Studii şi Cercetări Ştiinţifice 2019, **20** (2), pp. 209 – 218
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry *ISSN 1582-540X*

ORIGINAL RESEARCH PAPER

EFFECT OF HEAT TREATMENT ON PHYSICAL PROPERTIES OF WHEY PROTEIN BEVERAGES (WPB)

Anibal J. Barrios Quant*, Alberto R. Albis, Jose A. Perez

[1]*Universidad del Atlántico, Facultad de Ingeniería, Departamento de Ingeniería química, (Bloque H), 080007 Puerto Colombia, Colombia*

*Corresponding author: abarriosquant@fsu.edu

Received: September, 05, 2018
Accepted: May, 28, 2019

Abstract: There is a great interest in the production of heat-stable and clear beverages containing high levels of whey proteins. A challenge of incorporating whey proteins in sports beverages is hot-fill treatment (88 °C, 2 min). The objective of this research was to analyze the effects of thermal treatment on the profile of whey proteins in a whey protein beverage (WPB). WPB were prepared mixing 5 % whey protein with 0.04 % potassium sorbate and 0.5 M phosphoric acid was used to adjust *p*H to 3.0 and 7.0. The protein particle size and zeta-potential were tested using a spectrophotometer. Finally, the protein profile of beverages containing whey was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Hot-fill treatment had a negative impact on the physiochemical properties of whey proteins. The formation of protein-protein complexes produced an increase in particle size and absolute zeta potential in WPB formulations at both *p*H 3.0 and 7.0.

Keywords: *hot-fill treatment, particle properties, protein profile, SDS-PAGE, sport beverage*

INTRODUCTION

Whey represents a valuable by-product and can be directly processed to obtain a wide range of whey products: whey protein concentrate, whey protein isolate or individual whey proteins [1, 2]. Whey proteins are becoming increasingly employed in the sports field due to their contribution as a substantial amount of globular proteins for muscle growth and their utilization as a food ingredient in nutritional and sports supplements, especially beverages containing whey, due to their health and nutritional benefits to athletes [1]. Whey protein beverages (WPB) are functional drinks designed to provide essential amino acids for protein synthesis, support recovery, increase muscle strength, and increase training intensity and endurance [3]. The production of WPB with heat-stable and clear appearance characteristics is an increasing demand on the sports nutrition market [4]. A challenge of consolidate whey proteins beverages is the thermal treatments applied to prolong shelf life (hot-fill treatment at 88 °C, 2 min). Hot fill treatment produces denaturation and causes whey protein aggregates when the *p*H is close to the isoelectric point (*p*I), which is approximately 5.0, creating a white precipitate [5].

Whey proteins undergo denaturation over thermal processing, which means whey proteins suffer structural changes through unfolding of their initially folded state [6]. The major concern in the dairy industry is the denaturation suffered by whey proteins on processing [7]. When whey proteins are denatured they release narrow quantities of sulfur compounds. For example, hydrogen sulfide and methanethiol which are extremely flavorsome compounds cause cooked flavors in milk when heated [8]. Heat treatments usually affect the solubility and structure of whey proteins. The effect of the heat treatments over these characteristics fluctuates with the composition of the whey protein solution and the nature of the whey protein [9].

When whey proteins unfolding takes place with a loss of helical structure, the denaturation process is considered reversible, yet irreversible when whey protein aggregates appear involving sulfhydryl (–SH)/disulfide (S-S) interchange reactions or in the presence of hydrophobic and electrostatic interactions [10 – 12]. Whey protein aggregates and denaturation process are of appreciable interest to food scientists because of the potential to enhance the nutritional and properties of sports products [13]. The main purpose of this study was to analyze the effect of heat treatment (88 °C,2 min) on WPB, analyzing the changes of molecular size by sodium dodecyl sulfate-polyacrylamide electrophoresis (SDS-PAGE), and physiochemical properties of the beverage, solubility, zeta-potential and viscosity, with the aim of studying the negative consequences that may arise.

