

Investigating the effects of varying wall materials and oil loading levels on stability and nutritional values of spray dried fish oil

Hamed Khalilvandi-Behroozyar^{1*}, Mehdi Dehghan Banadaky², Mohammad Ghaffarzadeh³

¹ Department of Animal Science, Faculty of Agriculture, Urmia University, Urmia, Iran; ² Department of Animal Science, College of Agriculture and Natural resources, University of Tehran, Karaj, Iran; ³ Department of Organic Chemistry, Chemistry and Chemical Engineering Research Center of Iran, Tehran, Iran.

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Abstract

High oxidative capacity of polyunsaturated fatty acid rich oils is the main problem with their dietary application. The main objectives of this study were to determine the effects of different encapsulants and oil loading levels on nutritive value, fatty acid profile, and oxidative stability of microencapsulated fish oil powders. Four types of wall materials [glucose syrup and maltodextrin based Maillard reaction products (MRP) or equivalent non-reacted physical blends (Non-MRP)] were used along with the three levels of oil loadings (oil to wall ratio of 1:2; 1:1; 2:1 as low, medium and high oil loadings). Emulsions and resulting microcapsules were tested for fatty acid content and stability if fatty acids over time. Additionally, different oxidative parameters were used to assess the oxidative stability of the microcapsules. Results showed that high oil loading significantly increased the mean particle size of emulsions and resultant powders and concomitantly reduced microencapsulation efficiency (ME) and yield of capsules in all of the tested wall materials. However, MRP exhibited better performance. Maillard reaction products showed better protection efficiency against oil oxidation relative to non-MRP. Nevertheless, two types of MRP encapsulants showed different proficiency and glucose syrup-MRP, provided more protection than Maltodextrin-MRP. Maillard reaction had a positive correlation with the stability properties of emulsions and resulting microcapsules. Our results showed that microencapsulation with Maillard reaction products could be used as an efficient way to protect fish oil from oxidation.

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Introduction

Modern human communities are interested in fish oil omega-3 fatty acids [Docosahexaenoic acid (22:6n-3) and Eicosapentaenoic acid (20:5n-3)], with promotive health effects on insulin sensitivity and cardiovascular health. Also, anti-inflammatory and anti-cancer effects have been reported previously.¹⁻⁶ Nevertheless, some drawbacks such as high oxidative sensitivity and “fishy” smell and/or taste can be considered as the main reasons for the low consumption rate of fish oil in animal nutrition or manufacturing functional dairy products. High oxidative damage in fish oil may be related to the relatively high polyunsaturated fatty acid content. Several studies have demonstrated that diets rich in fish oil increase oxidative stress, organ dysfunction, aging promotion, and reduced cellular function.⁷ Therefore, preventing these fatty acids

from oxidation is indispensable in allowing them to consume in the physiological range.

Microencapsulation is a technique for packing small droplets of liquid or solid particles into a wall matrix.⁸ Different researchers showed the effectiveness of this technique to retard oxidation. However, in some cases, this technique was not able to prevent oxidation. Wall material composition as well as microencapsulation technique in practice, can affect this. Spray drying is a well-known and cost-effective tool for microencapsulation in the food industry.⁸ The wall materials used in spray drying have to exhibit high solubility, ability to make stable emulsions, rapid drying, and film-forming properties. Carbohydrates can improve the drying properties of the wall matrix by enhancing the formation of a dry crust around the drying droplets. Recently, Maillard reaction products (MRP) considered natural polymers with great potential in

*Correspondence:

Hamed Khalilvandi-Behroozyar. PhD
Department of Animal Science, Faculty of Agriculture, Urmia University, Urmia, Iran
E-mail: h.khalilvandi@urmia.ac.ir



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industrial applications. Protein-carbohydrate conjugates of Maillard reaction showed the potential to be used in the encapsulation of unsaturated oils.⁹ Protein-carbohydrate conjugates have superior emulsifying properties and higher stability compared to untreated proteins.^{10,11} Besides, the antioxidative properties of MRP can further improve the protection of unsaturated oils.^{9,12}

The present study aimed to determine the effects of dried glucose syrup (DGS) and maltodextrin based MRP of sodium caseinate on oxidative stability of emulsions, fresh and stored fish oil microencapsules. Fatty acid profiles and changes in response to storage conditions also were determined along with the general properties of emulsions and powders.

