

EMULSION PROCESS OPTIMIZATION OF BRAZIL NUT OIL WITH CHEESE WHEY FOR ENCAPSULATION

TANISE PILETTI¹, ADRIANI CRISTINA FELIPE dos SANTOS¹, DANIEL NEUTZLING LEHN²
and CLAUDIA FERNANDA VOLKEN de SOUZA³

¹ Centro Universitário UNIVATES, Laboratório de Biotecnologia de Alimentos

² Centro Universitário UNIVATES, Centro de Ciência Exatas e Tecnológicas

³ Centro Universitário UNIVATES, Programa de Pós-graduação em Biotecnologia

E-mail: tanisepiletti@hotmail.com

ABSTRACT – Brazil nut is the cornerstone for sustainable economic exploitation of the Amazon region. Its nutritional importance relies specially on its lipid part, which represents around 70% of its nut, and it is constituted of mainly polyunsaturated fatty acids. Brazil nut oil is a rich source of the omega family, essential for the perfect functioning of the human body. Cheese whey is a valuable nutritive product with versatile functional properties. At the same time, cheese whey is one of the main sources of organic contamination in the dairy industry. The objective of this work was to optimize the emulsion process of Brazil nut oil and cheese whey for future encapsulation, in order to avoid degradation and maximize its use in the food industry. An experimental design formulation was applied to prepare emulsions containing Brazil nut oil, cheese whey, Arabic gum and lecithin where time of agitation, oil and whey concentrations were the independent variables. Emulsions were analyzed according to its stability under different storage conditions, microscopic structure and turbidity. Results indicate that emulsions stored at 4 °C showed the best stability indexes. Emulsion containing 17.5% w/w of oil and 30% w/w of cheese whey concentrations was the most stable. Results demonstrate that it is possible to emulsify Brazil nut oil with cheese whey in order to encapsulate.

1. INTRODUCTION

Brazil nut (*Bertholletia excelsa* H. B. K.) is widely recognized as the cornerstone for sustainable economic exploitation of the Amazon Forest (Silvertown, 2004). It is perceived as one of the most nutritious human foods among all oleaginous seeds, due to its high levels of unsaturated lipids and essential minerals, specially selenium, an important antioxidant, associated to the cholesterol-lowering effect and to the prevention of heart disease and cancer. Around 70% of the nuts constituents are oil, composed of a complex mixture of triglycerides which the main ones are palmitic, oleic and linoleic. The fatty acid profile of the Brazil nut oil is an important source of the omega series: ω -3, ω -6 and ω -9. Many studies have shown that the consumption of these polyunsaturated fatty acids elicit numerous health benefits that help the proper functioning of the human body (Yang, 2009).

Cheese whey is a byproduct of the dairy industry. Around 85-95% of the milk volume used during the cheese making process results in whey which retains 55% of milk nutrients, that contain in

its most part lactose (4.5-5% w/v), soluble proteins (0.6-0.8% w/v), lipids (0.4-0.5% w/v) and mineral salts (8-10% of dried extract). The estimated worldwide production of cheese whey is around 145×10^6 ton/year, half of which is not treated, but discarded as effluent. Therefore, at the same time that cheese whey represents a valuable and nutritive product, it also represents an environmental problem due to its high volumes produced and its high organic load, constituting one of the main sources of organic contamination in the dairy industry (Siso, 1996).

Encapsulation is a technology in which a physical barrier (wall material) is applied to protect the bioactive components (active agents) of a system against undesirable environmental conditions (Nedovic *et al.*, 2013). Considering that Brazil nut oil is rich in polyunsaturated fatty acids and that unsaturated molecules may easily degrade and lose their sensory and nutritional values, encapsulation is a technique that can be applied to protect those unsaturated fatty acids, increasing their shelf life (Aghbashlo *et al.*, 2012). Even though many techniques to encapsulate food ingredients have been developed, spray drying is the most common one used in the food industry (Gharsallaoui *et al.*, 2007). The main wall materials used in the food industry are polysaccharides, starches, celluloses, gums and proteins, which can be used alone or in combination (Turchiuli *et al.*, 2014).