MATERIALS AND METHODS

Materials

Whey protein isolate (WPI) was obtained from MyProtein (Cheshire, UK); it had a proximate composition of 96.0 % (w/w) protein (N × 6.38), 2.5 % carbohydrate, 0.3 % fat, and 0.5 % salt (data provided by supplier). It was used to prepare a stock solution (100 mg·mL^{-1} protein) using deionized (DI) water and potassium sorbate 0.04 % (w/w) as a preservative. The stock solution was diluted to a final concentration of 50 mg·mL^{-1}

and allowed to rehydrate overnight at 4 °C.

Sample preparation

Protein stock solutions (10 % w/w) were prepared by dissolving the WPI powder in water with 0.04 % potassium sorbate (Table 1). WPI solutions were stirred continuously overnight 4 °C to ensure complete rehydration. For studies of protein physiochemical properties, denaturation and production of soluble protein aggregates, a 5 % (w/w) WPI solution was prepared. Finally, the *p*H of the stock solution was adjusted to 3.0 and 7.0 with 0.5 M of phosphoric acid. All of the reagents were pure grade [14].

Table 1. *Model WPB formulation*

Ingredient	% [w/w]	Final concentration [mM]
Water	95.91	
WPI	5.00	0.76 - 0.15
Potassium sorbate	0.04	
H_3PO_4 (1 M)	0.13	9.2
Red 40	0.02	

During sample preparation, all manipulations were performed at 4 °C. All samples were homogenized at 11.00 rpm for 1 min twice using an ULTRA-TURRAX T10 basic homogenizer (IKA Works, Wilmington, NC, USA). The samples were divided into heated (H) and unheated (U); the heated samples were prepared at 88 °C/600 rpm for 2 min using a Thermomixer F0.5 (Eppendorf, Hamburg, Germany), mixed in the Analog Vortex Mixer (Fisher Scientific, Hampton, NH, USA) and finally transferred to a pipette to prepare the sample used to load the electrophoresis gel, prior characterization [15].

Reducing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

Reducing SDS-PAGE was performed to study the protein profile of WPB using a Mini-PROTEAN Tetra Cell (Bio-Rad) equipped with a PowerPAC 300 power supply (Bio-Rad Laboratories, Hercules, CA, USA). The resolving gels contained 4 % and 15 % of polyacrylamide, respectively [16, 17].
WPB loading masses were 2 µg/lane and 10 µg/lane. WPB solutions were mixed with Laemmli sample buffer and diluted samples were prepared for reduction with β-mercaptoethanol (2-ME). This was used as a dissociating reagent to obtain degradation of co-aggregates formed by disulfide interactions. The protein profiles on the gel were visualized by Coomassie Blue. All images from reducing SDS-PAGE were captured by the Azure C400 Imaging System and analyzed using the AzureSpot software (version 14.2, Azure Biosystems Inc., Dublin, CA, USA [18].

Heat treatment

Samples were heated using hot-fill conditions (88 °C, 2 min) as used by the beverage industry to prevent yeast and mold growth in shelf stable juices and other acidic (*p*H ≤ 4.6) beverages [19]. Heated samples were either not filtered or passed through a 0.1 µm pore size syringe filter (Durapore, Millipore, Bedford, MS, USA) within the

same day of heating. The experiments were carried out, at least, in triplicate.

Determination of particle size & zeta-potential

Size distribution of WPB was performed using a Malvern Zeta-Sizer (ZS90) (Malvern Instruments Ltd., United Kingdom) to measure the mean size and the size distribution of WPB particles by dynamic light scattering. For size measurement, WPB was introduced into disposable sizing cuvettes at *p*H 3.0 and 7.0. The temperature of the cell was maintained at 25 ± 0.5 °C during measurement. Average diffusion coefficients were determined by the method of cumulants fit and were translated into average particle diameters (Z-value) using the Stokes-Einstein relationship [20].
Polydispersity index (PDI) derived from cumulants analysis of the DLS measurements was also evaluated; it describes the width or the relative variance of the particle size distribution. Each sample was measured 11 times to obtain average particle size and its distribution using Zeta-sizer operation software (PCS: zeta mode v 1.51, Malvern Instruments Ltd., Malvern, UK). Zeta-potential was measured using 1 mL of WPB. Both measurements were carried out, at least, in triplicate.

