.063	0.144	6.83 × 10 ⁻¹	0.745	0.096	1.47 × 10 ^{-3*}
X ₂	-17.300	5.374	3.23 × 10 ^{-2*}	1.397	0.056	1.53 × 10 ^{-5*}	1.415	0.056	1.51 × 10 ^{-5*}	-3.665	0.177	3.18 × 10 ^{-5*}	-1.288	0.117	3.90 × 10 ^{-4*}
X ₃	-32.400	6.206	6.42 × 10 ^{-3*}	0.503	0.065	1.47 × 10 ^{-3*}	0.535	0.065	1.20 × 10 ^{-3*}	0.112	0.204	6.13 × 10 ^{-1*}	0.558	0.135	1.45 × 10 ^{-2*}
X ₂ X ₃	26.600	8.369	3.36 × 10 ^{-2*}	1.223	0.087	1.50 × 10 ^{-4*}	1.196	0.088	1.69 × 10 ^{-4*}	2.390	0.275	9.63 × 10 ^{-4*}	0.153	0.182	4.50 × 10 ⁻¹
X ₃ X ₃	18.667	9.745	1.28 × 10 ⁻¹	0.299	0.102	4.20 × 10 ^{-2*}	0.281	0.102	5.18 × 10 ⁻²	-0.450	0.320	2.32 × 10 ⁻¹	-0.155	0.212	5.07 × 10 ⁻¹
X ₁ X ₂	-10.600	5.374	1.20 × 10 ⁻¹	0.029	0.056	6.34 × 10 ⁻¹	0.039	0.056	5.24 × 10 ⁻¹	-0.185	0.177	3.54 × 10 ⁻¹	-0.952	0.117	1.24 × 10 ^{-3*}
X ₁ X ₃	5.667	6.206	4.13 × 10 ⁻¹	0.032	0.065	6.48 × 10 ⁻¹	0.026	0.065	7.09 × 10 ⁻¹	0.038	0.204	8.60 × 10 ⁻¹	0.432	0.135	3.32 × 10 ^{-2*}
X ₂ X ₃	17.050	7.601	8.83 × 10 ⁻²	1.368	0.079	6.59 × 10 ^{-5*}	1.351	0.080	7.17 × 10 ^{-5*}	0.153	0.250	5.74 × 10 ⁻¹	0.355	0.166	9.88 × 10 ⁻²
Startup viscosity × peak time interval															
Factor	Temperature difference			Initial peak time			Final peak time			Peak time interval			Startup viscosity × peak time interval		
	Coeff (6)	Error (6)	P (6)	Coeff (7)	Error (7)	P (7)	Coeff (8)	Error (8)	P (8)	Coeff (9)	Error (9)	P (9)	Coeff (10)	Error (10)	P (10)
Constant	13.002	0.235	6.34 × 10 ^{-7*}	12.701	0.064	3.77 × 10 ^{-9*}	16.391	0.039	1.91 × 10 ^{-10*}	3.690	0.060	4.10 × 10 ^{-7*}	687.160	24.692	9.92 × 10 ^{-6*}
X ₁	0.682	0.147	9.75 × 10 ^{-3*}	0.112	0.040	4.87 × 10 ^{-2*}	-0.260	0.024	4.40 × 10 ^{-4*}	-0.371	0.037	5.82 × 10 ^{-4*}	-82.559	15.469	5.94 × 10 ^{-3*}
X ₂	2.378	0.180	1.90 × 10 ^{-4*}	-1.018	0.049	3.12 × 10 ^{-5*}	-1.039	0.030	4.07 × 10 ^{-6*}	-0.021	0.046	6.70 × 10 ⁻¹	-132.319	18.946	2.21 × 10 ^{-3*}
X ₃	0.447	0.208	9.81 × 10 ⁻²	0.034	0.056	5.79 × 10 ⁻¹	0.295	0.034	1.02 × 10 ^{-3*}	0.261	0.053	7.88 × 10 ^{-3*}	-65.281	21.876	4.06 × 10 ^{-2*}
X ₂ X ₂	-2.238	0.280	1.33 × 10 ^{-3*}	0.638	0.076	1.10 × 10 ⁻³	0.740	0.046	9.11 × 10 ^{-5*}	0.102	0.071	2.26 × 10 ⁻¹	-367.210	29.501	2.40 × 10 ^{-4*}
X ₃ X ₃	0.295	0.326	4.17 × 10 ⁻¹	-0.092	0.088	3.55 × 10 ⁻¹	-0.088	0.054	1.79 × 10 ⁻¹	0.004	0.083	9.66 × 10 ⁻¹	23.080	34.352	5.38 × 10 ⁻¹
X ₁ X ₂	-0.768	0.180	1.30 × 10 ^{-2*}	-0.049	0.049	3.72 × 10 ⁻¹	0.425	0.030	1.41 × 10 ^{-4*}	0.473	0.046	4.96 × 10 ^{-4*}	-67.197	18.946	2.39 × 10 ^{-2*}
X ₁ X ₃	0.393	0.208	1.31 × 10 ⁻¹	0.009	0.056	8.85 × 10 ⁻¹	-0.016	0.034	6.60 × 10 ⁻¹	-0.025	0.053	6.65 × 10 ⁻¹	193.768	21.876	8.97 × 10 ^{-4*}
X ₂ X ₃	0.203	0.255	4.71 × 10 ⁻¹	0.049	0.069	5.17 × 10 ⁻¹	-0.002	0.042	9.56 × 10 ⁻¹	-0.051	0.065	4.75 × 10 ⁻¹	-183.908	26.793	2.36 × 10 ^{-3*}

