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Rheological Characteristics of Yogurt Enriched with Microencapsulated Fish Oil

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ABSTRACT

This study was aimed at evaluating the flow behavior characteristics of yogurt enriched with fish oil (FO) microcapsules prepared by complex coacervation method. FO was microencapsulated in gelatin-acacia gum coacervates. Then, the microcapsules were dried, and yogurt was produced from the milk enriched with microcapsules powder. Rheological characteristics (as measured using a rotational viscometer) of yogurt were evaluated in the shear rate range of 0.262-7.86 s^{-1} at 6˚C during 21 days of storage. Power Law model was used for calculation of consistency coefficient and flow behavior index of yogurt. As compared to the control, the enriched yogurt had higher apparent viscosity. Consistency coefficients of the enriched and the control yogurts were in the range of 24.42-28.82 and 15.31-17.76 Pa s^n, respectively. Yogurt samples showed a non-Newtonian shear-thinning flow behavior. Addition of FO microcapsules to yogurt may be useful for improving its health-promoting effect and consistency.

Keywords: Complex coacervation, Encapsulation, Functional foods, Marine omega-3 oil, Yogurt flow behavior.

INTRODUCTION

The beneficial effects of long chain omega-3 polyunsaturated fatty acids (LCn-3PUFAs), i.e. eicosapentaenoic and docosahexaenoic acids (EPA and DHA), on human health have been well documented (Asadi *et al*., 2012). Since they have high unsaturation degree, these fatty acids are very susceptible to oxidization. Therefore, food enrichment with those sources has an undesirable influence on shelf-life, consumer acceptability, functionality, and safety of enriched foods (Arab-Tehrany *et al*., 2012). It has been demonstrated that microencapsulation protects LCn-3PUFAs against oxidation (Barrow and Shahidi, 2008). Currently, spray drying of emulsions is the most common technology for this purpose. There are several studies that discuss the enrichment of dairy products (e.g. milk, yogurt) with an emulsion of marine omega-3 oil (Chee *et al*., 2005; Let *et al*., 2005; Nielsen *et al.*, 2007). Since complex coacervation technique produces microcapsules with low surface oil, high oil content, high stability (Barrow and Shahidi, 2008; Madene *et al.*, 2006), water insolubility, heat resistant, and controlled release possibility (Mendanha *et al*., 2009), recently, a number of food products have been launched on the markets that contain microencapsulated fish oil (FO) produced by complex coacervation technique (Jin *et al.*, 2007). Briefly, microencapsulation by complex coacervation is accomplished by phase separation of at least two biopolymers from the initial solution and, subsequently, deposition of the newly formed coacervate phase around the active ingredient suspended or emulsified in the same reaction media (Mendanha *et al*., 2009). A prerequisite for this phenomenon is mixing of biopolymers at a pH and temperature or an inorganic salt electrolyte concentration wherein they have

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opposing charge. Gelatin, as a poly-cation, and acacia gum, as a poly-anion, are the most common encapsulants for this purpose in food industry (Barrow and Shahidi, 2008; Lakkis, 2007; Madene *et al*., 2006), presumably because of availability, low cost, and compatibility with the encapsulation of a wide range of (bio-)active materials.

Recently, we investigated the effect of formula composition on characteristics of FO microcapsules prepared in gelatin-acacia gum coacervates (Tamjidi *et al*., 2013), and examined the physicochemical characteristics, including pH, titratable acidity, whey separation, water-holding capacity, gel firmness, apparent viscosity in a shear rate, color, peroxide value and release of FO from the incorporated microcapsules, and sensory properties of enriched yogurt with FO-microcapsules. Until the end of storage period (on 22nd day), the increase in peroxide value of FO for yogurt enriched with free-FO was 3.6 times higher than the microcapsules-enriched ones (Tamjidi *et al*., 2012). This result showed that complex coacervation provides better protection of LCn-3PUFAs against oxidation, compared to emulsion technique. Whereas examination of rheological properties is crucial for process design and control of product quality, the present work was aimed to study the flow behavior of yogurt enriched with FO microcapsules.