Materials and Methods

The ingredients used for the preparation of microcapsules were food grade. Sodium caseinate (Cas; 83.00% crude protein) was obtained from Iran Caseinate Company (Tehran, Iran). Glucose monohydrate (Glu) was from Iran Glucosan Company (Tehran, Iran) and dried glucose syrup (DGS; Dextrose Equivalent = 26 - 30) and maltodextrin (Mal; DE=18) kindly donated by Dextrose Iran Company (Tehran, Iran). Fish oil was not supplemented with antioxidants and kept in -20.00 °C until use. All of the chemical reagents and solvents used in the evaluation of microcapsules and preparation of fatty acid methyl esters (FAME) were of analytical grade and solvents were re-distilled before use.

Preparation of encapsulants and emulsions. The encapsulants were developed using maltodextrin/glucose/casein and DGS/glucose/casein (1:1:1 ratio). Accordingly, casein was dispersed in deionized water (60.00 °C) via a high shear mixer. Afterward, an aqueous mixture of glucose and maltodextrin or DGS was added and gently mixed with casein. Maillard reaction products were produced with the heating of the pH adjusted solution (7.5; 1 M NaOH; Merck, Darmstadt, Germany) at 98.00 °C for 30 min. In the case of non-MRP encapsulants, pH adjustment and heating were omitted. Encapsulant mixtures were sonicated for one minute and cooled to 40.00 °C. Final emulsions were prepared by mixed up pre-warmed fish oil (40.00 °C) to create three oil to encapsulants proportions (1:2, 1:1, 2:1 as low, medium, and high oil loadings). The dry matter content of emulsions was adjusted to 30.00% (w/w) with deionized water and sonicated for three min. Protein, total carbohydrates, and oil content of the emulsions were 6.30, 13.70, and 10.00% for 1:2 oil loading; 5.00, 10.00 and 15.00% for 1:1 oil loading and 3.40, 6.60 and 20.00% for 1:2 oil loading.

Emulsion viscosity, droplet size, and Maillard reaction extent. The apparent viscosity of emulsions was measured at 25.00 °C using a stress-controlled rheometer (Physica RheolabMC 100; Paar Scientific, London, UK)

fitted with concentric cylinder geometry (MS-Z1 DIN) at a shear rate of 100 per sec. Particle size distributions were determined by a laser diffraction technique (Mastersizer S; Malvern Instruments, Worcs, UK). The calculation of the particle size distribution was based on a relative refractive index of 1.11 and absorption of 0.0001. Distilled water was used as the dispersing medium. The light absorbance spectrophotometer at 465 nm (UV-1201V; Shimadzu Corp., Kyoto, Japan) of the emulsions and re-dispersed microcapsules (1:20 dilution in deionized water) was considered as the Maillard reaction extent.⁹

Production and analysis of microcapsules. Emulsions were continuously stirred at 60.00 °C through a drying process and dried using a lab-scale spray-dryer (Mini spray-dryer B-290; Büchi laborotechnik AG, Flawil, Switzerland) under manufacturer's rules. Inlet and outlet temperatures were set to 130°C and 65.00 °C, respectively. For each type of the emulsions, three drying runs were applied and considered as the replications. Produced microcapsules were packed under the argon gas blanket and maintained at -20.00 °C until further analysis. The total yield of microcapsules was determined by weighing collected samples in cyclone separated jar and expressed as a weight percentage of emulsion's dry matter content.