¹Quadratic coefficient.
*Significant values within 95% confidence interval.

95°C/5 min. Region II consists of experiments 1 to 5 and 16 to 20; these were conducted at pH 5.5 for all samples under 85°C/15 min and 95°C/5 min heat treatment conditions. Table 12 summarizes this information. Figure 7 shows the weight chart on PC1 for every collected and calculated analytical parameter, which clearly shows that parameter 9, the peak time interval (Δt), has the least influence on the PC1.

The grouped experiments in I and II exhibit opposite behaviors under the simulated RVA conditions. Although group I has the highest trends for ΔV startup viscosities and $\Delta V/\Delta t$ values, II has the lowest. This behavior may possibly be attributed to the pH and MPC:WPC ratio, in particular. Graphic representations of the association between the experimental $2 \times 3 \times 5$ mixed planning matrix and the results of the explor-

Figure 6. Principal component (PC)1 versus PC2 scores graph and samples graph versus PC1 scores plot using the 10 collected analytical parameters from the heating ramp of the viscographic profiles of the whey protein concentrate samples: region I samples C, D, and E at pH 7.0 and thermal treatments 85°C/15 min and 95°C/5 min (■); region II samples A, B, C, D, and E at pH 5.5 and heat treatments of 85°C/15 min and 95°C/5 min (▼); and region III consisting of other samples (●). Color version available online.

Table 12. Summary of exploratory analysis groups¹

Group	Thermal treatment	pH	Samples
Region I	85°C/15 min 95°C/5 min	7.0	C
			D
			E
Region II	85°C/15 min 95°C/5 min	5.5	A
			B
			C
			D
			E

¹Proportions of milk protein concentrate:whey protein concentrate: A: 0:100; B: 7.5:92.5; C: 15:85; D: 22.5:77.5; E: 30:70.

atory analysis are presented in Supplemental Figures S6 and S7 (<http://dx.doi.org/10.3168/jds.2015-9300>).

Region III included all the experiments carried out at pH 6.5, regardless of the heat treatment or the PS composition, plus experiments 11, 12, 26, and 27 for samples A and B, which were heat-treated at 85°C/15 min or 95°C/5 min and pH 7.0. These results indicate that the synergistic effects at this pH are differentiated from those with the 15:85 MPC:WPC compositions (samples C, D, and E).

**Interpretation and Discussion
of Observed Behaviors**

We identified 2 main viscographic profiles within the experiments, which are presented in Figure 8. Based on the exploratory analysis, it is clear that the viscographic profile behavior of the samples in the heating ramp is

related to the variations in the pH, MPC:WPC ratios, and the heat treatment applied in the levels studied.

The data confirm that, upon heating, whey proteins undergo competitive and interdependent reactions; their relative importance is determined by the heating conditions, the composition/concentration of each type of protein, the chemical environment, or any combination thereof. Considering the composition of WPC, a reaction of particular importance is whey protein denaturation, and the resulting interactions between these and other proteins.

The structural characteristics of proteins in foods are affected by their interactions with water through hydrogen bonds and hydrophobic interactions. Thus, when a larger proportion or exposure of hydrophobic side-chain AA occurs, the proteins have lower hydration capacities than when their side chains consist of hydrophilic AA, which can form hydrogen bonds more easily with water (Ordóñez et al., 2005). However, the water begins to show a lower mobility due to the exposure of the hydrophobic groups, and thus viscosity increases, giving rise to the phenomenon known as tilting viscosity. Likewise, the protein-water interactions affect the stabilities of protein molecules and clusters, such as CN micelles, and the functionality of the proteins in raw materials and foodstuffs (Damodaran, 1996).