MATERIALS AND METHODS

Materials

Gelatin powder (type A; 260-265 bloom), acacia gum powder, and FO (containing ~18% EPA, ~12% DHA and 200 ppm α-tocopherol) were kindly supplied by Tasty Food Industry (Tehran, Iran), Colloides Naturels International (Rouen, France) and Nooshdarudarya Co. (Mazandaran, Iran), respectively. The peroxide value of FO was 1±0.1 meq O kg^{-1} according to AOAC standard method (AOAC, 1995). Cow milk was obtained from the research farm of Isfahan University of Technology (Isfahan, Iran). Skimmed milk powder (SMP) was purchased from Pegah Dairy Co. (Mashhad, Iran). Three packages (Codes: YO-A, YO-B, YO-S) of freeze-dried Direct Vat Set (DVS) yogurt starter cultures *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus* were purchased from Proquiga Biotech, SA (La Coruña, Spain). Acetic acid, n-hexane, and sodium dodecyl sulfate (SDS) were purchased from Merck Co. (Darmstadt, Germany). Double-distilled and deionized water was used.

Preparation of Microcapsules

According to the previously described protocol, a combination of gelatin (3.12% w/w), acacia gum (1.25% w/w), FO (6.25% w/w) and water (89.37% w/w) was used to prepare FO microcapsules with high oil content and high encapsulation efficiency (Tamjidi *et al*., 2013). Initially, aqueous solutions of gelatin (10.0% w/w) and acacia gum (2.0% w/w) were prepared separately at 50˚C. Then, 18.75 g FO (20˚C) was emulsified in 93.7 g of the gelatin solution using a laboratory mixer at 3,000 rpm for 5 min (Heidolph, Germany), and 187.5 g of the acacia gum solution was added to the emulsion. Subsequently, temperature of the emulsion was adjusted at 50°C, and its pH was reduced to 4 using an aqueous solution of acetic acid (8.7M). Then, the emulsion was stirred at 600 rpm for 15 min and, finally, cooled slowly down (0.5°C min^{-1}) to 4-7°C under continuous stirring.

Morphology, Particle Size, and Oil Content of Microcapsules

A portion of 0.15 g of the microcapsules suspension was diluted in 150 mL SDS solution (0.1% w/v), and manually stirred using a spatula within a water bath (35°C) for 20 min to disrupt flocculated particles (SDS facilitates disruption of flocculated particles).

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Then, the morphology and particle size of the microcapsules was observed by an optical microscope (Nikon Eclipse E600, Tokyo, Japan).

The microcapsules mixture was lyophilized (Heto Holten freeze drier, Allerød, Denmark) at –30°C and 0.8 mbar. The dried mixture obtained from whole of the formulation was weighed, ground using a laboratory mill, and sieved (Mesh: 100) to produce a fine powder. The oil content of microcapsules (OCM) was determined according to Westergaard method (Westergaard, 2004) with minor modifications. Five to 10 g of the powder was slowly mixed with n-hexane at ratio of 1:10 (w/w) for 15 minutes, subsequently, the dispersion was filtered (Whatman filter paper; Grade 42). This action was done twice at room temperature, the solvent of filtrate evaporated and the residual oil weighed as non-encapsulated oil. The OCM was determined using Equation (1).

$$OCM\ (\%) = [a/(a+b)] \times 100 \quad [1]$$

Where, a is weight of the non-extractable oil from the sampled powder of FO microcapsules and b is sum of gelatin and acacia gum in the sampled powder.

Starter Culture Preparation

One gram of the DVS culture (0.33 g of each package) was rehydrated at 1 L of sterile skimmed cow milk.

Yogurt Production

After extraction of surface oil, the OCM was ~50%. Therefore, by enriching a serving of yogurt (~150 g) with 0.8 g of the microcapsules powder and consuming one serving per day, amount of 0.12 g EPA+DHA will be provided, which is approximately half of the recommended value (0.2 g EPA+DHA day^{-1}) as stated by the European Academy of Nutritional Sciences (EANS).