Chemical analysis packed bulk density, and water activity. Dry matter was determined by drying the samples at 105 °C overnight and organic matter content determined by igniting the samples in a muffle furnace,¹³ total oil, and nitrogen (N) contents were measured by acid treatment,¹³ and Kjeldahl method (Kjeltec 1030 Auto-analyzer; Foss Tecator AB, Hogans, Sweden), respectively. Crude protein was calculated as N × 6.25. All chemical analysis was carried out in triplicates. Packed bulk density was determined by hand tapping 2.00 g of microcapsules into a 10-mL graduated cylinder.¹⁴ The water activity of the samples was measured by using an AquaLab water activity meter at 25.00 °C (Series 3; Decagon Devices Inc., Pullman, USA).

Microencapsulation efficiency and particle size analysis. Microencapsulation efficiency (ME) was calculated with the estimation of the solvent extractable and total fat content of microcapsules. Total fat was determined after HCl (Merck) digestion concomitant with ethanol (96.00%, w/v; Merck) and diethyl ether (Merck) assisted extraction and expressed as the weight percentage of the microcapsules. Petroleum ether was used to determine the non-encapsulated oil.¹⁵ Powder particle size was determined by laser diffraction (Malvern Mastersizer) after the dispersion of the dried emulsion sample in Propan-2-ol.¹⁶

Oxidative status and fatty acid profiles. One gram of the microcapsules was weighed and poured into 20.00 mL glass tubes, sealed and placed in different temperatures (4.00, 25.00, and 60.00 °C) for 15, 30, and 45 days in triplicates. Oil extraction from emulsions and reconstituted microcapsules was done using sodium hexametaphosphate

Triton X-100 (Merck) under the argon gas blanket and the oil was removed by light centrifuge. Nonadecanoic acid was used as an internal standard for GC analysis and stored at $-20.00\text{ }^{\circ}\text{C}$. Peroxide (Cd 8-53; AOCS, 2004) and p-anisidine (Cd 18-90; AOCS, 2004), were determined in triplicates.¹⁷ One microliter of fatty acid methyl esters was injected in the split mode (50:1) into a FID equipped gas chromatograph (CP-Sil 88 capillary column: $100\text{ m} \times 250\text{ }\mu\text{m} \times 0.20\text{ }\mu\text{m}$; Varian CP-3800; Chrompack, Middelburg, Netherlands).¹⁸ The injector and detector temperatures were set at $250\text{ }^{\circ}\text{C}$ and N_2 was used as the carrier gas with a constant flow of 1.00 mL per min . The oven temperature was held at $70.00\text{ }^{\circ}\text{C}$ for 1 min and then increased $5.00\text{ }^{\circ}\text{C per min}$ up to $100\text{ }^{\circ}\text{C}$ and maintained for 2 min. After that, the column temperature was increased $10.00\text{ }^{\circ}\text{C per min}$ up to $175\text{ }^{\circ}\text{C}$ and kept for 35 min and then increased $4.00\text{ }^{\circ}\text{C per min}$ up to $225\text{ }^{\circ}\text{C}$ and maintained for 35 min. Individual peaks were determined according to a FAME standard mixture.

Statistical analysis. A complete randomized design (CRD) was used for statistical analysis of all of the measured variables except for oxidative stability that was analyzed by CRD based factorial design. PROC GLM of SAS (version 9.1; SAS Institute, Cary, USA), was used for statistical analysis. Least square (LS) means were adjusted and compared to Tukey and PDIFF options, respectively. Data were shown as LS means and corresponding SEM and a significant difference was declared at $p < 0.05$. Proc corr was used for correlation coefficients.

Results

The chemical composition of emulsions and resultant microcapsules is presented in Table 1. Fiber and indigestible protein fractions of the microcapsules were increased as a result of the Maillard reaction which might be related to the nutritional unavailability of carbo-hydrates and proteins. Dried glucose syrup-based Maillard encapsulants had the lowest particle size in all of the oil loading levels.

