

DEVELOPMENT OF OIL-IN-WATER EMULSIONS BASED ON RICE BRAN OIL AND SOYBEAN MEAL AS THE BASIS OF FOOD PRODUCTS ABLE TO BE INCLUDED IN KETOGENIC DIETS

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Development of oil-in-water emulsions based on rice bran oil and soybean meal as the basis of food products able to be included in ketogenic diets

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Abstract

The aim of this work was to develop rice bran oil-in-water emulsions stabilized with proteins and polysaccharides from soybean meal as the basis of food products able to be included in ketogenic diet. The effect of the formulation (ketogenic ratios and oil mass fractions) and the high-pressure homogenization conditions (number of homogenization cycles) on the properties of the resulting O/W emulsions was evaluated. All freshly prepared emulsions showed multimodal particle size distributions and shear-thinning behaviour. At a fixed ketogenic ratio, all emulsions had the same oil to emulsifiers + stabilizers proportion but increasing their oil mass fraction resulted in systems composed by smaller particles with greater interfacial area, and apparent viscosity. The same effect was observed by increasing the number of homogenization cycles. Meanwhile, increasing the ketogenic ratio (at a fixed oil mass fraction) diminished its apparent viscosity. Most of the studied emulsions were stable for seven days of quiescent refrigerated storage, although some changes in its particle size distributions were observed. Only the stored emulsions with the highest ketogenic ratio and the lowest oil mass fraction presented gravitational separation but no phase separation. Emulsions prepared after five homogenization cycles presented greater stability to the coalescence than those prepared in one cycle.

Keywords: O/W emulsion, rice bran oil, soybean meal, valve high-pressure homogenizer, ketogenic diet.

1. Introduction

The close relationship between the diet of consumers and their health is well known (Bleich, Jones-Smith, Wolfson, Zhu, & Story, 2015). In recent years, the ketogenic diet has gained popularity as a diet to lose weight (Barry et al., 2018). The ketogenic diet is characterized by high-fat, moderate-to-low protein, and very-low carbohydrate content.

In this diet, the conventional fat to carbohydrate-plus-protein ratios (ketogenic ratios, KR) are 5:1, 4:1 and 3:1 (w/w) so that the highest caloric intake comes from lipids (between 87 and 92% of total calories) (Oliveira et al., 2018). The basic foods included in the ketogenic diet are cream, butter, mayonnaise, oil, cheese, bacon and eggs, and are complemented with meat, fish, nuts, fruits and vegetables. However, to achieve the appropriate proportion of macrocomponents, the quantities and nutritional composition of each ingested foods must be known (Armeno et al., 2014).

In parallel, the ketogenic diet has emerged as a potential metabolic therapy and it is used as a non-pharmacological treatment for people suffering from drug-resistant epilepsy, Alzheimer's disease, Parkinson's disease, brain traumas and different types of cancer, among others (Brouns, 2018; McDonald; Cervenka, 2018; Oliveira et al., 2018; Włodarek, 2019). The ketogenic diet is a currently accepted therapeutic alternative for the treatment of some of the diseases mentioned above but, at the same time, it is a restrictive and strict diet which is difficult to accept and continue for the patient (Armeno et al., 2014). The development of food products and ingredients that respect the ketogenic ratios, in particular, the formulation of emulsions is presented as a challenge for the food industry.

Oil-in-water (O/W) emulsions consist of oil droplets dispersed in an aqueous phase. O/W emulsions are thermodynamically unstable systems, but it is possible to form emulsions that are kinetically stable for a reasonable period (a few days to years) by including stabilizers. The physicochemical properties of O/W emulsions, such as their rheology, global stability and sensory properties are highly dependent on the characteristic of their droplets. In turn, these characteristics are determined by the emulsion composition (e.g. the type and concentration of emulsifier used or the phase ratio) and the homogenization conditions (e.g. intensity and duration of energy input) (McClements, 2016). Therefore, it is important to evaluate the effect of these factors on the properties of the emulsion-based products during their development.

Soybean meal is the most relevant by-product generated by soybean oil processing, with high protein and carbohydrate contents and some bioactive compounds such as poly-oligosaccharides and polyphenols (Gerliani, Hammami, & Aider, 2019). The popularity of

soybean proteins as emulsifiers is increasing given the drive to find vegetable-based alternatives to dairy proteins, due to their good nutritional value and functional properties (Tang, 2015). Numerous studies have shown the emulsifying properties of soybean protein concentrates, isolates and purified fractions (Di Giorgio, Salgado, & Mauri, 2019; Molina, Papadopoulou, & Ledward, 2000; Palazolo, Mitidieri, & Wagner, 2003; Puppo et al., 2011; Zhang et al., 2019). Simultaneously, soybean polysaccharides have been used as emulsifiers in acidic emulsion-based beverages (Nakamura, Maeda, & Corredig, 2004; Porfiri et al., 2017; Tran & Rousseau, 2013). Even so, their application as stabilizers in practical formulations is still very limited possibly due to the fact that their properties are highly dependent composition of the products, processing history (especially thermal treatment, drying and fractionation conditions), and even a number of environmental parameters, including ionic strength, pH and temperature (Tang, 2015). In this sense, the use of whole soybean meal, a natural proteins-polysaccharides mixture, could be a more effective and economical alternative than the use of particular fractions.

On the other hand, rice bran oil, a by-product of rice milling process, is a rich source of oleic and linoleic fatty acids, and also has antioxidants such as γ -oryzanol, tocopherols, tocotrienols, polyphenols, squalene and phytosterols that have been beneficial for the prevention of cardiovascular diseases (Friedman, 2013; Kowalska, 2016). Rice bran oil is regarded as healthy vegetable oil, but it has a more desirable flavour profile than fish and flaxseed oils (Murali, Kar, Patel, Mohapatra, & Krishnakumar, 2017). This oil has been used in a wide variety of food, pharmaceutical, and cosmetic products (Angkuratipakorn, Sriprai, Tantrawong, Chaiyasit, Singkhonrat, 2017; Orthoefer, 2005). A further application that has received much attention is O/W emulsions, which is simple and low cost in terms of production and provides better sensory properties than the oil alone (Piriyaprasarth, Juttulapa, Sriamornsak, 2016).