Experiments design

The preparation of the different WPB formulations was based on the concentration of WPI mixtures in which the independent variables were different ratios of the *p*H and the heating conditions (heated and unheated) (Table 1). Eight different samples were tested. Analysis of variance was performed using PROC GLM in PC SAS version 9.2 (SAS, Cary, NC, USA). A significant treatment effect was indicated by a significant F test (P 0.05). Differences between means were determined using the Tukey-Kramer multiple means comparison test.

RESULTS AND DISCUSSION

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE profiles of WPB samples (Figure 1) were divided into two zones depending on the loading concentration used on the gel (10 and 2 µg/lane), the *p*H (3.0 - 7.0), and the treatment conditions (heated and unheated samples). There were no appreciable differences in the WPB profiles at the two different loading concentrations; the high loading concentration (10 µg/lane) presented more intense bands and was used to analyze the profiles in this study.

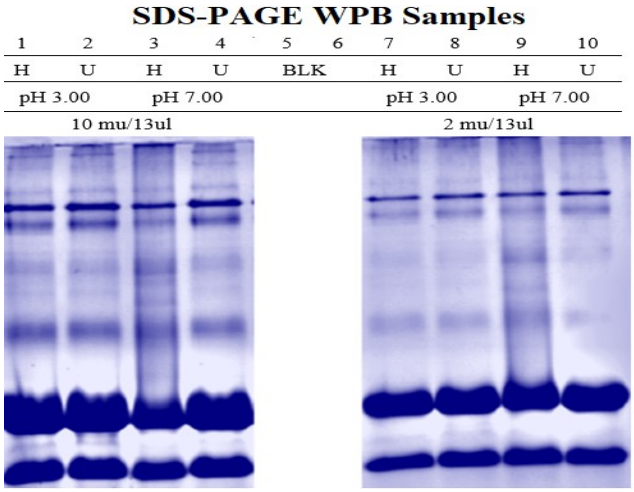

Figure 1. *SDS-PAGE gels of WPB samples*
*(H) heated samples at 88 °C, 2 min; (U) sample not heated

SDS-PAGE analysis clearly indicated that the heat treatment of WPB had an effect at *p*H 7.0 and showed that at *p*H values well below the *p*I of WPB, the proteins remained stable. Whey proteins are currently formulated below *p*H 3.5 in order to maintain clarity and stability [21]. At a *p*H value above the *p*I (*p*H 7.0), samples become turbid and unstable after heat treatment.
The degree of denaturation of dried whey proteins is related to the temperature used in the spray dryer [22]. β-LG precipitates rapidly and selectively at high temperature (70-120 °C) and *p*H near neutral (*p*H 7.0), α-LA precipitates and aggregates more at acidic *p*H (3.5-5.5) and moderate temperatures (50-65 °C) with long reaction times; this is usually accompanied by the precipitation of bovine serum albumin (BSA), immunoglobulins (Ig) and lactoferrin (Lf), while β-LG and casein-macropeptide (CMP) remain soluble [23 – 25].
Bands of whey proteins after heat treatment (H) at *p*H 7.0 on both concentration loadings produced smeared bands indicating a range of molecules of different molecular weights. Also, the big stain that belongs to the β-lactoglobulin present in the line (4) corresponding to unheated WPB at *p*H 7.0 was reduced in size when the sample was heated (88 °C for 2 min) and the other bands at 18 kDa and 66 kDa, could be attributed to SDS-monomers of beta-lactoglobulin (β-LG) and bovine serum albumin (BSA) respectively. The beta-LG and alpha-LA bands were located at probably ~18,000 and ~14,000 respectively [26 – 28].

Particle size in relation with *p*H

Particle size changes occurred due to heat treatment (H) at *p*H 3.0 and 7.0 (Figure 2). WPB particles were larger after hot-fill treatment; these results are shown in Table 1**.** The heated samples showed lower electrophoretic mobility than the unheated samples, which demonstrated the influence of the larger particles on the brownian movement and the velocity to reflect the scattering light inside the Zeta NanoSizer; the influence of heating at 88 °C for 2 min was evident in the WPB particle size data. The heated WPB at *p*H 3.0 showed a wide size distribution (polydispersity index 0.570) with a maximum near 190 nm.

