Effect of **ultrasonication**, pH and heating on stability of apricot gum-lactoglobuline two layer nanoemulsions

Omid Shamsara^{ab}, Zayniddin Kamarovich Muhidinov^a, Seid Mahdi Jafari^{b*}, Jamshed Bobokalonov^c, Abduvaly Jonmurodov^a, Mostafa Taghvaei^b, Mont Kumpugdee-Vollrath^d

^a Chemistry Institute of the Tajikistan Academy of Sciences, Dushanbe 734063, Tajikistan

^b Department of Food Materials and Process Design Engineering, Faculty of Food Science and Technology, University of Agricultural Sciences and Natural Resources, Gorgan, Iran

^c Kaunas University of Technology, Kaunas 44029, Lithuania

^d Beuth University of Applied Sciences, Department of Pharmaceutical Technology, Berlin 13353, Germany

*Corresponding author: Pishro Food Technology Research Group, Tel/fax: +98 17 32426432

Email address: smjafari@gau.ac.ir (SM Jafari)

Abstract

The objectives of this study were to evaluate the effect of apricot gum-lactoglobuline (AG/LgC) ratio, thermal treatment, **sonication with different times and amplitudes** and pH, on double layer sunflower oil in water emulsion stability. The emulsion stability **was determined** by the evaluation of **emulsion performance indices including** particle size, zeta potential, creaming and emulsion volume stability during 10 days of storage. Applying AG and LgC with the **ratio of 12.5:1 AG:LgC**, in order to obtain double layer oil in water emulsion, could result in a completely stable nano-emulsions during 10 days storage in room temperature. The ultrasound treatment significantly increased the emulsion stability. A 10 minutes ultrasound treatment with the amplitude of 25% was the optimum conditions for ultra-sonication. The best temperature for thermal treatment and the best pH, in order to improve the emulsion's stability, was 50°C and 3, respectively.

Keywords: Emulsion stability; Apricot gum; Lactoglobuline.

1. Introduction

The protein-polysaccharide interaction in liquid environment offers the opportunity of designing a new interfacial structure for emulsions with possible applications in food and pharmaceutical industries. The interaction of protein and polysaccharide molecules may occur chemically through covalent bonds or physically through electrostatic interactions [1, 2]. Theoretical and experimental studies indicate that various molecular and thermodynamic factors can be taken into consideration to optimize the effectiveness of covalent conjugates and electrostatic complexes for stabilizing emulsions [3, 4]. Maillard type protein-polysaccharide conjugates have excellent emulsifying and steric stabilizing properties, especially under conditions where the protein alone is poorly soluble [5]. Charged polysaccharides form soluble complexes or coacervates with proteins depending on pH, ionic strength, and biopolymer charge distribution [6].

The structure and stabilizing properties of protein-polysaccharide layer depends on the adsorption sequence of biopolymers to the interface [7]. There is a good potential for use of interfacial protein-polysaccharide complexes in the nano-scale engineering such as nano-encapsulation and delivery of nutrients [8], and protection of adsorbed proteins and emulsified lipids against enzymatic breakdown during digestion. Additionally, the polysaccharide complex formation could be used to improve plant protein functionality. For example, it has been shown that complex formation of seed globulins and anionic polysaccharides improve the globulin solubility in the vicinity of their isoelectric points (pI) and at lower pH values [9]. The interactions between other proteins and hydrocolloids in aqueous solutions have been vastly investigated [3, 5, 10, 11, 12, 13].

Emulsions can be formed using a two-step procedure called layer-by-layer technique. First, a primary emulsion is formed using an ionic emulsifier that facilitates the formation of small droplets during homogenization. Then, a secondary emulsion is formed by adding an oppositely charged polymer that adsorbs onto the droplet surfaces. The resulting droplets are coated by a two-

layer interfacial membrane that may provide improved emulsion stability and physicochemical properties [14, 15].