High-pressure homogenizers are probably the most common instrument used to produce fine emulsions in the food industry. In this process, the combination of intense shear, cavitation and turbulent flow conditions leads to disruption of the oil droplets present in coarse emulsion (McClements, 2016). The decrease in the average size of the oil droplets reduces the creaming velocity and increases the stability of the O/W emulsion (<https://www.sciencedirect.com/science/article/pii/S0260877412000362> Desrumaux & Marcand, 2002). It is well known that increasing the homogenization pressures or homogenization cycles decreases the droplet size to a certain level (Juttulapa, Piriyaprasarth, Takeuchi, & Sriamornsak, 2017), but also increases the energy input required to form the

emulsion, thereby increasing manufacturing costs (McClements, 2016). However, this processing can also produce structural changes in the components used as stabilizers, which may be favorable or not.

The aim of the present research was to develop rice bran oil-in-water emulsions stabilized with soybean proteins and polysaccharides as the basis of food products able to be included in ketogenic diets. In particular, the effect of the formulation (ketogenic ratios and oil mass fractions) and the high-pressure homogenization conditions (number of homogenization cycles) on the properties of the resulting O/W emulsions was evaluated. To the best of our knowledge, rice bran oil-in-water emulsions stabilized with soybean meal proteins and polysaccharides prepared by high-pressure homogenization have not been studied yet.

2. Materials and Methods

2.1. Materials

Commercial rice bran oil (Saman S.A., Uruguay) and soybean meal (kindly supplied by América Pampa S.A., Argentina) were used as raw materials. All reagents were of analytical grade and distilled water was used for the preparation of all emulsions.

2.2. Characterization of the raw materials

The fatty acids profile of the rice bran oil was determined by gas chromatography (GC) according to the standardized IUPAC method 2.302. The theoretical iodine index and saponification value were calculated according to fatty acid composition. The centesimal composition of soybean meal was determined according to AOAC methods (AOAC, 2002). Protein content was determined by Kjeldahl method (Nx5.7). Lipids were quantified by the Soxhlet method using n-hexane as solvent. Moisture and ashes content were determined by gravimetric method at 105 °C and 550 °C respectively. Fibers content was measured by the ceramic filter method and carbohydrates content was estimated by difference. The anti-tryptic activity of soybean meal was measured according to the method described by Sobral and Wagner (2009).

2.3. Preparation of oil-in-water emulsions

O/W emulsions were formulated with rice bran oil and aqueous dispersions of soybean meal. Emulsions with different ketogenic ratios (KR = 3:1, 4:1 and 5:1 w/w) and oil mass fractions (Φ_m = 0.20, 0.25 and 0.30) were prepared. **Table 1** shows the amounts of rice bran oil, soybean meal and distilled water used to prepare 300 mL of each emulsion. Before

emulsification, the amount of soybean flour was hydrated with distilled water keeping the dispersion in magnetic stirring at 1180 rpm (Wisd, MSH-20D, Germany) for 1 h at room temperature. Then, the amount of rice bran oil needed for each formulation was added. Coarse emulsions were prepared with a high-speed blender UltraTurrax T18 using a S18N-19G dispersing tool (Janke & Kunkel GmbH, Germany) at 18,000 rpm for 90 s at room temperature. In order to obtain fine emulsions, coarse emulsions were homogenized in a twovalve high-pressure homogenizer (Panda 2000; GEA Niro Soavi, Italy) at 150 bar and with two different number of homogenization cycles (1 and 5 cycles). Fine emulsions were subjected to tight storage at 4 °C and protected from the light. Antimicrobial agents were not added. Two individually prepared replicates were assayed for each condition.

Table 1. Formulations of rice bran oil-in-water emulsions prepared with different ketogenic ratios (KR= 3:1, 4:1, and 5:1 w/w) and oil mass fractions (Φ_m = 0.20, 0.25, and 0.30) processed in a high-pressure homogenizer.

	<i>KR= 3:1</i>			<i>KR= 4:1</i>			<i>KR= 5:1</i>		
	$\Phi_m=0.20$	$\Phi_m=0.25$	$\Phi_m=0.30$	$\Phi_m=0.20$	$\Phi_m=0.25$	$\Phi_m=0.30$	$\Phi_m=0.20$	$\Phi_m=0.25$	$\Phi_m=0.30$
Rice bran oil^a (g)	58.34	72.92	87.51	58.75	73.44	88.13	59.00	73.75	88.51
Soybean flour^b (g)	25.94	32.43	38.91	19.46	24.32	29.18	15.56	19.46	23.35
Water (g)	239.17	223.96	208.75	239.38	224.22	209.07	239.50	224.38	209.25

Ketogenic ratio (KR) is defined as the ratio of grams of fat to grams of carbohydrate plus protein.

^a Rice bran oil density: 0.916 g/mL.

^b Chemical composition of soybean meal: 49.81 ± 0.44% protein, 27.22 ± 0.05% carbohydrates, 6.45 ± 0.01% lipids, 7.24 ± 0.65% fibers, 6.06 ± 0.17% ashes, and 3.22 ± 0.27% moisture.

2.4 Characterization of oil-in-water emulsions

2.4.1 Morphology

Morphology of the emulsions was observed by optical microscopy. A drop of emulsion (20 µL) was placed on a glass slide and covered with a cover slip, and immediately examined with an optical microscope (Leica MDC100, Bensheim, Germany) equipped with a digital camera (Leica MC190 HD, Bensheim, Germany) at 100X magnification.

2.4.2. Particle size distribution and mean diameters

The particle size distribution was measured by static light scattering (SLS) using a Malvern Mastersizer 2000E analyzer (Malvern Instruments Ltd., Worcestershire, UK). About 1 mL of the emulsion was suspended directly in the water bath of

the dispersion system (600 mL) and the pump speed in dispersion unit was set at 2000 rpm (Hydro 2000MU, Malvern Instruments, Worcestershire, UK) reaching an obscuration of 10–20%. The refractive indexes of the dispersed and continuous phases were 1.40 and 1.33 respectively (Orthoefer, 2005). Volume-weighted ($D_{[4,3]}$) and surface-weighted ($D_{[3,2]}$) mean diameters of the emulsion particles were determined as follow:

$$D_{[4,3]} = \frac{\sum n_i \cdot d_i^4}{\sum n_i \cdot d_i^3} \quad [1]$$

$$D_{[3,2]} = \frac{\sum n_i \cdot d_i^3}{\sum n_i \cdot d_i^2} \quad [2]$$

Where: n_i is the number of particles of diameter d_i .