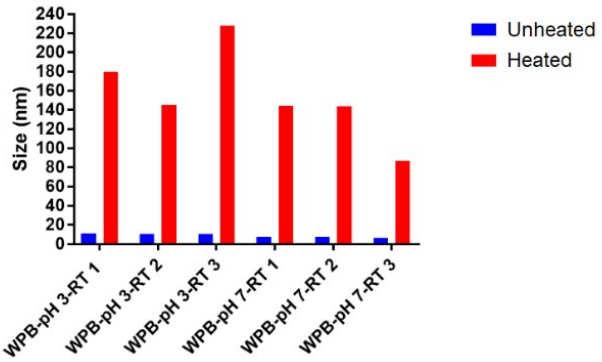

Figure 2. *Particle size distribution of WPB with 5 % protein (WPI) samples on Unheated and Heated conditions*

* RT means room temperature and the following number means the replicate times

As the sample preparation procedure was strictly the same for all 3 assays, the remarkable difference in average size of particles is attributed to the emulsifying properties of the protein near its isoelectric point; this property is apparently poorer at *p*H 3.40 than at any other *p*H away from the isoelectric point. The particle sizes of the heated samples (H) at *p*H 3.0 and 7.0 were higher than those of the unheated samples; this behavior is attributable to the protein-protein complexes formed in the heat treatment as a result of protein denaturation, according to [29].

Zeta- potential

The unheated samples showed lower Zeta-Potential than the heated samples (Figure 3). At *p*H 3.0, WPB exhibits a high positive net charge with an electrophoretic mobility corresponding to the whey protein particles for unheated and heated respectively (Table 1). At *p*H 7.0, on the other side of the isoelectric area, a similar negative mobility is observed. At this *p*H, whey proteins assume a net negative charge. As the *p*H increases, the net negative charge of the protein rises and thus the absolute mobility of the WPB significantly increases. The highest positive value observed is 13.2 mV at *p*H 3.0 whereas the highest negative value is -7.39 mV at *p*H 7.

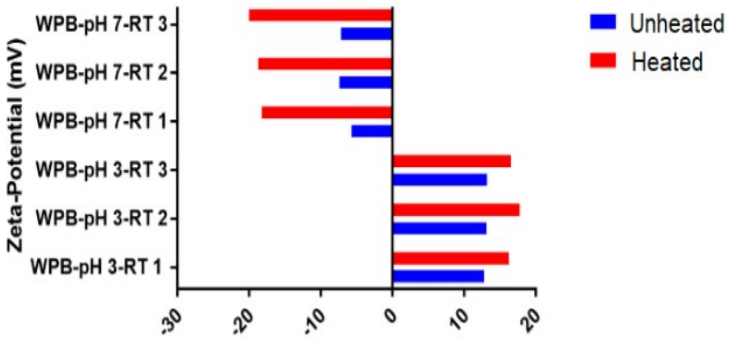

Figure 3. *Zeta potential of WPB as a function of pH. Data are means of three separate experiments with three measurements per pH, zeta potential data were experimentally measured at pH 3.0 and 7.0*

* RT means room temperature and the following number means the replicate times

The variations of the zeta potential under heated and unheated conditions based on electrophoretic mobility measurements were shown in Figure 3. The highest positive value observed is 17.8 mV at *p*H 3.0 whereas the highest negative value is - 20 mV at *p*H 7. These values were higher than the unheated conditions showing the effect of the heat treatment on the physiochemical properties of the whey protein beverages.
The physicochemical changes related to the particle size and zeta potential were analyzed by a One-Way ANOVA analysis to assess whether the different conditions of the WPB models yielded statistically different results for those variables evaluated (Table 2). These results were useful for determining the variability of particle size and zeta potential [30].