In the case of maltodextrin based encapsulants, differences between MRP and non-MRP were significant in high oil loading level. Oil content in microcapsules was increased with increasing oil level in the emulsions, however, not affected by the wall materials type. On the other hand, ME is a function of the oil loading level and wall material type. Results of re-dispersion behavior reflect the negative impacts of high oil loading on the encapsulation ability of the wall systems. A high negative correlation between the re-dispersed droplet size and ME was also found. In addition to the initial higher ME of MRP encapsulants compared to non-MRP counterparts, MRP powders kept higher ME during storage (Table 2), which might be an explanation for higher oxidative stability of these products. The effects of storage time on ME was also affected by oil loading levels. Maillard reaction extent was increased as a function of carbohydrates DE. Dried powders had the highest Maillard reaction extent compared to encapsulants and not dried emulsions (data not shown).

The oxidative state of emulsions and spray-dried microcapsules are represented in Table 3. Emulsions made with non-MRP encapsulants have slightly higher peroxide values than Maillard encapsulants in identical oil levels, however, p-anisidine value was remained unchanged. High oil loading resulted in increased peroxide value and significant differences were distinguished between DGS and maltodextrin based emulsions. Fish oil peroxidation in emulsions can be partially explained by heating in different steps such as sonication and homogenization. Heating in spray-dryer increased peroxide values compared to emulsions. The oil loading level and wall material type significantly affected the oxidation state instantly after spray-drying. Maillard wall materials produced powders with lower peroxide values. Oxidative stability indexes of microencapsulated oil are shown in Table 4. Increased storage temperature, incubation time, and oil loading levels amplified oxidative indexes in all of the wall materials.

Table 1. Chemical composition and production efficiency of microcapsules from different encapsulation systems.

Encapsulation system	Oil to wall ratio	Dry matter (g 100g ⁻¹)	Ash (g 100g ⁻¹ DM)	Fat (g 100g ⁻¹ DM)	Crude protein (g 100g ⁻¹ DM)	Insoluble fiber (g 100g ⁻¹ DM)	ME (%)	Yield (%)	
Mal	MRP	1:2	97.61	0.49	32.47 ^c	22.88 ^a	15.47 ^{cd}	98.01 ^c	98.37 ^a
		1:1	97.74	0.36	51.53 ^b	16.42 ^b	14.77 ^d	99.05 ^b	94.45 ^b
		2:1	97.24	0.24	66.45 ^a	11.37 ^c	16.78 ^c	62.48 ^g	78.1 ^d
	Non-MRP	1:2	97.62	0.49	32.15 ^c	22.99 ^a	4.49 ^f	89.45 ^d	93.13 ^c
		1:1	97.52	0.35	52.01 ^b	16.26 ^b	4.61 ^f	54.66 ^h	67.57 ^e
		2:1	97.38	0.22	68.64 ^a	10.63 ^c	4.93 ^f	42.25 ⁱ	48.83 ^f
DGS	MRP	1:2	97.27	1.49	32.56 ^c	22.83 ^a	22.61 ^{ab}	98.12 ^{bc}	99.23 ^a
		1:1	97.33	1.19	52.56 ^b	16.38 ^b	21.71 ^b	98.97 ^a	95.32 ^b
		2:1	97.61	1.74	67.48 ^a	12.34 ^c	24.28 ^a	72.27 ^f	78.65 ^d
	Non-MRP	1:2	97.59	1.47	32.18 ^c	22.94 ^a	11.62 ^e	89.75 ^d	94.01 ^{bc}
		1:1	97.63	1.79	52.04 ^b	16.22 ^b	11.76 ^e	76.14 ^e	67.62 ^e
		2:1	97.96	0.89	68.67 ^a	10.60 ^c	11.95 ^e	53.76 ⁱ	48.87 ^f
SEM	-	0.574	0.124	1.012	0.953	0.754	0.391	1.104	

DM: Dry matter; Mal: Maltodextrin; MRP: Maillard reaction products; DGS: Dried glucose syrup; and ME: Microencapsulation efficiency. Different letters in each column determines statistical significant difference ($p < 0.05$).