A number of studies have shown that it is possible to improve emulsion stability by combining the beneficial attributes of different types of emulsifiers by forming emulsions containing droplets stabilized by multilayered membranes [16]. It has been demonstrated, for example, that whey protein isolate (WPI) is capable of forming water-soluble complexes with certain hydrocolloids such as xanthan, guar and locust bean gums [17]. The thermodynamic aspects of protein-polysaccharide complex formation as well as their structural and functional properties were recently reviewed by Turgeon, Schmitt and Sanchez [18].

It has been shown that cross-linked beet pectin improved the stability of emulsions and was superior to simple deposition on the surface of lipid droplets [19]. In layer by layer deposition of protein, followed by cross-linking by laccase, emulsions showed better stability against NaCl and creaming and it was suggested that thick polymer layer decreases van der Waals attraction with increasing steric repulsion between droplets. As stabilizer in food emulsions, some gums were found to migrate slowly to air-water and oil-water interfaces and exhibited some surface and interfacial activities [20]. Jafari and his co-workers [21, 22], and Abbasi and Azarikia [23] have suggested that gums participate or adsorb onto oil droplets and satirically stabilize emulsions against flocculation and coalescence. However, it was shown that both adsorbing and non-adsorbing hydrocolloids are capable of stabilizing the system [6, 24]. The term 'adsorbing' is related to charged polysaccharides, which can interact with proteins via electrostatic forces and the interaction is highly dependent on pH and ionic strength of solution. In case of using this type of hydrocolloids, stability of system is due to electrostatic repulsion, steric repulsion or both of them. On the other hand, 'non-adsorbing' hydrocolloids can prevent serum separation by increasing the viscosity of continuous phase, entrapping water in a network and immobilizing the particles [23, 25, 26].

Gums are a complex of polymeric structures contained mainly, as monomers, pentoses, hexoses and uronic acids [27]. The molar ration of galactose, arabinose and xlylose found in apricot gum (AG) was 14:10:1 [28]. The structures of two aldobiouronic acids, 2-O- β -D-glucopyranosylurono-D-mannopyranose and 6-O- β -D-glucopyranosylurono-D-galactopyranose, have been confirmed by interpreting their ^{13}C NMR spectra [28]. AG as a plant gum may contain arabinogalactan proteins (AGPs); some plant glycoproteins which composed of a core-protein backbone O-glycosylated by one or more complex carbohydrates consisting galactan and arabinose as main components. Arabinogalactan proteins might be soluble, or might act as modulators and coreceptors of apoplastic morphogens; their amphiphilic molecular nature makes them prime candidates of mediators between the cell wall, the plasma membrane, and the cytoplasm [29].

In this study, a cationic protein (β -lactoglobulin) and anionic polysaccharide (apricot gum) emulsifiers, with different ratios, were used to produce a stable oil in water emulsion. The objective of the present study was to investigate the ability of theses natural emulsifiers, on stabilizing sunflower oil in water nano-emulsions and the effect of pH, thermal treatments and ultrasound treatments on the emulsion stability.

2. Materials and methods

2.1. Sample preparation

The emulsions were prepared using whey lactoglobuline concentrate (LgC) which contained 37.35% of β -LgA, 52,9% of β -LgB and 9.7% of α -LgA [13]. The polysaccharide of AG from apricot tree, growing in Tajikistan, was isolated by 2 times extraction with water-alcohol solution. Sodium chloride, hydrochloric acid and sodium hydroxide were purchased locally and potassium monobase phosphate (KH_2PO_4) was obtained from Sigma Aldrich (USA) in order to prepare dissolution media. Pectin solutions (0.5 wt%) were centrifuged to remove microgel particulates and all the other solutions were filtered through 0.45 μm ultrafilter (Millipore, USA) before preparation of emulsions.