Table 2. *ANOVA analysis*

Source of variation	± SD	F-value	p-value	R-square	Is it significant (p-value < 0.05)
Particle-Size - *p*H 3.0 Unheated & Heated	2.754	2.603	0.221	0.6345	Yes
Particle-Size - *p*H 7.0 Unheated & Heated	8.754	0.07681	0.9278	0.04871	Yes
Zeta-Potential - *p*H 3.0 Unheated & Heated	2.166	0.05572	0.0184	0.03581	Yes
Zeta-Potential - *p*H 7.0 Unheated & Heated	6.746	0.01867	0.9816	0.0123	Yes

Results showed significant differences ($p < 0.05$) for both physicochemical properties (particle size and zeta potential) when the WPB models were compared at the same *p*H on unheated or heated samples. This difference is attributed to the formation of aggregates as shown on the top of the WPB in Figure 4, which probably interrupted the mobility of the particles inside the cuvettes, and to the protein-protein complexes, resulting from protein denaturation, formed during heating [31].

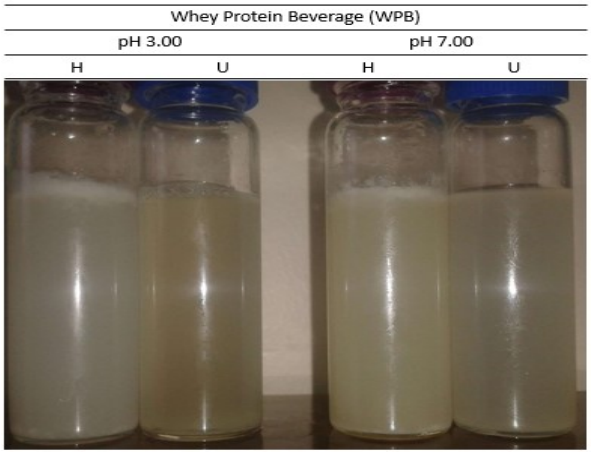

Figure 4. *Physical appearance of WPB*
Left to right: *(1) WPB pH 3 unheated, (2) WPB pH 3 unheated, (3) WPB pH 7 unheated, (4) WPB pH 7 unheated*

The unheated samples displayed in Figure 4 showed more clarity and transparency than heated samples; the unheated sample at *p*H 3.0 was less turbid that the unheated sample at *p*H 7.0. Also, the heated samples presented foam at the top of the WPB; this pattern is attributed to protein denaturation and the formation of soluble aggregates. The samples that showed a layer of foam on the top of the WPB were the samples heated (Table 3). These presented bigger particle sizes, higher zeta-potentials, and more turbidity. Greater turbidity was associated with larger particles in the heated samples.

Table 3. *Surface properties of whey protein beverage*

	Physical properties of whey protein beverage			
*p*H	3.00		7.00	
Condition	U	H	U	H
Mean particle size [nm]	13.3	189.4	16.5	134.098
Zeta-potential [mV]	11.3	17.1	-26.3	-28.7
Polydispersity index [PdI]	0.2	0.7	0.2	0.6
Electrophoretic mobility [$\mu m \cdot cm \cdot V^{-1} \cdot s^{-1}$]	0.9	0.2	-0.9	-1.7
Conductivity [$mS \cdot cm^{-1}$]	15.8	16.3	10.5	11.7

*Temperature of measurements: 25 °C

According to [32] whey protein should have a mean particle size between 12.675 ± 3.2 and 22.03 ± 0.91 nm which matches our data on unheated samples at *p*H 3.0. The increase in the mean particle size of the beverage samples during the heat treatment can be explained by the formation of soluble aggregates, as shown by [33]. In that study, when whey protein beverages were heated statically at 90 °C for 10 min, soluble aggregates were formed at *p*H 7.5. They also reported that the beverage with soluble aggregates had lower turbidity and viscosity and smaller aggregate size, and that the aggregates had a more negative zeta potential as the results presented in this study related to heated samples. It is important to highlight that the conductivity of the samples increased when the WPB was heated and that this behavior was attributed to the larger surface area of the heated particles, which is related to their capacity to conduct electricity.