The specific surface area (SSA) was calculated according to the following equation:

$$SSA = \frac{6 \cdot \Phi}{D_{[3,2]}} \quad [3]$$

Where: SSA is the specific surface area ($\text{m}^2 \text{g}^{-1}$), Φ is the dispersed-phase volume fraction, and $D_{[3,2]}$ is the surface-weighted mean diameter.

Additionally, to determine the width of the particle size distribution, the polydispersity index (Span) was calculated from the following equation:

$$Span = \frac{D_{0.9} - D_{0.1}}{D_{0.5}} \quad [4]$$

Being $D_{0.1}$, $D_{0.5}$ and $D_{0.9}$ the fractions of droplets with diameters smaller than 0.1, 0.5 and 0.9, respectively. The particle size distribution was measured immediately after preparation of emulsions and after 7 days of refrigerated storage at 4 °C. Measurements were carried out in duplicate.

2.4.3. Rheological behavior

Rotational rheological tests were carried out on a Discovery HR-1 controlled stress oscillatory rheometer (TA Instrument Inc., USA) using a concentric cylinders sensor system with 5923.44 μm gap between sensors. The samples were subjected to a logarithmic increasing shear rate with a continuous ramp from 1 to 500 s^{-1} in 120 s. Flow behaviour of emulsions was described by fitting the experimentally measured data to the Ostwald de Waele model (Steffe, 1996) as follow:

$$\tau = k \cdot D^n \quad [5]$$

Where: τ is the shear stress (Pa), D is the shear rate (s^{-1}), k is the flow consistency index (Pa s^n) and n is the flow behavior index (dimensionless). The apparent viscosity was calculated at 100 s^{-1} . All measurements were performed in duplicate at constant temperature (20 ± 0.5 °C).

2.4.4. Global emulsion stability

The emulsions were transferred into cylindrical glass test tubes immediately after the emulsification. They were subjected to quiescent storage at 4 °C for 7 days. The stability of the emulsions was determined by measurements with a Vertical Scan Analyzer Turbiscan MA 2000 (Formulation, Toulouse, France) according to Cabezas, Madoery, Diehl & Tomás (2012). This equipment allows the optical characterization of any type of dispersion using an electro-luminescent diode in the nearinfrared ($\lambda=850$ nm) as light source. The transmission and backscattering (BS) profiles as a function of the sample height (ca. 65 mm) were collected at room temperature (20 °C). Transmission is useful for analyzing non-opaque samples whereas backscattering is useful for analyzing opaque samples (such as emulsions) (Mengual, Meunier, Cayré, Puech, & Snabre, 1999). Any change due to a variation of the droplet size (flocculation, coalescence) or a local variation of the volume fraction (migration phenomena: creaming, sedimentation) is detected (Pan, Tomás, & Añón, 2002). Measurements were carried out immediately after preparation of the emulsions and after 7 days of refrigerated storage. Determinations were conducted at least in duplicate.

2.4.5. Quantification of accelerated oil phase separation

Freshly prepared emulsions (20 mL) were transferred into a 50 mL centrifugal plastic tube and centrifuged at 2600×g for 15 min at 25 °C (Borco, Model C28, Germany). After the test, the emulsions were separated into optically opaque “cream layer” at the top, a turbid “serum layer” at the middle, and a “precipitate layer” at the bottom. The total height of the emulsions (HE) and the height of the serum layer (HS) were measured with a Vernier caliper. Creaming index (CI) was calculated as follow:

$$CI = \left(\frac{HS}{HE} \right) * 100 \quad [6]$$

Measurements were carried out at least in duplicate.

2.5. Statistical analysis

Results were expressed as mean $\pm$ standard deviation and were analyzed by analysis of variance (ANOVA). Means were evaluated with the Tukey's HSD (Honestly Significant Difference) test for paired comparison, with a significance level $\alpha=0.05$, using the Statgraphics Plus version 5.1 software (Statgraphics, USA).

3. Results and Discussion

3.1. Characterization of raw materials

Fatty acid composition of the rice bran oil used in this work is shown in **Table 2**. This oil is a source rich in unsaturated fatty acids ($\approx 75\%$ w/w). Oleic and linoleic acids were the main mono- and poly-unsaturated fatty acids presented in the rice bran oil. On the other hand, palmitic and stearic acids were the most abundant saturated fatty acids ($\approx 20\%$ w/w). This fatty acid profile agrees with those described by Murali et al. (2017) and Pal & Pratap (2017). The theoretical iodine index and saponification value were 95 g I₂/g oil and 193 mg KOH/g oil respectively, being under the ranges established to the rice bran oil by the current legislation (FAO, 2015; Orthoefer, 2005).

Soybean meal had high protein content ($49.81 \pm 0.44\%$) and also had appreciable amounts of carbohydrates ($27.22 \pm 0.05\%$) and lipids ($6.45 \pm 0.01\%$). These results are in agreement with those reported by Al Loman & Ju (2016). Information about the chemical composition of soybean meal was taken into account to formulate the O/W emulsions with different ketogenic ratios (KR) and oil mass fractions (Φ_m), as shown in **Table 1**. It should be noted that the soybean meal used in this study did not present antitryptic activity and this is important since it is proposed as a raw material in the food emulsion development.

Table 2. Fatty acids profile of rice bran oil.