2.2. High-performance size exclusion chromatography (HPSEC)

Determination of molecular weight and size distributions of AG was performed by coupling on line to a high-performance size exclusion chromatography, a differential refractometer and a differential viscosimeter using universal calibration, as shown in Fig 1 [30]. Physico-chemical characterization of pectic polysaccharides from various sources obtained by steam assisted flash extraction (SAFE). 15 mg AG dissolved into 10 mL 50 mM NaNO₃ buffer containing 0.02 wt% NaN₃ as preservative were filtered through 0.22 µm Millex HV filter (Millipore Corp., Bedford, MA), and injected on a Waters HPSEC system constituted of a two PL-Aquagel size exclusion columns (OH-60 and OH-40) which used in series. The samples were eluted at 0.8 mL min⁻¹ with a 50 mM NaNO₃ solution. On-line molecular weight and intrinsic viscosity determinations were performed at room temperature using a ViscoStar model differential pressure viscometer (Wyatt Technology, USA), and a differential refractometer (Waters 2410, USA).

2.3. Emulsion preparation

A primary emulsion was prepared by homogenizing 4 mL sunflower oil with emulsifier solutions containing 0.2 wt% LgC or AG for 15 minutes, using a high-speed homogenizer model IKA T-25 (Ultra Turrax, Ika-Werke GmbH & Co.Kg, Germany) at 12000 rpm and 60°C [31]. The system was kept under mechanical stirring in order to achieve a fine emulsion and to form a polymer envelop around the oil droplet, which was stabilized by adding appropriate volume of apricot gum solutions to form secondary emulsions with varying AG/LgC ratio. The immediate capsulation resulted in the formation of a microcapsule milky colloidal suspension. The system was left at room temperature under continuous stirring (6000 rpm) until the temperature of solution reached 30°C. The emulsions were then stored at room temperature for 24 h before being analyzed. All experiments were carried out using freshly prepared emulsions. The optimization of the preparation process was assessed by measurements of particle size, zeta potential and visual observation of milky stabile dispersion.

2.4. Droplet size and zeta potential measurement

The particle size distribution and zeta potential of the emulsions were measured using Zetasizer Nano instrument (Malvern Instruments Ltd., Malvern, UK).

2.5. Emulsion stability

To study the kinetics of phase separation, immediately after addition of the gum or protein, emulsion mixtures (18 mL) were poured into 25 mL-graduated glass tubes. The tubes were sealed and stored quiescently at room temperature. **The volume of apparently stable emulsion without any creaming was monitored** daily for 10 days [32].

2.6. Ultrasonic treatment

Before the ultrasound treatments, the aqueous and oil phase were premixed together in a beaker using a magnetic stirrer. 5 mL aliquot of the mixture was introduced into a round bottom glass. Sonication was carried out using an ultrasonic processor (EW-04714-53, Cole Parmer, USA), with the operating frequency of 20 KHz and power output of 130 W. A tapered titanium sonotrode of 3 mm in diameter was used for sonication. The tip of sonotrode was placed 1 cm below the surface of mixture [33]. All samples were sonified for 10, 15, 20 and 25 minutes with the amplitude of 25, 50, 75 and 100 percent, in thermostatic water bath. All ultrasound experiments were conducted in duplicates. One of the two samples was used to measure emulsion stability and particle size measurement.

2.7. Statistical analysis

All experiments were performed using three freshly prepared samples (replicates). The results presented are the averages and standard deviations that were calculated using Student's "t" test for independent samples in Microsoft Excel software.

Fig. 1

3. Results and discussion

3.1. Analysis of the extracted apricot gum

Analysis of the 40 wt% water alcohol solution fractions of apricot gum by **HPSEC** (Fig. 1) showed that each contained similar proportions of the various sugars and differed essentially in their molecular masses and protein contents. The bulk of the gum (73.26 wt% of the total) was shown to be comprised by an AG fraction with a weight average molecular weight (M_w) of $4.082 \times 10^5 \text{ g mol}^{-1}$ and a low protein content (0.35 wt%). The second major fraction (13.88 wt% of the total) was identified as an AGP with a molecular weight of $5.521 \times 10^6 \text{ g mol}^{-1}$ and contained a greater proportion of protein (9.8 wt%).