The enriched yogurt was prepared by the following traditional process. Cow milk was standardized by adding SMP and skimmed milk according to Pearson's Square method (Tamime and Robinson, 1999). The standardized milk (Total solid: 13%; Fat content: 2.5%) was warmed to 60°C, homogenized at 20 MPa (Behsaz-Machine homogenizer, Tehran, Iran), and heated at 90°C for 10 minutes (Batch heating condition). Later, 0.8 g of FO microcapsules was added to 150 g heated milk. Then, the enriched milk was cooled down to 45°C in an ice bath, and inoculated by 2% (w/w) of the rehydrated starter culture. The cultured milk, in sterile polystyrene containers, was incubated at 43°C until its pH decreased to 4.7 and, subsequently, transferred to 4°C. The microcapsule-free yogurt (control) was produced in the same manner as above.

Rheological Properties of Yogurt Samples

Rheological analysis was carried out after 24 hours of yogurt production and each week during 3 weeks of storage. Before measurements, yogurt samples were gently stirred with a spatula for 40 seconds to ensure homogeneity. This is the most common method to perform rheological tests on yogurt, probably because it is difficult to find and standardize a mechanical and reproducible method to stir yogurts without breaking substantial amount of their structure (Vercet *et al*., 2002).

The rheological properties of samples were measured at 6°C by a rotational viscometer (Model RVDV-II, Brookfield Engineering, Inc., USA) equipped with spindle model RV4. Samples were sheared at an increasing order of spindle speed (N) at 1, 3, 5, 10, 15, 20, and 30 rpm, and the apparent viscosity (η_{app}) was recorded in centipoises (cP) after 50 seconds of shearing. The rpm values mentioned above correspond to apparent shear rates of 0.262, 0.786, 1.31, 2.62, 3.93, 5.24, and 7.86, respectively, based on the empirical

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Equation (2) provided in the manufacturer's instruction:

$$\dot{\gamma} = N \times 0.262 \quad [2]$$

Where, N and $\dot{\gamma}$ are the rotational speed of spindle and the corresponding shear rate, respectively. Then, shear stress (τ) was calculated by the Equation (3):

$$\tau = \eta_{app} \times \dot{\gamma} \quad [3]$$

Flow behavior of yogurt samples was described by fitting the experimental data to the Power Law model [Equation (4)], and the goodness of fitting was quantified by calculating the *R*-squared (r^2).

$$\tau = K\dot{\gamma}^n \quad [4]$$

Where, K is the consistency coefficient (Pa s^n) and *n* is the flow behavior index (dimensionless).

The parameter of viscosity ratio (λ) was calculated as Equation (5).

$$\lambda = \eta_{appE}/\eta_{appC} \quad [5]$$

Where, η_{appE} and η_{appC} are apparent viscosities of the enriched and the control yogurts, respectively.

Statistical Analyses

Statistical analyses were performed using SAS version 9.0 (SAS Institute Inc., Cary, NC). An ANOVA was performed using the general linear models procedure to determine significant differences among the samples. Means were compared by using Fisher's least significant difference (LSD) procedure. Differences of $P \leq 0.05$ were considered to be significant. All measurements were performed in triplicate.

RESULTS AND DISCUSSION

Microcapsules: Formation and Morphology

Gelatin as the emulsifier stabilizes the initial FO emulsion. Since the isoelectric point (pI) of gelatin type A is 7-9 (Lakkis, 2007), its net charge is positive at pH= 4. Conversely, the charge of acacia gum at this pH is negative. In this condition, a liquid–liquid phase separation, called coacervation, occurs. The coacervate phase is rich in macromolecules and is in equilibrium with the relatively dilute macromolecular liquid phase (Bungenberg-de-Jong, 1949). The coacervate phase participates in a complex coacervation encapsulation system, and the microcapsule shell forms in this phase (Lakkis, 2007). By cooling the system below the gel point of coacervate, the microcapsule shell solidifies (Barrow and Shahidi, 2008) and the final form of