Table 2. Differences in encapsulation efficiency after storage at 25.00 °C.

Encapsulation system	Oil to wall ratio	Microencapsulation efficiency (%)				SEM	
		Initial	15 days	30 days	45 days		
Mal	MRP	1:2	98.01 ^{cW}	89.16 ^{bX}	87.13 ^{cY}	83.12 ^{cZ}	0.235
		1:1	99.05 ^{bW}	86.13 ^{cX}	84.11 ^{dY}	83.09 ^{eY}	0.364
		2:1	62.48 ^{gW}	55.12 ^{hX}	47.18 ^{iY}	40.03 ^{gZ}	0.216
	Non-MRP	1:2	89.45 ^{dW}	73.12 ^{eX}	64.06 ^{fY}	53.16 ^{fZ}	0.672
		1:1	54.66 ^{hW}	43.16 ^{iX}	38.26 ^{jY}	32.12 ^{hZ}	0.537
		2:1	42.25 ^{jW}	39.94 ^{iX}	28.11 ^{kY}	21.67 ^{iZ}	0.363
DGS	MRP	1:2	98.12 ^{bcW}	96.12 ^{aW}	93.17 ^{aX}	89.54 ^{aY}	0.775
		1:1	98.97 ^{aW}	95.15 ^{aX}	89.15 ^{bY}	87.18 ^{bZ}	0.442
		2:1	72.27 ^{fW}	67.43 ^{fX}	59.12 ^{hY}	56.18 ^{eZ}	0.811
	Non-MRP	1:2	89.75 ^{dW}	81.15 ^{dX}	76.37 ^{eY}	68.14 ^{dZ}	0.563
		1:1	76.14 ^{eW}	65.13 ^{gX}	60.14 ^{gY}	55.17 ^{eZ}	0.892
		2:1	53.76 ^{iW}	42.13 ^{iX}	38.53 ^{jY}	32.17 ^{hZ}	0.372
SEM	-	0.391	0.562	0.467	0.327	-	

Mal: Maltodextrin; MRP: Maillard reaction products; DGS: Dried glucose syrup.

Different lowercase letter in each column determines the statistical difference among different supplements ($p < 0.05$), and different uppercase letter in each row determines the statistical difference among different days ($p < 0.05$).

Table 3. Oxidative state of dried microcapsules and emulsions prepared with different wall material systems.

Encapsulation system	Oil to wall ratio	Powders		Emulsions		
		Peroxide (mEq kg ⁻¹)	p-anisidine*	Peroxide (mEq kg ⁻¹)	p-anisidine*	
Mal	MRP	1:2	1.78 ^{dX}	7.53 ^{dX}	1.64 ^{bX}	2.98 ^{dY}
		1:1	1.76 ^{dX}	7.13 ^{deX}	1.60 ^{bX}	3.05 ^{dY}
		2:1	6.23 ^{bX}	7.95 ^{cdX}	2.07 ^{bY}	4.38 ^{bY}
	Non-MRP	1:2	1.80 ^{dX}	8.67 ^{cX}	1.65 ^{bX}	3.60 ^{cY}
		1:1	3.10 ^{cX}	8.28 ^{cX}	2.98 ^{abX}	3.93 ^{bcY}
		2:1	10.48 ^{aX}	19.48 ^{aX}	3.37 ^{aY}	5.86 ^{aY}
DGS	MRP	1:2	1.60 ^{dX}	6.29 ^{eX}	1.34 ^{bX}	2.82 ^{dY}
		1:1	1.59 ^{dX}	5.89 ^{eX}	1.45 ^{bX}	2.90 ^{dY}
		2:1	6.08 ^{bX}	7.75 ^{dX}	1.98 ^{bY}	4.26 ^{bY}
	Non-MRP	1:2	1.67 ^{dX}	7.32 ^{dX}	1.94 ^{bX}	3.49 ^{cdY}
		1:1	2.93 ^{cX}	5.85 ^{eX}	2.21 ^{bX}	3.79 ^{cY}
		2:1	10.30 ^{aX}	10.61 ^{bX}	3.21 ^{aY}	5.72 ^{aY}
SEM	-	0.4613	0.4383	0.3742	0.2641	

Mal: Maltodextrin; MRP: Maillard reaction products; DGS: Dried glucose syrup.