In order to encapsulate oil into matrixes of wall material using the spray drying technique it is first necessary to go through an emulsification process which has to be carefully designed to enhance the oil encapsulation. Emulsion is a dispersion of one liquid in another. This type of dispersion system is unstable by nature unless an amphiphilic substance is present in the interface of the two phases, such as proteins that spontaneously migrate to an oil-aqueous interface (Damodaran *et al.*, 2010). Eventually, an emulsion will physically destabilize and undergo phase separation. The phenomena that can be observed are: flocculation, creaming, sedimentation, Ostwald ripening, coalescence (Bendjaballah *et al.*, 2010). In order to facilitating the formation and enhancing the stability of emulsions, emulsifiers can be added to the formula. Gums and lecithins are the most commonly used emulsifiers for food processing. They reduce the oil-water interfacial tensions and form a protective layer around the oil droplets to prevent them from aggregating (Chuah *et al.*, 2009).

The processing of emulsions can be better controlled by means of an experimental design. One advantage of such a system is the possibility to evaluate individual effect of each parameter adopted on the emulsification process and the significant interactions among them (Bendjaballah *et al.*, 2010). Another advantage is the reduction of the number of tests or their repetition, enhancing the quality of the information obtained. As the interactions amongst the independent variables are analyzed simultaneously, it is possible to verify and quantify their synergetic and opposite effect. This methodology is a fundamental and indispensable tool in the search of consistent results and optimal processes (Rodrigues and Lemma, 2009).

The objective of this work was to study the influence of Brazil nut oil and cheese whey concentrations on the formation and stability of emulsions, in the search of an optimized emulsion process for future encapsulation by spray drying. Stability under different storage conditions, microstructure and turbidity of emulsions were analyzed as responses.

2. MATERIALS AND METHODS

2.1. Materials

Brazil nut oil was purchased from Pазze Indústria de Alimentos Ltda (Panambi/RS, Brazil). Cheese whey powder was donated by BRF S.A. (Lajeado/RS, Brazil). Arabic gum was purchased from Synth (São Paulo/SP, Brazil). Soy lecithin was purchased from Bremil (Lajeado/RS, Brazil). Citric acid was acquired in a local store. Sodium Dodecyl Sulphate (SDS) was bought from Vetec (Rio de Janeiro/RJ, Brazil).

2.2. Experimental Design

Emulsions were systematically carried out accordingly to a rotatable central composite design (2^3), considering three factors (independent variables): agitation time (10-60 minutes), oil (10-30% w/w) and cheese whey powder (2-30% w/w) concentrations. Five levels of each variable were chosen for the trials, including the central point and two axial points, giving a total of 17 combinations (Table 1). Arabic gum (2% w/w) and soy lecithin (0.5% w/w) concentrations were kept constant through all the experiments. Distilled water was used to complete 100% w/w.

Table 1 – Experimental design for emulsion preparation.