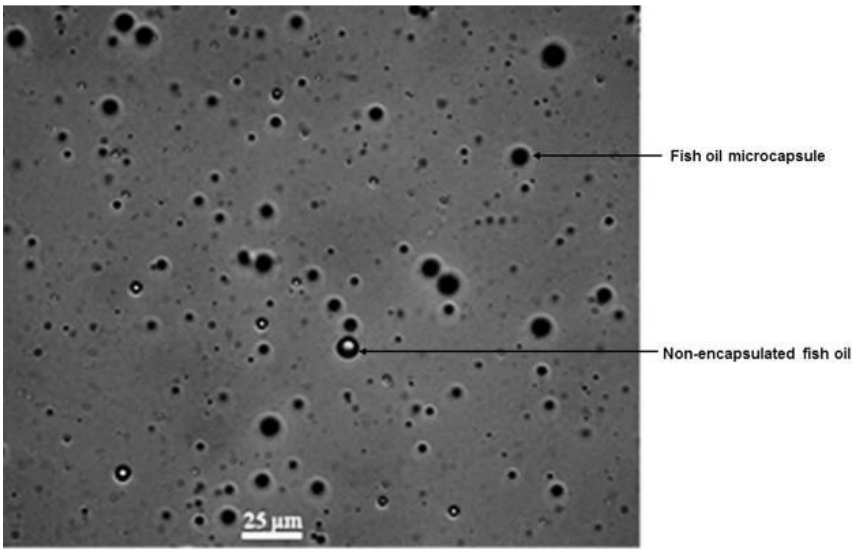

Figure 1. Optical photograph (400X) of microcapsules. The scale bar represents 25 µm.

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microcapsule completes. The optical photograph given in Figure 1 indicates the deposition of the coacervate onto the core droplets to form the microcapsule shell. The microcapsules have round form and their diameter is in the range of ~0.5–10 microns. Previously, similar forms were observed when employing complex coacervation technique for microencapsulation process (Mendanha *et al*., 2009). Moreover, Figure 1 clearly shows the presence of non-encapsulated oil droplets.

Flow Properties of Yogurts

Figure 2 shows η_{app}–$\dot{\gamma}$ plot of yogurt samples for all storage periods measurements. As shown in this figure, the η_{app} of the yogurt samples decreases with increase in shear rate. Such behavior is typical for a non-Newtonian shear-thinning flow behavior and implies that the fluid does not have a true viscosity. Apparent viscosity of yogurt is influenced by aggregate size (Parnell-Clunies *et al*., 1988), and disruption of protein aggregates increases with shear rate. This flow behavior can, therefore, be attributed to combined effects of breakdown of weak linkages between the milk proteins and/or between the milk proteins and the added ingredients (i.e. FO powder), and of reformation of such linkages as a result of Brownian motion and molecular collisions (Tang *et al*., 1993). A decrease in the η_{app} with the increase in shear rate was also noted in previous studies for yogurt fortified with wheat-bran (Aportela-Palacios *et al*., 2005) or inulin (Paseephol *et al*., 2008). The maximum and minimum values of η_{app} were found to be 89.4±5.0 (on 14th day) and 4.9±0.3 Pa s (on 21st day) for the enriched yogurt, and 49.2±3.8 (on 21st day) and 3.0±0.2 Pa s (on 7th and 14th days) for the control yogurt, respectively.

By increasing shear rate from 0.262 to 0.786 s^{-1}, the enriched yogurt showed a greater decrease in η_{app} as compared to the control for all measurements during storage (Figure 2), indicating faster disruption of protein aggregates in the enriched yogurt.