The interactions of various functional groups, such as thiols or hydrophobic moieties exposed on the surfaces of protein molecules, lead to the formation of covalent or noncovalent intermolecular bonds, which result in protein aggregation, polymerization, or both. Such molecular changes alter the functional characteristics

Figure 7. Graph of weight on principal component (PC)1 versus the 10 collected analytical parameters from the heating ramps of the viscographic profiles of the whey protein concentrate samples.

Figure 8. Interpretation of the viscographic profiles of experiments in (A) region I plus the breaking point in the heating ramp, and (B) region II plus the breaking point in the cooling ramp. Color version available online.

of proteins, including hydration, solubility, solution viscosity, film formation, gelation, and adsorption at the interface between aqueous and lipid phases (Damodaran, 1996).

The rapid and significant increases in the viscosity of the solutions during heating in the RVA, often obtaining tilting viscosity, can be interpreted with respect to the denaturation of whey proteins because they are considered to be cooperative changes: the breaking of some of the links within a molecule causes the remain-

ing bonds to become less stable, and many are broken simultaneously. This explains why denaturation occurs in a very narrow range of temperatures and concentrations (Walstra et al., 2001).

Dannenberg and Kessler (1998) showed how the kinetics of the thermal denaturation of whey proteins has a significant effect at temperatures above 90°C, especially for β -LG and α -LA. The change in protein conformation increases the available surface area and exposes formerly hidden peptides and AA side chains,

which can then interact with water, thus increasing viscosity. Therefore, one can attribute the increased viscosity of the solutions during processing to the thermal denaturation of whey proteins and to their mutual interactions, or to CN micelles when present in the medium (samples B to E).

In this study, we can infer that the behavior observed in the experiments classified into region I is greatly influenced by the chemical environment (pH 7.0). It is possible to see that the speed of the increase in peak viscosity and the presence of a breaking point (defined later) are related to intense protein denaturation, justified by the higher ionization and activity of SH (sulfhydryl) groups of the cysteines in promoting the deployment of conformations of globular whey proteins during heat treatment. For the peptide chain, the pK is about 9.5 at 25°C. This means that, on average, at pH 6.1, 6.4, and 6.7, approximately 0.04, 0.08, and 0.16% of the groups are decoupled, respectively (Walstra et al., 2006). Within the pH range used in the experiments (5.5 to 7.0), the net charge is negative in most of the whey proteins and increases with pH (Law and Leaver, 2000). At pH 7.0, a strong negative net charge is present, which induces intramolecular electrostatic repulsion and promotes the rapid unfolding of β -LG (mainly) and α -LA molecules during heating, while still allowing aggregation and potential precipitation as a result of hydrophobic intermolecular bonds. The resulting loss of uniformity in the RVA sample compartment is indicated by a sharp viscosity decrease; this phenomenon is designated herein as a breaking point.

The opposite trend was observed in the samples classified into region II. Because of the lower pH (5.5), denaturation is delayed, as evidenced by the higher temperature at the beginning of the peaks. Furthermore, aggregation interactions occur more slowly, as indicated by the viscosity plateaus after the increases in viscosity.

This same evaluation was observed by Law and Leaver (2000), who pointed out that the denaturation reactions of β -LG and α -LA are enhanced when the pH of milk is increased and delayed when the pH is lowered. Another remark regarding this protein group is the increased hydrophobic interactions occurring after denaturation, which is a major factor in loss of effect on the solubility of these proteins.

Based on the present data, it seems we can suggest some industrial or technological application for the RVA viscosity analysis, mainly for products containing dairy proteins. In this sense, depending on the pH conditions and of the desired viscosity of the product, the composition of the protein system can be set up to obtain the highest performance for the specific thermal process to be applied for the production technology.

CONCLUSIONS

In this study, we observed the influence of the synergistic effects of protein systems based on various combinations of MPC and WPC by interpreting the rheological responses captured during the startup viscosity ramp by RVA during thermal processing under the influence of pH. A pH value of 5.5 influenced the onset temperature of the viscosity peak, increasing it by at least 5°C compared with experiments at pH 6.5 and 7.0, and demonstrating the clear influence of this parameter in relation to the reactions and interactions of the proteins in PS subjected to different heat treatments. The type of protein present in the PS influenced the rheological response captured in the RVA, producing higher startup viscosity values in the 30:70 product with respect to MPC:WPC proportion at pH 7.0. Conversely, this same product presented the lowest startup viscosity values at pH 5.5.

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