The effect of apricot gum-lactoglobuline concentrate (AG/LgC) in varied weight ratios from 2 to 15, different pH values, thermal, and ultrasound treatments on the stability of double layer emulsions have been presented in the following sections.

3.2. The effect of AG/LgC ratio

Regarding the emulsion stability, **the best ratio was 12.5:1 AG:LgC (12.5)**, which has resulted in a completely stable emulsion during 10 days storage in room temperature (Fig. 2a and Fig. 3). Applying AG/LgC ratio of 7.5 also resulted in an emulsion with a good stability as its volume decreased only 1.6 mL (**due to creaming**) after 7 days and did not significantly changed ($P > 0.05$) until day 10. Other samples did not show a suitable stability as a sharp decrease was observed in their emulsion volume and **creaming happened** between day 2 and 5.

The **particle size** of emulsions prepared using AG/LgC emulsifiers with different ratios **has been shown** in Fig. 2b. The biggest and smallest particle size belonged to samples with 12.5 and 2.5 ratios, respectively. The higher the AG/LgC ratio, the bigger the particle size. Emulsions particle size did not change significantly ($P > 0.05$) during 10 days storage except for samples with AG/LgC ratio of 2.5. Their particle size increased significantly ($P < 0.05$) after day 5. Probably at high AG/LgC ratios, adsorption of more AG on the secondary wall may occur through both polysaccharides-protein and protein (Lg)- AGPs protein patches, which forms bigger particles.

Fig. 2c shows the zeta potential change of emulsions prepared using AG/LgC emulsifiers with

different ratios during 10 days storage. Samples with the ratio of 12.5, which had shown the best emulsion stability, showed the lowest zeta potential (-19 mV). The zeta potential of samples with the ratio of 7.5, 10 and 12.5 did not change significantly ($P>0.05$) after day 5, whereas, samples with the ratio of 2.5 and 5 showed a significant ($P<0.05$) increase after day 2. Samples with the ratio of 12.5, 10.0 and 7.5 showed the better zeta potential stability during storage. Increase of the emulsion zeta potential should result in the formation of stable emulsions. However in this system, highest zeta potential observed in the low stable emulsions with a AG/LgC ratio of 2.5 and 5.0 at the beginning. The reason for emulsion stability at higher AG/LgC ratio may be increase of viscosity of discontinuous phase due to unabsorbed AG molecules. Because of the formation of a thick surface on bilayer formed through polysaccharide-protein and protein-protein interactions by applying high concentration of AG in competition with smallest particles would slow down the interfacial potential, which might decrease zeta potential. The results of the present study reveal that at the low AG/LgC ratio, even highest surface negative potential formed by carboxyl group of AG can't induce repulsive forces to stabilize emulsions; the decreased double layer thickness seems to be the basic factor initiating the coagulation. Once such coagulation is triggered, further changes in zeta potential with ageing can be attributed to the fall of surface coverage of Lg adsorbed on oil droplets.

Fig. 2

Fig. 3

In recent years it has been observed that higher zeta potentials do not necessarily lead to emulsion stability. The rate of decrease in zeta potential, as a matter of fact, controls emulsion stability. Increase in the rate of decay of zeta potential will reduce emulsion stability. This indicates that emulsion stability is governed by the rate in which desorption of emulsifier from interfacial film takes place during ageing. If the interfacial film is strong, then lesser would be the zeta potential decay rate and high would be the stability of emulsion. This reveals that emulsion

stability is primarily governed by the strength of interfacial film [34, 35, 36].