Fatty acid	Common name	Percentage (%)
C14:0	Myristic acid	$0,22 \pm 0,02$
C16:0	Palmitic acid	$16,70 \pm 1,11$
C16:1 N-7 CIS	Palmitoleic acid	$0,18 \pm 0,05$
C18:0	Stearic acid	$1,91 \pm 0,36$
C18:1 N-9 CIS	Oleic acid	$40,18 \pm 2,05$
C18:2 N-6 CIS	Linoleic acid	$31,51 \pm 1,63$
C18:3 N-3 CIS	α -Linolenic acid	$1,11 \pm 0,05$
C18:3 N-6 CIS	γ -Linolenic acid	$0,71 \pm 0,13$
C20:0	Arachidic acid	$0,13 \pm 0,01$
C20:1 N-9 CIS	Eicosenoic acid	$0,53 \pm 0,03$
C20:3 N-6 CIS	Dihomo- γ -linoleic acid	$0,30 \pm 0,14$
C24:0	Lignoceric acid	$0,46 \pm 0,18$

Reported values are means $\pm$ standard deviation ($n=2$).

3.2. Characterization of O/W emulsions

3.2.1. Initial characteristic of O/W emulsions

Table 3 shows the volume-weighted mean diameter ($D_{[4,3]}$), polydispersity index (Span), and specific surface area (SSA) of the O/W emulsions prepared after one cycle of homogenization. All freshly prepared emulsions showed multimodal particle size distributions (**Supplementary Figure**) with intermediate span values (2.4-6.8). The formulation and the number of homogenization cycles affected the particle size distributions in different ways. The emulsions with the lowest ketogenic ratio (KR =3:1) and oil mass fraction (Φ_m = 0.20) were characterized by $SSA = 0.42 \text{ m}^2 \text{ g}^{-1}$, $D_{[4,3]} \approx 30 \text{ }\mu\text{m}$, and $\text{span} = 2.8$. Increasing the oil mass fraction, a slight decrease in $D_{[4,3]}$ was observed, but their SSA and span were significantly increased. Emulsions with intermediate ketogenic ratio (KR=4:1) and $\Phi_m = 0.20$ and 0.25 presented the highest particle size ($D_{[4,3]} \approx 40 \text{ }\mu\text{m}$). A slight decrease in $D_{[4,3]}$ accompanied by an increase in SSA and span values was observed while the Φ_m increased to 0.30. These results suggests that, in general terms, increasing the oil mass fraction in the formulation produces emulsions composed by smaller particles with greater interfacial area, but with greater polydispersity. These results seem to be contradictory with those reported by other authors (McClements, 2016; Tadros, 2010), however it should be noted that in this work, all emulsions at fixed KR had the same oil to emulsifiers + stabilizers proportion, regardless of their Φ_m . On the other hand, the oil to emulsifiers + stabilizers proportion in emulsions increased with KR at fixed Φ_m . Thus, $D_{[4,3]}$ increased when KR augmented from 3:1 to 4:1. These results could be attributed to the increase in the emulsion oil concentration, in accordance with results reported by Tadros (2010) and McClements (2016). However, the 5:1 emulsion presented similar $D_{[4,3]}$ to those with KR=3:1. The microscopic appearance of O/W emulsions prepared after one homogenization cycle is shown in Fig. 1A.

Table 3. Specific surface area (SSA), De Broucker ($D_{[4,3]}$) mean diameter and the polydispersity index (Span) of particles of freshly prepared and stored (4°C, 7 days) rice bran oil-in-water emulsions formulated with different ketogenic ratios (KR = 3:1, 4:1 and 5:1 w/w) and oil mass fractions (Φ_m = 0.20, 0.25 and 0.30), obtained after one cycle of homogenization in a high-pressure homogenizer at 150 bar.

Emulsion formulation		Freshly prepared emulsions			Stored emulsions (4°C, 7 days)	
Ketogenic ratio (KR)	Oil mass fraction (Φ_m)	SSA ($m^2 g^{-1}$)	$D_{[4,3]}$ (μm)	Span	$D_{[4,3]}$ (μm)	Span
3:1	0.20	0.42 ± 0.04^b	$30.73 \pm 1.01^{c/Z}$	$2.83 \pm 0.02^{a/Z}$	$30.74 \pm 1.40^{e/Z}$	$4.91 \pm 0.06^{a/Y}$
	0.25	0.75 ± 0.03^d	$24.85 \pm 0.74^{a,b/Y}$	$4.83 \pm 0.02^{c/Z}$	$18.60 \pm 1.00^{b,c/Z}$	$22.34 \pm 0.96^{c/Y}$
	0.30	0.89 ± 0.03^e	$21.76 \pm 1.71^{a/Z}$	$6.80 \pm 0.41^{d/Z}$	$17.19 \pm 0.09^{a,b/Z}$	$19.86 \pm 1.72^{c/Y}$
4:1	0.20	0.30 ± 0.01^a	$42.38 \pm 1.55^{d/Y}$	$2.55 \pm 0.05^{a/Z}$	$24.27 \pm 2.11^{d/Z}$	$4.37 \pm 0.19^{a/Y}$
	0.25	$0.46 \pm 0.01^{b,c}$	$37.73 \pm 0.60^{d/Y}$	$2.44 \pm 0.05^{a/Z}$	$22.47 \pm 0.52^{c,d/Z}$	$5.21 \pm 0.44^{a/Y}$
	0.30	$0.52 \pm 0.02^{b,c}$	$29.71 \pm 2.39^{b,c/Z}$	$4.34 \pm 0.20^{b,c/Z}$	$25.31 \pm 1.24^{e/Z}$	$5.89 \pm 0.45^{a/Y}$
5:1	0.20	0.56 ± 0.03^c	$27.88 \pm 1.07^{b,c/Y}$	$2.81 \pm 0.02^{a/Z}$	$13.62 \pm 1.37^{a/Z}$	$16.80 \pm 0.52^{b/Y}$
	0.25	0.76 ± 0.02^d	$24.51 \pm 0.22^{a,b/Y}$	$3.70 \pm 0.19^{b/Z}$	$15.92 \pm 0.16^{a,b/Z}$	$5.31 \pm 0.47^{a/Y}$
	0.30	0.87 ± 0.04^e	$25.32 \pm 2.27^{a,b,c/Z}$	$2.82 \pm 0.03^{a/Z}$	$23.85 \pm 0.81^{d/Z}$	$3.84 \pm 0.54^{a/Y}$

Ketogenic ratio (KR) is defined as the ratio of grams of fat to grams of carbohydrate plus protein.