Different lowercase letter in each column show the statistical difference among different supplements ($p < 0.05$), and different uppercase letter in each row indicate the statistical difference between powders and emulsions in each of the measured parameters ($p < 0.05$).

*The p-anisidine value is defined by convention as 100 times the optical density measured at 350 nm in a 1.00 cm cuvette of a solution containing 1.00 g of the oil in 100 mL of a mixture of solvent and reagent according to the method described.

Table 4. Oxidative stability of dried microcapsules prepared with different wall material systems and different Incubation days.

Encapsulation system	Oil to wall ratio	peroxide values (mEq kg ⁻¹)			p-anisidine values			
		15 days	30 days	45 days	15 days	30 days	45 days	
Mal	MRP	1:2	3.11 ^f	5.35 ^{ef}	7.10 ^h	11.66 ^{ef}	19.78 ⁱ	26.49 ^g
		1:1	2.95 ^f	6.15 ^e	9.90 ^g	11.26 ^e	20.18 ⁱ	25.69 ^{gh}
		2:1	6.10 ^d	8.07 ^d	13.90 ^f	14.85 ^c	23.96 ^g	35.01 ^d
	Non-MRP	1:2	3.90 ^f	10.40 ^c	20.50 ^{cd}	12.69 ^{de}	35.43 ^d	28.77 ^f
		1:1	3.90 ^f	9.28 ^c	15.30 ^e	13.08 ^d	26.75 ^f	29.56 ^f
		2:1	10.60 ^b	11.90 ^b	23.00 ^b	31.46 ^a	56.08 ^b	62.15 ^b
DGS	MRP	1:2	3.21 ^f	4.50 ^f	6.90 ^h	9.92 ^f	16.94 ^j	22.64 ⁱ
		1:1	3.21 ^f	5.30 ^{ef}	6.90 ^h	9.52 ^f	17.21 ^j	21.84 ⁱ
		2:1	4.94 ^e	7.09 ^{de}	12.75 ^f	13.33 ^{cd}	21.67 ^h	30.95 ^e
	Non-MRP	1:2	3.24 ^f	8.19 ^{cd}	14.96 ^e	10.98 ^f	23.25 ^g	24.78 ^h
		1:1	3.36 ^f	8.33 ^{cd}	21.32 ^c	9.64 ^f	29.21 ^e	22.21 ⁱ
		2:1	8.70 ^c	8.85 ^{cd}	19.16 ^d	20.45 ^b	41.53 ^c	48.47 ^c
Non encapsulated Oil	-	12.57 ^a	43.71 ^a	69.35 ^a	32.537 ^a	64.275 ^a	73.14 ^a	

Mal: Maltodextrin; MRP: Maillard reaction products; DGS: Dried glucose syrup.

Different letter in each column determines the statistical difference between among different supplements ($p < 0.05$).

Presented data are means of three measurements in each of the three replicates. The main effects of microencapsulate type, incubation day along with their interactions were analyzed. Overall SEM for peroxide and p-anisidine values were 0.476. and 0.512, respectively.

Correlation coefficients between quality indices of microencapsules indicated a strong negative correlation among oil loading level and encapsulation efficiency, the yield of microcapsules, and oxidative state (-0.758, -0.752 and -0.738, respectively).