Tests	Coded setting levels			Actual levels			Creaming Index (CI) (%)		
	x_1	x_2	x_3	X_1	X_2	X_3	4 °C	25 °C	55 °C
1	-1.00	-1.00	-1.00	20	10	8	72.21	72.21	79.31
2	-1.00	-1.00	1.00	20	10	24	37.73	57.40	55.55
3	-1.00	1.00	-1.00	20	25	8	40.67	57.62	55.93
4	-1.00	1.00	1.00	20	25	24	00.00	07.14	08.62
5	1.00	-1.00	-1.00	50	10	8	67.79	68.42	67.24
6	1.00	-1.00	1.00	50	10	24	33.33	55.35	58.92
7	1.00	1.00	-1.00	50	25	8	32.20	58.33	53.44
8	1.00	1.00	1.00	50	25	24	00.00	10.71	10.52
9	-1.68	0.00	0.00	10	17.5	16	53.70	58.62	54.38
10	1.68	0.00	0.00	60	17.5	16	35.59	59.32	65.00
11	0.00	-1.68	0.00	35	5	16	71.92	66.66	68.96
12	0.00	1.68	0.00	35	30	16	01.66	38.33	28.81
13	0.00	0.00	-1.68	35	17.5	2	65.51	69.49	67.79
14	0.00	0.00	1.68	35	17.5	30	00.00	03.63	00.00
15	0.00	0.00	0.00	35	17.5	16	37.28	58.92	62.50
16	0.00	0.00	0.00	35	17.5	16	42,85	60.71	60.71
17	0.00	0.00	0.00	35	17.5	16	36.66	57.62	55.73

x_1 , x_2 and x_3 are coded values. X_1 , X_2 and X_3 are the actual values.

X_1 = time (min); X_2 = oil (% w/w); X_3 = cheese whey powder (% w/w)

2.3. Preparation of Emulsions

Arabic gum was dispersed in distilled water at 60 °C under magnetic stirring for 3 minutes. The solution was subsequently cooled to 20 °C before the addition of the cheese whey powder (2-30% w/w) under magnetic stirring for 3 minutes. The solution was placed in a cold shaker (MA 830, Marconi) at 300 rpm for 12 h at 20 °C to ensure complete hydration. The resulting mixture was adjusted to pH 4.2 using citric acid 50% w/v. The oil phase containing the Brazil nut oil (10-30% w/w) and the soy lecithin was then blended with the above aqueous solution using a stirring-type homogenizer (Heidolph Diax 900, fitted with a 10 G homogenizer tool), under a constant speed rotation at 20,000 rpm in different agitation times (10-60 min). During the homogenization process, the mixture was kept under ice-water bath, at a controlled temperature around 1 ± 0.5 °C.

2.4. Stability under Storage

After each emulsion test, three test tubes were filled with 10 g of samples immediately after homogenization. Subsequently, the tubes were tightly capped to prevent evaporation and the height of the total emulsion was measured (centimeters units). One tube was stored vertically at 4 °C, one at 25 °C, and one at 55 °C, and all tubes were observed at time + 24 h when a new measurement was taken. The stability of emulsions against creaming or separation was determined visually from the height of serum. The extent of creaming was characterized and computed for each emulsion using the following equation:

$$CI\% = \frac{H_s}{H_g} \times 100 \quad (1)$$

where *CI* is the creaming index, *H_s* is the height of the serum layer, and *H_g* is the total height of the emulsion.

2.5. Microstructure Analysis

The microstructure of the emulsions was analyzed using an optical microscope (Leica DM 500), right after homogenization at room temperature. In some emulsions clumping of particles was apparent, so they were diluted 1:2 from the original concentration to avoid the particles from clumping together. A drop of the emulsion (both diluted and undiluted) was placed on a microscope slide and covered with a cover slip. Subsequently, it was observed at an objective magnification of 40X. Images of the emulsion structures were captured using digital image processing software (Leica Application Suite EZ). The particle sizes were later compared visually from the images.

2.6. Measurement of Turbidity

The measurement of turbidity was conducted according to the method described by Pearce and Kinsella (1978). A volume of 0.1 mL of each emulsion sample was diluted with 4.9 mL of 1 g L⁻¹ solution of sodium dodecyl sulphate (SDS), right after emulsification process. The absorbance of the diluted emulsions was determined at a wavelength of 500 nm in a spectrophotometer (SP-2000UV, Bel Photonics). Four replicates of each measurement were made. Emulsion stability (ES) was estimated by the durability of emulsion over 10 minutes, and expresses as