Reported values for each emulsion are means $\pm$ standard deviation. Different small letters in the same column indicate significant differences ($p < 0.05$) among the different emulsions for the same storage time, according to Tukey's test. Different capital letters in the same line indicate significant differences ($p < 0.05$) among the different refrigerated storage time for the same emulsion, according to Tukey's test.

In all freshly prepared emulsions, significant decreases in $D_{[4,3]}$ were observed increasing the number of homogenization cycles (from 1 to 5 cycles) (Table 4). This fact could be corroborated observing the microscopic appearance of the studied emulsions (Fig. 1B). Significant increases in SSA were also observed in the KR=3:1 and KR=4:1 emulsions while the number of homogenization cycles increased (Table 4). These emulsions prepared after five homogenization cycles were composed by smaller particles with greater interfacial area than those prepared after one cycle of homogenization. On the other hand, the opposite behaviour was observed in the SSA of KR=5:1 emulsions (Table 4). It could be attributed to depletion of emulsifier or loss of its functionality due to overprocessing (Jafari, Assadpoor, He, & Bhandari, 2008). It should be noted that these emulsions had the highest oil to surfactants + stabilizers proportion (and the highest KR). In particular, emulsions with the lowest KR and highest Φ_m (KR=3:1, Φ_m =0.30) had the smallest mean particle size ($D_{[4,3]} \approx 12 \mu m$) but the highest polydispersity index (span ≈ 22). On the other hand, emulsions with

intermediate KR and Φ_m (KR=4:1, Φ_m =0.25) presented the highest $D_{[4,3]} \approx 30 \mu\text{m}$. The particle size distributions of these O/W emulsions are shown in **Supplementary Figure**. These differences in particle size distributions could be important since it is believed that the droplet size is the most important factor determining such properties of the emulsion as rheology, shelf-life stability, colour and taste (Kowalska, 2016).

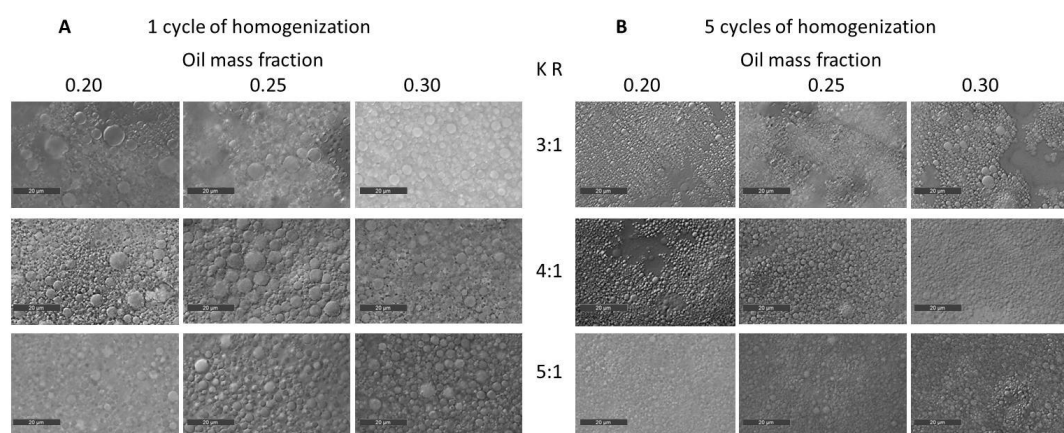


Figure 1: Optical microscope images (100X) of the rice bran oil-in-water emulsions prepared at different ketogenic ratios (KR = 3:1, 4:1 and 5:1 w/w) and oil mass fractions (Φ_m = 0.20, 0.25 and 0.30) obtained after one (**A**) or five (**B**) cycles of homogenization in a high-pressure homogenizer at 150 bar. Scale bar=20 μm . Ketogenic ratio (KR) is defined as the ratio of grams of fat to grams of carbohydrate plus protein.

Table 4. Specific surface area (SSA), De Broucker ($D_{[4,3]}$) mean diameter and the polydispersity index (Span) of particles of freshly prepared and stored (4°C, 7 days) rice bran oil-in-water emulsions formulated with different ketogenic ratios (KR = 3:1, 4:1 and 5:1 w/w) and oil mass fractions (Φ_m = 0.20, 0.25 and 0.30), obtained after five cycles of homogenization in a high-pressure homogenizer at 150 bar.

Emulsion formulation		Freshly prepared emulsions			Stored emulsions (4°C, 7 days)	
Ketogenic ratio (KR)	Oil mass fraction (Φ_m)	SSA ($m^2 g^{-1}$)	$D_{[4,3]}$ (μm)	Span	$D_{[4,3]}$ (μm)	Span
3:1	0.20	$0.63 \pm 0.04^{b,c}$	$18.56 \pm 1.36^{c,d/Z}$	$3.47 \pm 0.10^{b,c/Z}$	$15.29 \pm 0.47^{c/Z}$	$4.11 \pm 0.22^{a/Z}$
	0.25	0.82 ± 0.04^d	$19.77 \pm 0.47^{d/Y}$	$4.45 \pm 0.09^{d/Z}$	$17.28 \pm 0.62^{d/Z}$	$5.37 \pm 0.21^{a/Y}$
	0.30	1.24 ± 0.01^e	$11.60 \pm 0.07^{a/Y}$	$22.56 \pm 0.64^{f/Z}$	$10.78 \pm 0.07^{a/Z}$	$23.25 \pm 0.05^{d/Z}$
4:1	0.20	$0.71 \pm 0.02^{c,d}$	$14.12 \pm 0.10^{a,b/Z}$	$4.00 \pm 0.05^{c,d/Z}$	$12.80 \pm 0.68^{b/Z}$	$17.38 \pm 1.16^{c/Y}$
	0.25	$0.75 \pm 0.02^{d,e}$	$30.29 \pm 2.16^{e/Y}$	$2.84 \pm 0.09^{a,b/Z}$	$21.69 \pm 0.07^{e/Z}$	$5.18 \pm 0.16^{a/Y}$
	0.30	$0.77 \pm 0.01^{d,e}$	$17.64 \pm 0.04^{b,c,d/Z}$	$5.69 \pm 0.19^{e/Y}$	$17.10 \pm 0.55^{d/Z}$	$5.31 \pm 0.36^{a/Z}$
5:1	0.20	0.48 ± 0.02^a	$15.84 \pm 1.49^{b,c,d/Z}$	$2.37 \pm 0.04^{a/Z}$	$12.08 \pm 0.43^{a,b/Z}$	$4.04 \pm 0.21^{a/Y}$
	0.25	0.60 ± 0.01^b	$14.63 \pm 0.39^{a,b,c/Y}$	$4.45 \pm 0.06^{d/Z}$	$10.52 \pm 0.10^{a/Z}$	$11.28 \pm 0.06^{b/Y}$
	0.30	0.83 ± 0.01^d	$19.21 \pm 0.68^{d/Z}$	$3.30 \pm 0.05^{b,c/Z}$	$18.29 \pm 0.03^{d/Z}$	$3.81 \pm 0.18^{a/Z}$