3.3. The effect of sonication amplitudes

In order to assess the effect of ultrasound treatment with different amplitudes on emulsion stability, a 10 minutes sonication with amplitude of 25, 50, 75 and 100 percent were applied. Regarding the fact that emulsion samples prepared with AG/LgC ratio of 5 did not show a suitable stability (see section 3.2), that ratio was chosen to find out if ultrasound treatment could improve its stability. As it has been shown in Fig 4a, the volume of blank emulsion (without any sonication) showed a sharp decrease after day 4, whereas, other samples with ultrasound treatment were much more stable. There was no significant difference ($P>0.05$) between the stability of samples with different ultrasound amplitudes. It could be concluded that sonication for 10 minutes could significantly improve the emulsion stability and ultrasound amplitude had no effect on its stability. The data presented in Fig. 4a shows that the more the ultrasound amplitude, the less the emulsion's particle size. Sonication caused a decrease in particle size which was stable during 10 days and it could be attributed to the disaggregation of aggregates formed between different fractions of AG polysaccharides and its protein parts as the reason for positive effect of sonication on emulsion stability. The particle size also decreased at higher ultrasound amplitude but regarding Fig. 4a, this decrease was not high enough to impact the emulsion's stability. The ultrasound treatment also decreased the zeta potential of emulsions (Fig. 4c). The zeta potential of blank sample was less stable than other samples during 10 days of storage [24, 31, 32, 33].

Fig. 4

3.4. The effect of sonication time

The effect of sonication time on the emulsion stability was evaluated by applying 10, 15, 20 and 25 minutes ultrasound treatments. Similar to Fig. 4a, the time of ultrasound treatment had no significant ($P>0.05$) effect on emulsion stability during 14 days storage (Fig. 5a). It could be concluded that sonication can significantly improve the emulsion stability as was stated above by

disrupting the aggregates particles and this influence is not affected by time of treatment.

As it has been shown in Fig. 5b, sonication time had a significant ($P<0.05$) effect on decreasing the emulsion's particle size. The higher treatment times of the ultrasound, the smaller the particle size. There were no significant differences between the particle size of emulsions treated for 20 and 25 minutes by ultrasound. These results concurred with results from section 3.3.

The zeta potential of emulsions decreased at higher sonication times (Fig. 5c). The ultrasound has also made the zeta potential to be more stable during the storage and this effect was not influenced by ultrasound treatment time. These results also concurred with results from section 3.3.

3.5. The effect of thermal treatments

A 30 minutes incubation time at 50, 60, 70, 80 and 90°C, right after preparation of emulsions was applied in order to assess the effect of thermal treatments on emulsion's parameters. As it has shown in Fig. 6a, the lowest emulsion particle size (132 nm) was achieved by applying a thermal treatment at 50°C. The zeta potential results (Fig. 6b) also revealed that the lowest zeta potential could be obtained by applying a thermal treatment at 50°C. The shape of both diagrams in Fig. 6 was to some extent similar where the highest particle size and the lowest zeta potential belonged to thermal treatment at 70°C and after that a decreasing trend was observed at higher temperatures.

This could be due to denaturation and aggregation of lactoglobuline at 70°C.

3.6. The effect of pH

In order to assess the effect of pH on emulsion's parameters, emulsions prepared using AG/LgC emulsifiers with the ratio (w/w) of 5, were kept in solutions with the pH of 2, 3, 4, 5, 6 and 7 for 24 hours at 25°C. As it has shown in Fig. 6a, the lowest particle size was obtained using pH of 4, whereas pH of 7 showed the highest particle size. The results of zeta potential experiment (Fig. 7b) clearly showed that AG was able to stabilize emulsions by lowering interfacial potential between -29 mV and -31mV in a wide range of pH around isoelectric point of LgC proteins (5.2).

The lowest zeta potential obtained from samples kept in pH of 3. Regarding both droplet size and zeta potential results, the pH of 4, where both biopolymers are oppositely charged could be the optimum pH in order to achieve an emulsion with the best stability. In fact at this pH, which is lower than isoelectric point of LgC, it has a positive charge and AG with a negative charge and the most governing stabilizing phenomenon could be due to electrostatic mechanism.