CONCLUSION

Hot-fill treatment of a model whey protein beverage had a negative impact on the physiochemical properties of the whey proteins. The formation of protein-protein complexes produced an increase in the particle size and the absolute zeta potential in the whey protein beverage formulations at both *p*H 3.0 and 7.0. The heat treatment caused protein denaturation and produced high turbidity and precipitates in the whey protein beverage. These results indicate the necessity to develop different paths to ensure shelf life of whey proteins such as the use of preservatives. Finally, what is important for beverage stability is not that particle size increases by heating (as it will at any food-relevant concentration), but the absolute size and reactivity of the particles formed by heating. In this case, the absolute value of the zeta potential increased and the largest particle size was ~ 190 nm. This would suggest a stable beverage, when WPB were formulated in *p*H 3.0.

REFERENCES

1. Kilara, A., Vaghela, M.N.: Chapter 4: Whey proteins, *Proteins in Food Processing (Second Edition)*, **2018**, 93-126;
2. Grosu, L., Alexa, I.C., Patriciu, O.I.: Study concerning the production of biomass protein by valorisation of by-products from the dairy industry, S*cientific Study & Research - Chemistry & Chemical Engineering, Biotechnology*, **2015**, **16**, 169-172;
3. Laclair, C.E., Etzel, M.R.: Ingredients and *p*H are Key to clear beverages that contain whey protein, *Journal of Food Sciences*, **2010**, **75**, 21-27;
4. Lee, C.A., Vickers, Z.M.: The astringency of whey protein beverages is caused by their acidity, *International Dairy Journal*, **2008**, **18** (12), 1153-1156;
5. Etzel, M.R.: Manufacture and use of dairy protein fractions, *The Journal of Nutrition*, **2004**, **34** (4), 996-1002;
6. Wijayanti, H.B., Bansal, N., Deeth, H.C.: Stability of whey proteins during thermal processing: A Review*, Comprehensive Reviews in Food Science and Food Safety*, **2014**, **13** (6), 1235-1251;
7. Anema, S.G.: The whey proteins in milk: Thermal denaturation, Physical interactions, and effects on the functional properties of milk, *Milk Proteins: from Expression to Food, 2nd Edition*, **2014**, 269-318;
8. Al-Attabi, Z., D'Arcy, B.R., Deeth, H.C.: Volatile sulphur compounds in UHT milk, *Critical Reviews on Food Science & Nutrition*, **2009**, **49**, 28-47;
9. Dewit, J.N., Klarenbeek, G.: Effects of various heat-treatments on structure and solubility of whey proteins, *Journal of Dairy Science*, **1984**, **67** (11), 2701-2710;
10. Vasbinder, A.J., De Kruif, C.G.: Casein-whey protein interactions in heated milk: the influence of *p*H, *International Dairy Journal*, **2003**, **13** (8), 669-677;
11. Hoffmann, M.A.M., Van Mil, P.: Heat-induced aggregation of β-lactoglobulin: role of the free thiol group and disulfide bonds, *Journal of Agricultural Food Chemistry*, **1997**, **45** (8), 2942-2948;
12. Anema, S.G., Li, Y.: Further studies on the heat-induced, *p*H-dependent dissociation of casein from the micelles in reconstituted skim milk, *Lebensm Wiss Technology - Food Science & Technology*, **2000**, **33** (5), 335-343;
13. Ashokkumar, M., Lee, J., Zisu, B., Bhaskarcharya, R., Palmer, M.: Hot topic: sonication increases the heat stability of whey proteins, *Journal of Dairy Science*, **2009**, **92** (11), 5353-5356;
14. Rittmanic, S.: U.S. Whey proteins in ready-to-drink beverages, *U.S. Dairy Export Council*, **2006**, 7-8;
15. Tokur, B.K., Sert, F., Aksun, E.T., Özoğul. F.: The effect of whey protein isolate coating enriched with thyme essential oils on trout quality at refrigerated storage (4 ± 2 °C), *Journal of Aquatic Food Product Technology*, **2016**, **25** (4), 585-596;
16. Brunelle, J.L., Green, R.: Chapter twelve - one-dimensional sds-polyacrylamide gel electrophoresis (1D SDS-PAGE), *Methods in Enzymology*, **2014**, **541**, 151-159;
17. Hosseinpour, S., Izadi, M., Aminlari, M., Ramezani, R., Tavana, M.: Changes in the solubility and SDS-PAGE profile of whey proteins during storage at different temperatures: A kinetic study, *Journal of Food and Agriculture Science*, **2011**, **1**, 15-21;
18. Qi, P.X., Xiao, Y.P., Wickham, E.D.: Changes in physical, chemical and functional properties of whey protein isolate (WPI) and sugar beet pectin (SBP) conjugates formed by controlled dry-heating, *Food Hydrocolloids*, **2017**, **69**, 86-96;
19. Laclair, C.E., Etzel, M.R.: Turbidity and protein aggregation in whey protein beverages, *Journal of Food Science*, **2009**, **74** (7), 526-535;
20. Amin, S., Barnett, G.V., Pathak, J.A., Roberts, C.J., Sarangapani, P.S.: Protein aggregation, particle formation, characterization & rheology, *Current Opinion in Colloid & Interface Science*, **2014**, **19** (5), 438-449;
21. Beecher, J.W., Drake, M.A., Luck, PJ., Foegeding, E.A.: Factors regulating astringency of whey protein beverages, *Journal of Dairy Science*, **2008**, **91**, 2553-2560;
22. Outinen, M., Tossavainen, O., Tupasela, T., Koskela, P., Koskinen, H., Rantamäki, P., Syväoja, E.L., Antila, P., Kankare, V.: Fractionation of proteins from whey with different pilot scale processes, *Food Science Technology*, **1996**, **29**, 411-417;
23. Plock, J., Spiegel, T., Kessler, H.G.: Influence of the lactose concentration on the denaturation kinetics of whey proteins in concentrated sweet whey, *Milchwissenschaft-Milk Science International*, **1998**, **53**, 389-393;