Ketogenic ratio (KR) is defined as the ratio of grams of fat to grams of carbohydrate plus protein.

Reported values for each emulsion are means $\pm$ standard deviation. Different small letters in the same column indicate significant differences ($p < 0.05$) among the different emulsions for the same storage time, according to Tukey's test. Different capital letters in the same line indicate significant differences ($p < 0.05$) among the different refrigerated storage time for the same emulsion, according to Tukey's test.

3.2.2. Rheological behavior of O/W emulsions

The experimental flow curves of rice bran oil-in-water emulsions were satisfactorily fitted to the Ostwald de Waele model ($r^2 > 0.99$). The resulting flow behavior index (n) and consistency index (k) are presented in **Figure 2.A** and **Figure 2.B**, respectively. All studied O/W emulsions showed a typical shear-thinning effect for pseudoplastic materials ($n < 1$). Pseudoplastic flow is the most common type of nonideal behavior exhibited by food emulsions (McClements, 2016). This rheological behavior was accentuated by the increase in Φ_m at a fixed KR, as observed by the decline in the flow behavior index (n) and the increase of the consistency index (k), especially in KR 4:1 and KR 5:1 emulsions.

The apparent viscosities of studied O/W emulsions decreased with increased shear rate due to being pseudoplastic fluids. It is known that the viscosity of concentrated emulsions ($0.05 < \Phi < 0.49$) is the result of the balance between Brownian motion, hydrodynamic effects and colloids interactions (Tadros, 2010; McClements, 2016). At low shear rates, the particles have

a random distribution because of their Brownian motion and the hydrodynamic forces are not large enough to disrupt the bonds holding the particles together, and so the flocs act like rigid particles with a fixed size and shape, resulting in relatively high viscosity. As the shear rate is increased, the hydrodynamic forces become large enough to cause the flocs to become deformed and disrupted, and so the particles become more ordered along the flow lines to form strings or layers of particles. As a consequence, there is a reduction in their effective volume fraction and less resistance to fluid flow, which causes a decrease in emulsion viscosity. The viscosity reaches a constant value at high shear rates, either because all of the flocs are completely disrupted so that only individual droplets remain, or because the number of flocculated droplets remains constant since the rate of floc formation is equal to that of floc disruption (McClements, 2016).

The apparent viscosities of the studied O/W emulsions at 100 s^{-1} of shear rate, considered as a typical value of food processes such as flow through a pipe, mixing and stirring, chewing and swallowing, pouring from a bottle (Steffe, 1996), are shown in **Figure 2.C**. Theoretically, the viscosity of a fluid emulsion is directly proportional to the viscosity of the continuous phase. Consequently, any alteration in the rheology of the continuous phase will have a corresponding influence on the rheology of the whole emulsion (McClements, 2016). In the present work, the apparent viscosity of studied O/W emulsions decreased as the ketogenic ratio increased at fixed oil mass fractions (**Figure 2.C**), due to it is diminished the content of solids presents in the continuous phase (carbohydrates and proteins from soybean meal) (see **Table 1**). On the other hand, as the oil mass fraction increased at a fixed KR, apparent viscosity exhibited an increasing trend (**Figure 2.C**) because the droplets increase the rate of energy dissipation due to friction associated with the fluid flow around them. McClements (2016) reported that emulsion viscosity increases linearly with Φ at low droplet concentrations, but viscosity increase is steeper at higher droplet concentrations. Above a critical disperse-phase volume fraction, the particles are packed so closely together that they cannot easily flow past each other. Above this droplet concentration, the emulsion gains gel-like properties. All the O/W emulsions studied in this work exhibited a higher internal rheological resistance, resulting in a storage modulus, G' , that is greater than its loss modulus, G'' ; and $\tan \delta < 1$ (data not shown) which indicates predominantly elastic behavior (Capitani et al., 2015).

Increasing the number of homogenization cycles (from 1 to 5 cycles) also increased the apparent viscosity of the resulting O/W emulsions (**Figure 2.C**). These increases in apparent viscosity are more noticeable for $\Phi_m=0.25$ and 0.30 emulsions, while the formulations with

the lowest oil mass fraction ($\Phi_m=0.20$) did not show significant differences in their apparent viscosities. Differences in the rheological behavior of the O/W emulsions, attributed to differences in their formulation, particle size distributions as well as to interactions between particles, could be condition their processing and determine, among other factors, their stability.