Fig. 5

Fig. 6

Fig. 7

4. Conclusion

Applying AG:LgC with the ratio of 1:8, in order to obtain double layer oil in water emulsion, could result in a completely stable emulsion during 10 days storage in room temperature. The ultrasound treatment significantly increased the emulsion stability. Both sonication time and amplitude had a significant effect on decreasing the particle size and zeta potential but their positive effect on emulsion stability was all the same. Hence, regarding the consumption of energy and time, a 10 minutes ultrasound treatment with the amplitude of 25% could be the optimum conditions for ultra-sonication. The best temperature for thermal treatment and the best pH was 50°C and 3, respectively. Obviously, it is necessary to perform further works to fully understand formation of stable emulsions using AG/LgC in different ratios. More knowledge about the fine structures of AG polymers will help us to understand the chemical interaction of AG polymers with LgC and interaction of AGPs with whey proteins, which could give further insight into the emulsion stabilization.

Acknowledgements

The ISTC T-1419 project funded by the USDA, Agriculture Research Service, is gratefully acknowledged for financial support. Postdoctoral fellowship is being funded by the European Union

References

- [1] A. Faridi, S. M. Jafari, E. Assadpour, & A. Mohammadi, Nano-encapsulation of saffron extract through double-layered multiple emulsions of pectin and whey protein concentrate, *Journal of Food Engineering*, 165 (2015), 149–155.
- [2] A. Mohammadi, S. M. Jafari, A. F. Esfanjani, S. Akhavan, Application of nanoencapsulated olive leaf extract in controlling the oxidative stability of soybean oil, *Food Chemistry*. 190 (2016), 513-519.
- [3] E. Dickinson, Interfacial structure and stability of food emulsions as affected by protein-polysaccharide interactions, *Soft Matter* 4 (2008) 932.
- [4] D. J. McClements, *Food Emulsions: Principles, Practices, and Techniques*, second ed., CRC Press, Boca Raton, Florida, 2004.
- [5] V. Tolstoguzov, Some thermodynamic considerations in food formulation, *Food Hydrocolloids* 17 (2003) 1–23.
- [6] S. M. Jafari, Y. He, & B. Bhandari, Effectiveness of encapsulating biopolymers to produce sub-micronemulsions by high energy emulsification techniques, *Food Research International*, 40 (2007), 862–873.
- [7] D. J. McClements, Non-covalent interactions between proteins and polysaccharides, *Biotechnology advances* 24 (2006) 621–5.
- [8] M. Raei, G. Rajabzadeh, S. Zibaei, S. M. Jafari, A. M. Sani, Nano-encapsulation of isolated lactoferrin from camel milk by calcium alginate and evaluation of its release, *International Journal of Biological Macromolecules*. 79 (2015), 669-673.
- [9] E. E. Braudo, I. G. Plashchina, K. D. Schwenke, Plant protein interactions with polysaccharides and their influence on legume protein functionality. A review, *Die Nahrung* 45

(2001) 382–4.

[10] A. Benichou, A. Aserin, N. Garti, Protein-Polysaccharide Interactions for Stabilization of Food Emulsions, *Journal of Dispersion Science and Technology* 23 (2002) 93–123.

[11] N. Larichev, A. Gurov, V. Tolstoguzov, Protein-polysaccharide complexes at the interphase. 1. Characteristics of decane/water emulsions stabilized by complexes of bovine serum albumin with dextran sulphate, *Colloids and Surfaces* 6 (1983) 27–34.

[12] R. Lutz, A. Aserin, Y. Portnoy, M. Gottlieb, N. Garti, On the confocal images and the rheology of whey protein isolated and modified pectins associated complex, *Colloids and Surfaces B: Biointerfaces* 69 (2009) 43–50.

[13] Z. Muhidinov, A. Jonmurodov, K. Teshaev, Lactoglobulins concentrate from whey protein and method for its isolation, *J. Zdravokhraneniya Tajikistana* 5 (2009) 44–49.

[14] L. S. Jourdain, C. Schmitt, M. E. Leser, B. S. Murray, E. Dickinson, Mixed layers of sodium caseinate + dextran sulfate: influence of order of addition to oil-water interface., *Langmuir: the ACS journal of surfaces and colloids* 25 (2009) 10026–37.