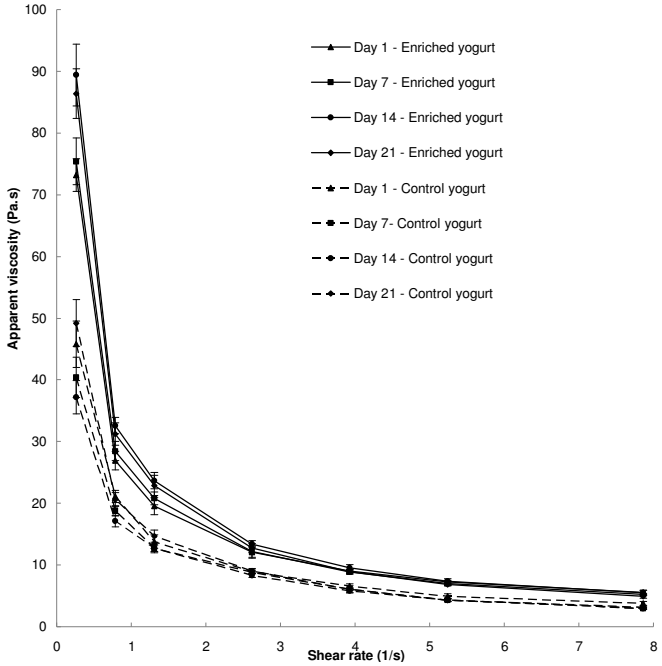

Figure 2. Viscosity-shear rate profile of microcapsule-enriched and control yogurts during storage time. Enriched yogurt contained 0.8 g of the powder of FO microcapsules. Some standard deviation (SD) bars lie within the data points.

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Presumably, the ingredients of FO powder in the enriched yogurt decreased the strength of particulate network of casein micelles linked together with denatured whey proteins in clusters. In other words, in the enriched yogurt, the total number and magnitude of the different kinds of bonds between the milk proteins and/or between the milk proteins and ingredients of the FO powder, decreased. However, the yogurt containing microcapsules had greater η_{app} than the control at each shear rate for all storage periods measurements ($P< 0.05$).

Figure 3 illustrates the effect of storage time on the viscosity ratio λ [Equation (5)] at different shear rates. As shown in this figure, λ increases with time until 14th day and reveals a decrease on 21st day. The increase of λ during storage is because of increasing η_{app} of the enriched yogurt and decreasing that of the control. Conversely, the decrease of λ on 21st day was due to decrease in η_{app} of the enriched yogurt and increase in that of the control. The maximum and minimum values of λ were found to be 2.40 (on 14th day) and 1.27 (on 1st day), respectively. Normally, by increasing $\dot{\gamma}$, λ values decreased and afterwards showed an increase.

Power Law model [Equation (3)] was used to describe the rheological behavior of yogurts and to calculate consistency coefficient (K) and flow behavior index (n). This model has widely been used by others to describe the flow behavior of yogurt in response to external shear stress (Aportela-Palacios *et al*., 2005; Pinto *et al*., 2012; Rezaei *et al*., 2011; Singh and Muthukumarappan, 2008; Staffolo *et al*., 2004). It should be noted that the Power Law model does not have a yield stress term while all stirred yogurts have yield stress, unless they have been sheared first and no recovery time allowed reconstructing some structures (Lee and Lucey, 2010).

This model satisfactorily fitted the experimental data for each type of yogurt and obtained a minimum coefficient of determination (r^2) of 0.84. Table 1 shows the