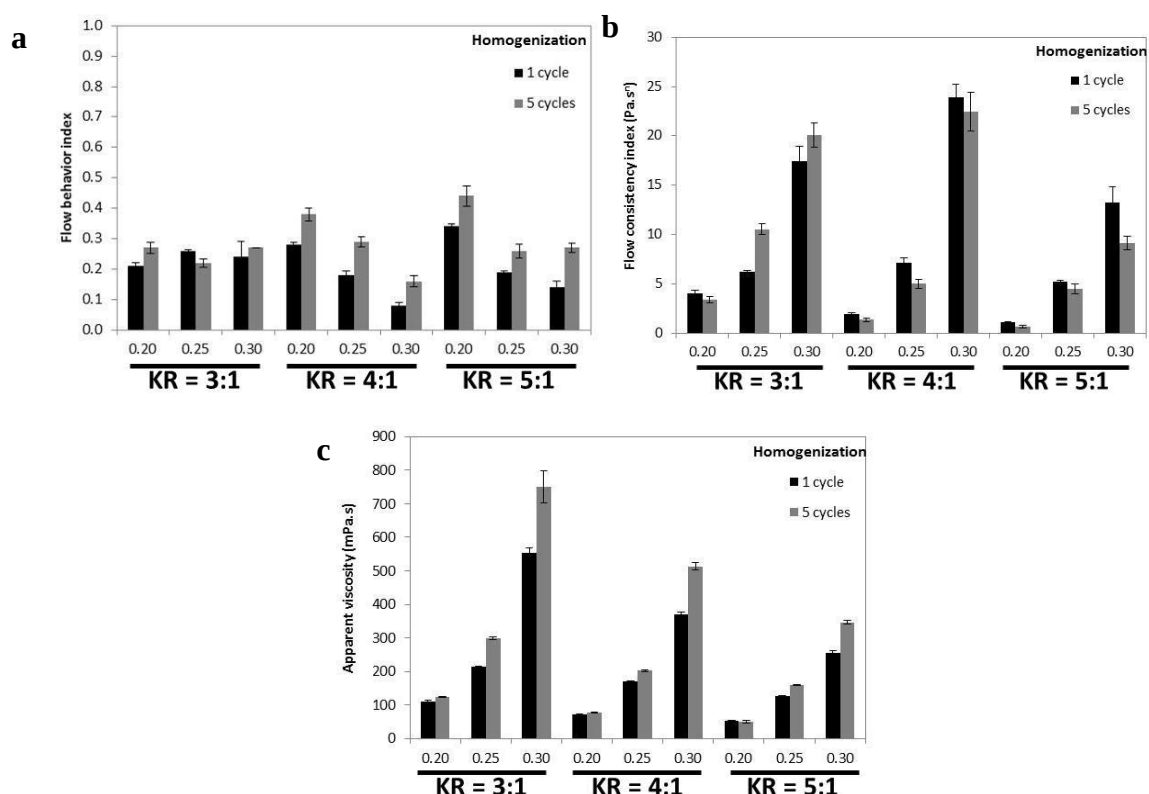


Figure 2: (A) Flow behavior index (n), (B) flow consistency index (k) and (C) apparent viscosity at 100 s^{-1} of freshly prepared rice bran oil-in-water emulsions formulated with different ketogenic ratios (KR = 3:1, 4:1 and 5:1 w/w) and oil mass fractions ($\Phi_m = 0.20, 0.25$ and 0.30), obtained after one (■) or five (▒) cycles of homogenization in a high-pressure homogenizer at 150 bar. Ketogenic ratio (KR) is defined as the ratio of grams of fat to grams of carbohydrate plus protein.

3.2.3. Stability of O/W emulsions

Table 3 and **4** also present the $D_{[4,3]}$, Span, and SSA of the stored O/W emulsions prepared after one or five homogenization cycles, respectively. In all the emulsions, some changes in $D_{[4,3]}$ and span values were observed after refrigerated storage (4°C , 7 days). Decreases in $D_{[4,3]}$ and increases in span were observed in most of the emulsions prepared by one homogenization cycle (**Table 3**). Meanwhile, these changes were less noticeable in the

emulsions obtained after five homogenization cycles (**Table 4**), suggesting that these emulsions had greater stability than those prepared in one cycle.

The global O/W emulsion stability was determined by comparing the percentages of mean backscattered light (%BS) of freshly prepared and stored (4°C, 7 days) emulsions. These results are shown in **Table 5**. All freshly prepared O/W emulsions showed homogeneous %BS throughout the tube height (%BS≈82.5-89.6%) regardless of the formulation studied. It is known that %BS depends on the oil droplet size and concentration (Cabezas, Madoery, Diehl, & Toma's, 2012). The O/W emulsions obtained after five cycles of homogenization had smaller particle size and higher %BS than those prepared after one homogenization cycle, but these differences did not become statistically significant. After seven days of quiescent refrigerated storage, all O/W emulsions exhibited high global stability, recording no significant changes in %BS. However, the stored emulsions with the highest KR and the lowest Φ_m (KR=5:1, $\Phi_m=0.20$) showed low %BS values, but high standard deviation and this implies that these emulsions do not present a homogeneous aspect. In these samples, the %BS =60-70% in the lower part of the tube (10-15 mm height) while %BS=82-85% in the upper part of the tube (55-60 mm height). This fact indicates the occurrence of gravitational separation. The migration of oil droplets from the bottom to the top of the emulsion sample could lead to a progressive reduction of oil droplet concentration at the bottom of the emulsion sample, and a concurrent decrease in the intensity of back- scattered light. Julio et al. (2016) reported that the high viscosity of chia oil-in-water emulsions reduced the mobility of the oil droplets and hence their upward movement according to the Stokes' law, resulting in stable systems. In the present work, the emulsions that showed the lowest apparent viscosity (KR=5:1, $\Phi_m=0.20$; **Figure 2.C**) were those that only showed gravitational separation. Even so, there was no evidence of phase separation.

The stability to coalescence can be determined by subjecting the O/W emulsions to a centrifugal acceleration for a specific length of time (Tcholakova, Denkov, Ivanov, & Campbell, 2006; van Aken & van Vliet, 2002). In these conditions, the oil droplets tend to move toward the axis of rotation of the centrifuge because of the difference in densities between oily and aqueous phases. Initially, the droplets form a cream layer where they are forced into proximity to one another, but they retain their original size. However, if the centrifugal force is sufficiently high, the droplets will be forced into even closer proximity, and the interfacial layers surrounding them will be ruptured, thereby releasing a layer of oil on top of the emulsion (McClements, 2016). Creaming index is shown in **Table 6**. All the emulsions obtained after one homogenization cycle showed CI≈0.60-0.74, but it could not be

related to their formulations. However, significant decreases in CI were observed increasing the number of homogenization cycles (from 1 to 5 cycles) (**Table 6**), due to this process diminished the mean size of emulsion particles (**Figure 1, Table 3 and 4**). These results indicate that the emulsions prepared after five homogenization cycles presented greater stability to the coalescence (evidenced by lower CI) than those prepared in one cycle. Nevertheless, accelerated coalescence tests should be treated with caution because they may not always give a good correlation with the long-term coalescence stability of an emulsion.