$$ES (\%) = \frac{Abs_{10min}}{Abs_{0min}} \times 100 \quad (2)$$

3. RESULTS AND DISCUSSION

Table 1 shows the emulsions stability obtained by observing the CI of the 17 tests after a period of 24 h of storage at different temperatures. Lower CI indicates higher emulsion stability. After homogenization, during storage, emulsions tend to separate into different layers. The upper part is the oil-rich phase, known as the creaming layer, and the lower part is the water-rich phase, known as the serum layer. It is possible to observe, by the CI, that temperature greatly influences the stability of emulsions. The ones stored at the lowest temperature showed a more stable character. It is also possible to observe, analyzing the index and the oil-whey composition of the emulsions (Table 1) that the samples containing the highest values of whey and oil were the ones kept stable for longer period of time: 25% w/w oil, 24% w/w cheese whey; and 17.5% w/w oil, 30% w/w cheese whey. It is valuable to observe that emulsion Tests 4, 8 and 14 stored at 4 °C were stable for a period superior of a month.

CI values were determined at 4, 25 and 55 °C, being the lowest values of this index observed at 4 °C. Therefore, the regression equations were constructed considering this storage temperature. The significance of each regression coefficient was determined by *t*-values and *p*-values listed in Table 2. The *p*-values for the negative coefficient for the linear effect of oil concentration ($p_{x_2} = 0.0025$) and the negative coefficient for the linear effect of cheese whey concentration ($p_{x_3} = 0.0025$) were highly significant. These results indicate that CI of Brazil nut oil in storage temperature at 4 °C decreases with the increase of oil and/or cheese whey concentrations. Therefore, the emulsion stability of Brazil nut oil in storage temperature at 4 °C increases with the increasing of the concentrations of those emulsion components. The model was simplified by the elimination of statistically insignificant terms. Then, the model should be reduced to:

$$Y = 39.0457 - 18.7693 x_2 - 18.4418 x_3 \quad (3)$$

where: *Y* is the predicted response to the CI (%) of Brazil nut oil in storage temperature at 4 °C, x_2 the oil concentration (% w/w) and x_3 is the cheese whey concentration (% w/w) as coded settings.

Statistics of the model was checked by the Fisher's test for analysis of variance (ANOVA). The computed F-value (106.81) was highly significant ($p < 0.0001$). The determination coefficient ($R^2 = 0.98$) implies that the sample variation of 98% for CI of Brazil nut oil in storage temperature at 4 °C is attributed to the independent variables, and can be explained by the model; the value of *R* (0.99) suggests an excellent representation of the process model and a good correlation between the experimental results and the theoretical values predicted by the model equation. This shows that equation 3 provides a suitable model to describe emulsion process of Brazil nut oil in storage temperature at 4 °C. In Figure 1 it is depicted the shape contour of oil concentration versus cheese whey concentration, showing that CI of Brazil nut oil in storage temperature at 4 °C tends to decrease with both high oil and cheese whey concentrations. The polynomial model gives the optimal levels for the three process variables to be: agitation time of 60 min, Brazil nut oil concentration of 30% w/w,

and cheese whey concentration of 30% w/w, with a predicted lowest CI of zero.

Table 2 – Effect and coefficient estimates by the regression model for optimization of emulsion process of Brazil nut oil in storage temperature at 4 °C

Independent variables	Effect	Coefficient	t-value	p-value
Intercept	39.0457	39.0457	19.6507	0.0026
x_1	-7.0161	-3.5080	-3.7595	0.0640
x_1, x_1	2.9237	1.4619	1.4234	0.2906
x_2^*	-37.5387	-18.7693	-20.1149	0.0025
x_2, x_2	-2.6271	-1.3135	-1.2790	0.3292
x_3^*	-36.8837	-18.4418	-19.7639	0.0025
x_3, x_3	-5.4555	-2.7278	-2.6560	0.1173
x_1, x_2	0.1250	0.0625	0.0513	0.9638
x_1, x_3	2.1250	1.0625	0.8715	0.4754
x_2, x_3	-0.9750	-0.4875	-0.3999	0.7279

x_1 , x_2 and x_3 are coded values of variables time (min), oil concentration (% w/w) and cheese whey concentration (% w/w), respectively. * Statistically significant at 99% of confidence level.