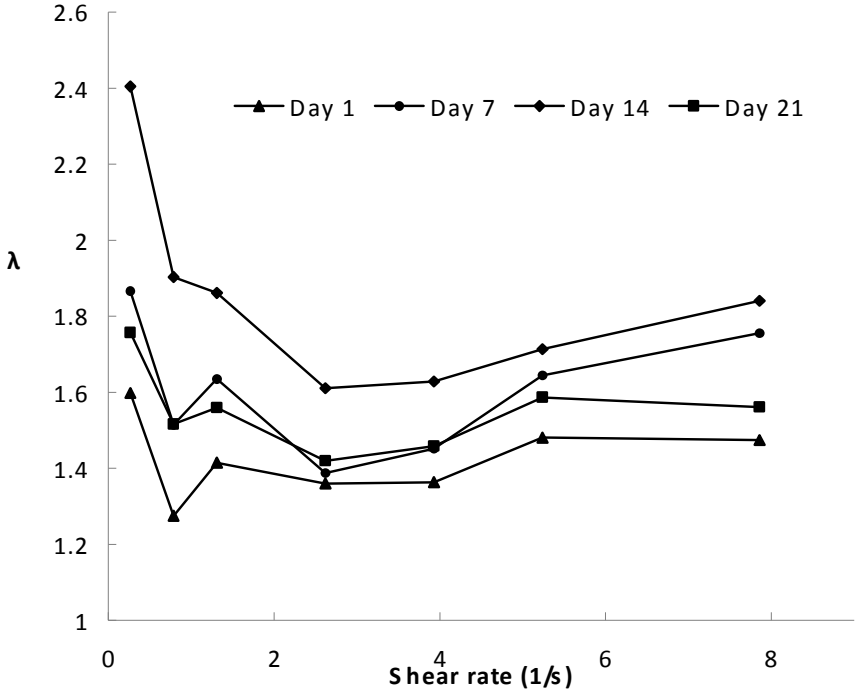

Figure 3. Variation of viscosity ratio (λ) during storage.

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Table 1. Power Law parameters for yogurt samples.*

Yogurt sample	Day	*pH±SD*	*K±SD* (Pa s^n)	*n±SD*	r^2
Enriched	1	4.62±0.01[a]	24.42±0.55[d]	0.27±0.03[a]	0.97
	7	4.42±0.02[b]	25.36± 0.47[c]	0.23±0.01[bc]	0.97
	14	4.26±0.01[c]	28.82±0.57[a]	0.19±0.03[cd]	0.95
	21	4.15±0.01[d]	27.64±0.86[b]	0.17±0.02[d]	0.92
Control	1	4.62±0.01[a]	17.47±0.65[e]	0.26±0.02[ab]	0.95
	7	4.40±0.02[b]	16.13±0.30[f]	0.22±0.02[bc]	0.86
	14	4.26±0.01[c]	15.31±0.33[f]	0.25±0.01[ab]	0.90
	21	4.15±0.00[d]	17.76±0.49[e]	0.18±0.01[d]	0.84

* Different superscripts in the same column are significantly different (P≤ *0.05*) with LSD test; r^2 shows the goodness degree of Power Law model and, SD: Standard Deviation.

values of K, n and r^2 for enriched and control yogurts during the 21 days of storage. The maximum and minimum values of K were found to be 28.82 (on 14th day) and 24.42 Pa s^n (on 1st day) for enriched yogurt, and 17.47-17.76 (on 1st and 21st day) and 15.31-16.13 Pa s^n (on 7th and 14th days) for the control yogurt, respectively. At all analysis times, the K of yogurt enriched with FO microcapsules was higher than that of the control, significantly. The K values are in agreement with literature data (Keogh and O'Kennedy, 1998; Staffolo *et al*., 2004).

There are a number of possible reasons for the increase of η_{app} and K i.e. resistance to flow, with the addition of FO powder: (a) the FO powder added to yogurt consists of microcapsules, and coacervated and non-coacervated biopolymers i.e. gelatin and acacia gum. The net charge of gelatin and acacia gum in the pH of yogurt is not zero, thus, they tend mainly to contribute to electrostatic interactions. Therefore, major interactions can occur between the FO microcapsules/biopolymers and the milk protein in the enriched yogurt. Presumably, these interactions increase the size of protein aggregates and thereby η_{app} and K; (b) the bigger particle size of microcapsules compared to aggregates of milk proteins can also increase η_{app} and K of the enriched yogurt; and (c) the higher total solids content of the enriched sample increases the water-binding capacity (WBC) of it (Tamjidi *et al*., 2012), and thereby η_{app}, and also K.