[15] J. M. Rodr'iguez Patino, A. M. Pilosof, Proteinpolysaccharide interactions at fluid interfaces, *Food Hydrocolloids* 25 (2011) 1925–1937.

[16] D. Guzey, D. J. McClements, Formation, stability and properties of multilayer emulsions for application in the food industry., *Advances in colloid and interface science* 128-130 (2006) 227–48.

[17] A. Benichou, A. Aserin, N. Garti, W/O/W double emulsions stabilized with WPI-polysaccharide complexes, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 294 (2007) 20–32.

[18] S. L. Turgeon, C. Schmitt, C. Sanchez, Protein-polysaccharide complexes and coacervates, 2007. doi:10.1016/j.cocis.2007.07.007.

[19] F. Littoz, D. J. McClements, Bio-mimetic approach to improving emulsion stability: Cross-

linking adsorbed beet pectin layers using laccase, *Food Hydrocolloids* 22 (2008) 1203–1211.

[20] N. Garti, HYDROCOLLOIDS AS EMULSIFYING AGENTS FOR OIL-IN-WATER EMULSIONS, *Journal of Dispersion Science and Technology* 20 (1999) 327–355.

[21] S. M. Jafari, P. Beheshti, and E. Assadpoor, Rheological behavior and stability of d-limonene emulsions made by a novel hydrocolloid (Angum gum) compared with Arabic gum, *Journal of Food Engineering* 109(1) (2012) 1-8.

[22] S. M. Jafari, P. Beheshti, and E. Assadpoor, Emulsification properties of a novel hydrocolloid (Angum gum) for d-limonene droplets compared with Arabic gum, *International Journal of Biological Macromolecules* 61(10) (2013) 182-188.

[23] F. Azarikia, S. Abbasi, On the stabilization mechanism of Doogh (Iranian yoghurt drink) by gum tragacanth, *Food Hydrocolloids* 24 (2010) 358–363.

[24] S. M. Jafari, E. Assadpoor, Y. He, and B. Bhandari, Re-coalescence of emulsion droplets during high-energy emulsification, *Food Hydrocolloids* 22 (2008) 119-1202.

[25] D. W. Everett, R. E. McLeod, Interactions of polysaccharide stabilisers with casein aggregates in stirred skim-milk yoghurt, *International Dairy Journal* 15 (2005) 1175–1183.

[26] Z. K. Mukhidinov, G. F. Kasymova, S. R. Usmanova, K. B. Murzagulova, M. E. Kim, A. V. Yanovich, Production of rifampicin-containing microcapsules based on apple pectin complexes with β -lactoglobulin, *Pharmaceutical Chemistry Journal* 46 (2012) 306–309.

[27] D. Boothby, Gummosis of stone-fruit trees and their fruits, *Journal of the Science of Food and Agriculture* 34 (1983) 1–7.

[28] J. Ros'ík, J. Kubala, M. Stanova', P. Lac'ok, Structural properties of polysaccharides from apricot-tree gum in dependence on infection of the tree by pathogens or application of 2-methyl-4-chlorophenoxyacetic acid, *Collection of Czechoslovak Chemical Communications* 33 (1968) 1943–1945.

[29] M. Saniewski, J. Ueda, M. Horbowicz, K. Miyamoto, J. Puchalski, Gum in apricot (*Prunus*

armeniaca L.) shoots induced by methyl jasmonate, *Acta Agrobotanica* 54 (2001) 27–34.

[30] Z. Muhidinov, K. Teshaev, A. Jonmurodov, D. Khalikov, M. Fishman, Physico-Chemical Characterization of Pectic Polysaccharides from Various Sources Obtained by Steam Assisted Flash Extraction (SAFE), *Macromolecular Symposia* 317-318 (2012) 142–148.

[31] S. M. Jafari, Y. He, and B. Bhandari, Production of sub-micron emulsions by ultrasound and microfluidization techniques, *Journal of Food Engineering* 82(4) (2007) 478-488.

[32] S.M. Jafari, Y. He, and B. Bhandari, Optimization of nano-emulsions production by microfluidization, *European Food Research and Technology*, 225 (2007), 733-741.