Discussion

As shown in Table 1, heating of the emulsions in the drying process was resulted in increased fiber and indigestible protein fractions. Originally, the reaction between amino acids and reducing sugars of feed materials during the heating process was resulted in the browning of the reaction mixture known as the Maillard reaction starting from the glycation of protein and progressing the reaction between glucose and amino groups of protein to form the Amadori products.¹⁹ The Maillard reaction may take place at room temperature, however, its intensity increases with an increase in time, temperature, moisture (maximum at 40.00 to 70.00%), and alkalinity (linear between pH 3.00 and 8.00).¹⁹ kinds of research showed that lysin is the most affected amino acid.²⁰ The Maillard reaction causes protein denaturation, reduction of ruminal protein solubility and degradation, and increased acid detergent insoluble nitrogen content of the heat-processed feedstuffs.²¹ However, differences between Maillard reaction products of DGS and maltodextrin can be related to the Maillard reaction extent (Table 3). Microencapsulated products had low moisture content and water activity as a result of the drying process and physical properties of the encapsulant materials.^{9,15} In agreement with our results, Kosaraju *et al.* did not find any difference in the DM content of MRP or non-MRP coated microcapsules.¹⁵ Other researchers showed that moisture content was not affected by the core to wall ratio.²¹⁻²³ However, the DM content of encapsulated products can be also related to properties of wall materials, type of spraying nozzle, differences between inlet and outlet temperatures, and relative humidity in the drying chamber. Most of the published papers used higher temperatures for microencapsulation, ranging from 150 to 180 °C. Aghbashlo *et al.* stated that the energy efficiency of fish oil microencapsulation is a function of applied temperature and lower drying temperature. It seems that lower energy consumption saves the core oil and nutrients of wall material.²⁴ Our results showed that 130 °C was sufficient for the production of stable products with high and desirable DM contents.²⁵ Aghbashlo *et al.* reported that oil peroxide values were increased as a result of higher temperature applied in the drying process.²⁶

As shown in Table 1, oil content in microcapsules was increased with increase in oil level in the emulsions, however, not affected by the wall materials type.²⁷ On the other hand, microencapsulation efficiency (ME) is a function of the oil loading level and wall material type.

Lower ME in high oil loaded microcapsules can be related to insufficient wall materials to encapsulate all of the oil.^{8,27-29} On the other hand, in the same oil loading levels, MRP wall materials have higher ME than non-MRP and two types of MRP materials showed significant differences. Augustin *et al.* concluded that MRP encapsulants with different protein sources had very high encapsulation ability.⁹ More recently, Kosaraju *et al.* concluded that casein and whey protein isolate (WPI) based MRP and non-MRP wall materials had high ME in the case of low and medium oil loadings.¹⁵ Microencapsulation efficiencies in this study, were comparable to the results of previous studies,^{9,15} in the case of MRP and non-MRP encapsulants and were higher compared to conventional wall materials reported elsewhere.^{10,27,30} However, microencapsulation efficiency is a function of temperature, wall material type, and emulsion properties and is not easy to compare among different studies. Higher ME for MRP wall materials in high oil loadings can be a result of emulsifying effects of MRP.³¹⁻³³ Also, Oliver *et al.* and Dickinson stated that protein-polysaccharide conjugates covalently bonded by the Maillard reaction had better surface activity properties and higher emulsion stabilization capacity than the corresponding proteins.^{10,11} Slightly higher ME values for MRP and non-MRP microcapsules of DGS compared to maltodextrin's can be related to higher DE of DGS. The positive effect of increasing carbohydrate dextrose equivalent on microencapsulation efficiency is consistent with results of studies including whey protein-carbohydrate,^{34,35} and casein - carbohydrate combinations.³⁶ In this case, smaller oligosaccharides in high DE powders formed a more uniform and less porous surface upon drying temperature, pH, and type of reducing sugars in a reaction medium that were reported as important factors in rate and extent of Maillard reaction.³⁷ Also, they concluded that the higher the carbohydrate DE, the higher the rate and extent of the Maillard reaction.