24. Kiesner, C., Clawin-Rädecker, I., Meisel, H., Buchheim, W.: Manufacturing of alpha-lactalbumin-enriched whey systems by selective thermal treatment in combination with membrane processes, *Lait*, **2000**, **80**, 99-111;
25. Fernández, A., Menendez, V., Riera, F.A., Álvarez, R.: Caseinomacropeptide behaviour in a whey protein fractionation process based on alpha-lactalbumin precipitation, *Journal of Dairy Research*, **2011**, **78**, 196-202;
26. Hong, Y., Creamer, L.K.: Changed protein structures of bovine β-*lactoglobulin* B and α-*lactalbumin* as a consequence of heat treatment, *International Dairy Journal*, **2002**, **12** (4), 345-359;
27. Peram, M.R., Loveday, S.M., Ye, A., Singh, H.: In vitro gastric digestion of heat-induced aggregates of β-*lactoglobulin*, *Journal of Dairy Science*, **2012**, **96** (1), 63-74;
28. Aich, R., Batabya, S., Joardar, S.N.: Isolation and purification of beta-lactoglobulin from cow milk, *Veterinary World*, **2015**, **8** (5), 621-624;
29. Le, T., Saveyn, P., Dinh, Hoa, H., Van der Meeren, P.: Determination of heat-induced effects on the particle size distribution of casein micelles by dynamic light scattering and nanoparticle tracking analysis, *International Dairy Journal*, **2008**, **18** (12), 1090-1096;
30. Bull, S.P., Hong, Y.C., Khutoryanskiy, V.V., Parker, J.K., Faka, M., Methven, L.: Whey protein mouth drying influenced by thermal denaturation, *Food Quality and Preference*, **2017**, **56**, 233-240;
31. Ryan, K.N., Foegeding, A.E.: Formation of soluble whey protein aggregates and their stability in beverages, *Food Hydrocolloids*, **2015**, **43**, 265-274;
32. Jambrak, A.R., Mason, T.J., Lelas, V., Paniwnyk, L., Herceg, Z.: Effect of ultrasound treatment on particle size and molecular weight of whey proteins, *Journal of Food Engineering*, **2014**, **121**, 15-23;
33. Ryan, K.N., Zhong, Q., Foegeding, A.E.: Use of whey protein soluble aggregates for thermal stability-a hypothesis paper, *Journal of Food Science*, **2017**, **78** (8), 1105-1115.