[33] S. M. Jafari, Y. He, and B. Bhandari, Nano-emulsion production by sonication and microfluidization- A comparison, *International Journal of Food Properties* 9(3) (2006) 475-485.

[34] M. Alkan, M. Karadağ, M. Doan, O. Demirbağ, Zeta potentials of perlite samples in various electrolyte and surfactant media, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 259 (2005) 155–166.

[35] B. J. Kirby, E. F. Hasselbrink, Zeta potential of microfluidic substrates: 2. Data for polymers, *Electrophoresis* 25 (2004) 203–13.

[36] J. Stachurski, M. Michałek, The Effect of the zeta Potential on the Stability of a Non-Polar Oil-in-Water Emulsion, *Journal of colloid and interface science* 184 (1996) 433–6.

Figure Captions:

Fig. 1. HPSEC elution profiles of water-alcohol fractions of Apricot gum

Fig. 2. The volume (a), droplet size (b), and zeta potential (c) of emulsions prepared using AG / LgC emulsifiers with the ratio (w/w) of 2.5 (●), 5.0 (■), 7.5 (▲), 10.0 (+) and 12.5 (×) during 10 days storage.

Fig. 3. Creaming stability of emulsions prepared by AG/LgC with different ratios (w/w) after 7 days of storage.

Fig. 4. The effect of 10 minutes sonication with amplitudes of 25 (×), 50 (+), 75 (●) and 100 (◆) percent, along with the blank sample (■), on volume (a), droplet size (b), and zeta potential (c) of emulsions prepared using AG / LgC emulsifiers with the ratio (w/w) of 5.

Fig. 5. The effect of 10 (×), 15 (●), 20 (+) and 25 (◆) minutes sonication (with amplitude of 75%) and the blank sample (■), on volume (a), particle size (b), and zeta potential (c) of emulsions prepared using AG / LgC emulsifiers with the ratio (w/w) of 5.

Fig. 6. The effect of a 30 minutes thermal treatment at different temperatures on droplet size (a) and zeta potential (b) of emulsions prepared using AG / LgC emulsifiers with the ratio (w/w) of 5.

Fig. 7. Droplet size (a) and zeta potential (b) of emulsions prepared using AG / LgC emulsifiers with the ratio (w/w) of 5 and kept in solutions with the pH of 2, 3, 4, 5, 6 and 7 for 24 hours at 25 °C

Fig. 1. HPSEC elution profiles of water-alcohol fractions of Apricot gum

Fig. 2. The volume (a), droplet size (b), and zeta potential (c) of emulsions prepared using AG / LgC emulsifiers with the ratio (w/w) of 2.5 (●), 5.0 (■), 7.5 (▲), 10.0 (+) and 12.5 (×) during 10 days storage.

Fig. 3. Creaming stability of emulsions prepared by AG/LgC with different ratios (w/w) after 7 days of storage.

Fig. 4. The effect of 10 minutes sonication with amplitudes of 25 (×), 50 (+), 75 (●) and 100 (◆) percent, along with the blank sample (■), on volume (a), droplet size (b), and zeta potential (c) of emulsions prepared using AG / LgC emulsifiers with the ratio (w/w) of 5.

Fig. 5. The effect of 10 (×), 15 (●), 20 (+) and 25 (◆) minutes sonication (with amplitude of 75%) and the blank sample (■), on volume (a), particle size (b), and zeta potential (c) of emulsions prepared using AG / LgC emulsifiers with the ratio (w/w) of 5.

Fig. 6. The effect of a 30 minutes thermal treatment at different temperatures on droplet size (a) and zeta potential (b) of emulsions prepared using AG / LgC emulsifiers with the ratio (w/w) of 5.

Fig. 7. Droplet size (a) and zeta potential (b) of emulsions prepared using AG / LgC emulsifiers with the ratio (w/w) of 5 and kept in solutions with the pH of 2, 3, 4, 5, 6 and 7 for 24 hours at 25 °C