Low ME reduced the yield of microcapsules, mainly due to the sticking effects of non-encapsulated oil. Similarly, Tan *et al.* reported that in high oil loaded starchy wall materials, ME and yield of microcapsules were reduced combined with the production of tacky microcapsules.²⁷ All the samples had low water activity in agreement with the literature.³⁸ However, higher oil loading has resulted in more reduced water activity. In the same oil loading levels, the difference among different encapsulants was not significant.

Bulk density is dependent on the water content of the product, shrinkage rate, and drying method.³⁹ However, we could not find any differences between types of wall materials, however, the oil content influenced the bulk density (data were not shown).

Dried glucose syrup-based Maillard encapsulants had the lowest particle size in all of the oil loading levels. In the

case of maltodextrin based encapsulants, differences between MRP and non-MRP was significant in high oil loading level and may be attributed to high-viscosity of emulsions, inefficient atomization or agglomeration particles due to high surface oil.^{21,36} Differences in particle size is more reasonable in dry and reconstituted powders. Results of re-dispersion behavior reflect the negative impacts of high oil loading on the encapsulation ability of the wall systems. A high negative correlation between the re-dispersed droplet size and ME was also found. In addition to initial higher ME of MRP encapsulants compared to non-MRP counterparts, MRP powders kept higher ME during storage (Table 2), which may be an explanation for the higher oxidative stability of these products. The effects of storage time on ME was also affected by oil loading levels.

The viscosity of emulsions was affected by encapsulant type and oil levels in the emulsions. Results showed that MRP encapsulants had higher emulsion viscosity. However, the viscosity of emulsions was reduced as a function of increased oil loading.^{36,40} Al-Hakkak and Al-Hakkak reported an increase in viscosity and a decrease in oil droplet diameter in oil-in-water emulsions as a result of Maillard conjugation of egg white protein/pectin mixtures.⁴¹ A positive correlation was found between emulsions viscosity and ME in fresh and stored samples. Aghbashlo *et al.* suggested that high emulsion viscosity increased the encapsulation efficiency due to limited oil penetration to the particle surface.²⁶

As shown in Table 3, emulsions made with non-MRP encapsulants had slightly higher peroxide values than Maillard encapsulants in identical oil levels, and Maillard wall materials produced powders with lower peroxide values. Aghbashlo *et al.* concluded that increased drying temperature was resulted in higher peroxide values in producing microcapsules.²⁶

Oxidative stability indices of microencapsulated oil are shown in Table 4. Increased storage temperature, incubation time, and oil loading levels amplified oxidative indices in all of the wall materials consistent with Kagami *et al.*⁴² and Hogan *et al.*⁴³ Microencapsulation process was shown to be effective process against oxidation compared to emulsions. Nevertheless, the type and composition of encapsulants impress the protecting ability of microencapsulation. Higher microencapsulation efficiency besides higher viscosity, emulsifying, and anti-oxidative properties can cause lower oxidative indices in MRP microcapsules.^{9,44,45} Higher protection provided by MRP encapsulants also can be explained by differences in particle size of emulsions, powders, and re-dispersed powders. Risch and Reineccius stated that in the microencapsulation process, smaller emulsion droplet size conferred advantages in terms of emulsion stability, oil retention in the dried powder and less extractable surface oil, which could reflect in higher ME and lower peroxide

values in encapsulated materials.⁴⁶ Differences in Maillard reaction extent can partially explain the differences between two MRP encapsulants ability to retard oxidation. Kosaraju *et al.* indicated that a positive relationship existed between the Maillard reaction extent and anti-oxidative capacity of Maillard reaction products.⁴⁶

According to the results, it could be concluded that microencapsulation with Maillard conjugates of protein and carbohydrates with medium oil loading levels had a strong ability in preventing encapsulated fish oil from oxidation. Also, it could be concluded that spray drying with inappropriate wall materials or high oil loading levels even in low inlet temperatures could lead the core oil to oxidation and reduce polyunsaturated fatty acid content.

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Conflict of interest

The authors report no conflict of interest.

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