Table 5. Backscattering (%BS) of freshly prepared and stored (4°C, 7 days) rice bran oil-in-water emulsions prepared at different ketogenic ratios (KR = 3:1, 4:1 and 5:1 w/w) and oil mass fractions (Φ_m = 0.20, 0.25 and 0.30) obtained after one or five cycles of homogenization in a high-pressure homogenizer at 150 bar.

Emulsion formulation		1 cycle of homogenization		5 cycles of homogenization	
Ketogenic ratio (KR)	Oil mass fraction (Φ_m)	Freshly prepared emulsion	Stored emulsions (4°C, 7 days)	Freshly prepared emulsion	Stored emulsions (4°C, 7 days)
3:1	0.20	82.5 ± 5.1 ^{a/Z}	79.1 ± 5.0 ^{a/Z}	87.4 ± 5.3 ^{a/Z}	84.2 ± 4.7 ^{a/Z}
	0.25	83.5 ± 3.2 ^{a/Z}	82.7 ± 2.9 ^{a/Z}	88.0 ± 2.0 ^{a/Z}	85.9 ± 1.5 ^{a/Z}
	0.30	84.0 ± 3.4 ^{a/Z}	82.0 ± 4.0 ^{a/Z}	87.0 ± 1.8 ^{a/Z}	84.5 ± 1.7 ^{a/Z}
4:1	0.20	85.8 ± 4.7 ^{a/Z}	81.4 ± 4.6 ^{a/Z}	87.6 ± 5.3 ^{a/Z}	81.5 ± 7.3 ^{a/Z}
	0.25	85.6 ± 4.8 ^{a/Z}	81.7 ± 4.5 ^{a/Z}	89.6 ± 4.7 ^{a/Z}	86.7 ± 4.6 ^{a/Z}
	0.30	84.6 ± 3.7 ^{a/Z}	82.5 ± 3.5 ^{a/Z}	88.0 ± 3.6 ^{a/Z}	85.7 ± 3.3 ^{a/Z}
5:1	0.20	82.9 ± 5.3 ^{a/Z}	71.0 ± 15.6 ^{a/Z}	86.4 ± 5.1 ^{a/Z}	79.2 ± 11.7 ^{a/Z}
	0.25	86.4 ± 5.0 ^{a/Z}	81.4 ± 7.1 ^{a/Z}	86.4 ± 6.9 ^{a/Z}	83.7 ± 6.3 ^{a/Z}
	0.30	86.1 ± 4.7 ^{a/Z}	82.7 ± 3.7 ^{a/Z}	88.7 ± 4.8 ^{a/Z}	84.6 ± 4.8 ^{a/Z}

Ketogenic ratio (KR) is defined as the ratio of grams of fat to grams of carbohydrate plus protein.

Reported values for each emulsion are means ± standard deviation ($n=2$). Different small letters in the same column indicate significant differences ($p<0.05$) among the different emulsions for the same storage time, according to Tukey's test. Different capital letters in the same line indicate significant differences ($p<0.05$) among the different refrigerated storage time for the same emulsion, according to Tukey's test.

Table 6. Creaming index of rice bran oil-in-water emulsions prepared at different ketogenic ratios (KR = 3:1, 4:1 and 5:1 w/w) and oil mass fractions (Φ_m = 0.20, 0.25 and 0.30) obtained after one or five cycles of homogenization in a high-pressure homogenizer at 150 bar.

Emulsion formulation		Number of cycles of homogenization	
Ketogenic ratio (KR)	Oil mass fraction (Φ_m)	1 cycle	5 cycles
3:1	0.20	$0.74 \pm 0.01^{d/Y}$	$0.54 \pm 0.03^{a/Z}$
	0.25	$0.76 \pm 0.01^{d/Y}$	$0.59 \pm 0.04^{a/Z}$
	0.30	$0.68 \pm 0.01^{c/Y}$	$0.50 \pm 0.02^{a/Z}$
4:1	0.20	$0.61 \pm 0.01^{a,b/Z}$	$0.59 \pm 0.01^{a/Z}$
	0.25	$0.65 \pm 0.01^{a,b,c/Y}$	$0.57 \pm 0.04^{a/Z}$
	0.30	$0.60 \pm 0.02^{a/Y}$	$0.53 \pm 0.04^{a/Z}$
5:1	0.20	$0.66 \pm 0.01^{b,c/Y}$	$0.58 \pm 0.01^{a/Z}$
	0.25	$0.60 \pm 0.01^{a/Z}$	$0.57 \pm 0.04^{a/Z}$
	0.30	$0.62 \pm 0.02^{a,b/Y}$	$0.50 \pm 0.01^{a/Z}$

Ketogenic ratio (KR) is defined as the ratio of grams of fat to grams of carbohydrate plus protein.

Reported values for each emulsion are means $\pm$ standard deviation. Different small letters in the same column indicate significant differences ($p < 0.05$) among the different emulsions, according to Tukey's test. Different capital letters in the same line indicate significant differences ($p < 0.05$) among the different number of homogenization cycles for the same emulsion formulation, according to Tukey's test.

4. Conclusions

Rice bran oil-in-water emulsions stabilized with proteins and polysaccharides from soybean meal were developed. The formulation and emulsifying processing affected the particle size distribution, rheological behaviour and stability of the resulting O/W emulsions. At a fixed ketogenic ratio, all emulsions had the same oil to emulsifiers + stabilizers proportion but increasing their oil mass fraction resulted in systems composed by smaller particles with greater interfacial area, and apparent viscosity. The same effect was observed by increasing the number of homogenization cycles. On the other hand, increasing the ketogenic ratio (at a fixed oil mass fraction) diminished its apparent viscosity due to the decrease in the content of solids of the continuous phase. Most of the studied O/W emulsions were stable for seven days of quiescent refrigerated storage. Only the stored emulsions with the highest ketogenic ratio and the lowest oil mass fraction presented gravitational separation but no phase separation. Emulsions prepared after five homogenization cycles presented greater stability to the coalescence than those prepared in one cycle. These O/W emulsions could be used as the basis of food products able to be included in ketogenic diets.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2019.108809>.

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