The n values of yogurt samples were below 1 (Table 1), meaning that they had shear-thinning/pseudoplastic behavior. In many studies, rheological behavior of yogurt was reported as non-Newtonian shear-thinning. The n values of the enriched and the control yogurts were found in the ranges of 0.17-0.27 and 0.18-0.26, respectively. These n values are comparable with some reported values in literature (Singh and Muthukumarappan, 2008; Vercet *et al*., 2002). Only on 14th day, the n of the enriched yogurt was significantly different from that of the control. Keogh and O'Kennedy (1998) also showed that the K value was more frequently influenced by addition of hydrocolloids (e.g. gelatin, locust bean gum/xanthan mixture) than the n.

Storage time was a significant factor for both K and n. For the enriched yogurt, K increased during storage until 14th day and decreased on 21st day, significantly, while the shear-thinning index (n) decreased during storage, continually. For the control yogurt, K decreased on 14th day and increased on 21st day, while n showed a decrease after 21 days of storage (Table 1). Singh and Muthukumarappan (2008) reported that K and n values of the control and calcium-fortified yogurts remained

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constant during 14 days of storage, while in other studies these parameters changed during storage (Aportela-Palacios *et al*., 2005; Velez-Ruiz *et al*., 2012). Usually, the net charge of milk proteins, gelatin, and acacia gum change with pH. Therefore, the changes of η_{app} and also K of yogurt samples during storage is presumably because of conformational changes and interactions of milk proteins and/or gelatin and acacia gum, as a consequence of the pH decrease during storage (Table 1).

CONCLUSIONS

Fish oil (FO) was microencapsulated in gelatin-acacia gum coacervates. The yogurt enriched with FO microcapsules and the control yogurt sample showed non-Newtonian flow behavior of shear-thinning type, well fitted by the Power Law equation ($0.84 \leq r^2$). The presence of FO microcapsules in yogurt increased its apparent viscosity and consistency coefficient. Excepting day 14, the flow behavior index was not influenced by addition of microcapsules. The addition of 0.2 g LCn-3PUFAs per serving to supplement yogurt appears to be a promising path for increasing LCn-3PUFAs intake. Both LCn-3PUFAs and yogurt itself are well known for their beneficial health effects, and together they constitute a functional food with commercial applications. However, the fish odor would make it necessary to add flavor components to modify yogurt formulation to match consumer preferences.

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خواص رئولوژیکی ماست غنی شده با روغن ماهی ریزپوشینه‌دار شده

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چکیده

این مطالعه به‌منظور ارزیابی خصوصیات رفتار جریان ماست غنی‌شده با میکروکپسول‌های روغن ماهی تولید شده با روش توده‌ای شدن مرکب، انجام شد. روغن ماهی در توده‌های ژلاتین- صمغ عربی ریزپوشینه‌دار شد. سپس میکروکپسول‌ها خشک شدند و از شیر غنی‌شده با پودر میکروکپسول‌ها ماست تولید شد. خواص رئولوژیکی ماست در محدوده سرعت برشی ۰/۲۶۲-۷/۸۶ بر ثانیه در دمای ۶ درجه سانتیگراد طی ۲۱ روز نگهداری (با گرانروی‌سنج چرخشی) اندازه‌گیری شد. مدل قانون توان برای محاسبه ضریب قوام و اندیس رفتار جریان ماست استفاده شد. ماست غنی‌شده در مقایسه با نمونه کنترل گرانروی ظاهری بیشتری داشت. ضریب قوام نمونه‌های ماست غنی‌شده و کنترل به ترتیب در دامنه‌های ۲۴/۴۲-۲۸/۸۲ و ۱۵/۳۱-۱۷/۷۶ $Pa.s^n$ بود. نمونه‌های ماست رفتار جریان غیر- نیوتنی رقیق شونده با برش داشتند. افزودن میکروکپسول روغن ماهی به ماست برای بهبود دادن اثر سلامتی‌بخشی و قوام آن مفید است.

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