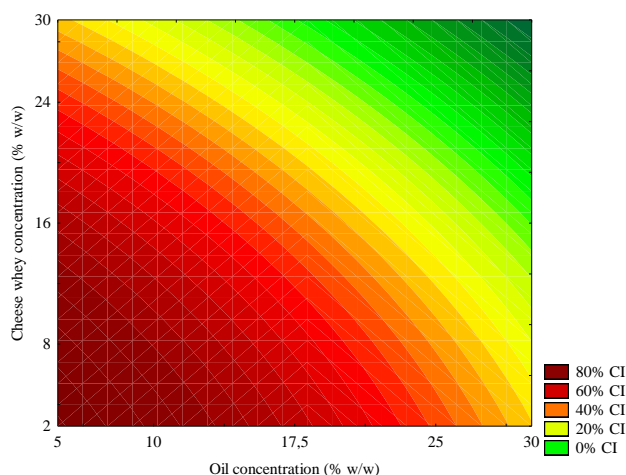


Figure 1 - Contour plot for the effect of oil concentration and cheese whey concentration on CI of Brazil nut oil in storage temperature at 4 °C. The variable that is not plotted is fixed at zero level.

The average particle size was smaller in the emulsions that presented a more stable character. This result comes in agreement with a generally accepted principle that indicates that smaller emulsions droplets are more physically stable than larger emulsions droplets. In other words, the instability of the system is the result of the formation of large particle size which, in turn, facilitates the coalescence process (Klaypradit and Huang, 2008). From the microscopic images of the emulsions droplets it is possible to observe that most of the samples containing the highest concentrations of cheese whey and the lowest concentrations of oil were the ones that presented the smallest particle sizes (Figure 2). Bylaite *et al.* (2001) studying the emulsification properties of caraway oil with whey

proteins and lecithin also found that the droplet size decreased with the increasing protein and lecithin concentrations. Besides, Tonon *et al.* (2011), in their studies of the influence of emulsion composition on the microencapsulation of flaxseed oil, concluded that a higher concentration of oil resulted in a lower emulsion viscosity and led to a larger droplet size what may influence emulsion stability. The same phenomena could be observed in most of the samples of this study with certain differences among them related probably to the different time of agitation they were submitted.

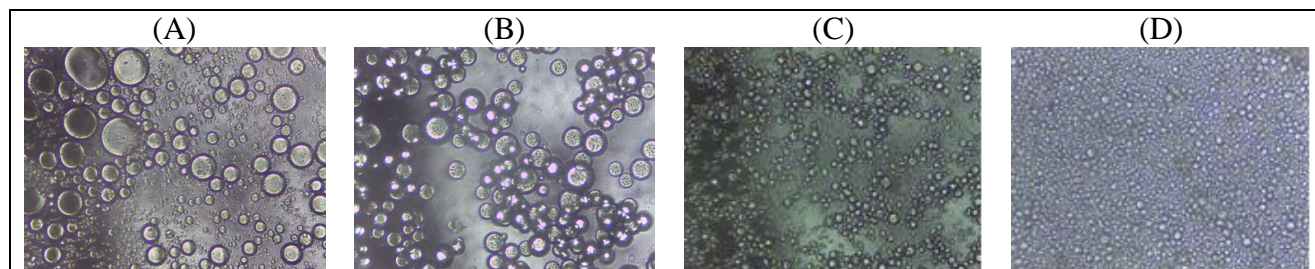


Figure 2 – Microscopic images of emulsions prepared with different oil and cheese whey concentrations (A) Test 1: 10% w/w oil, 8% w/w cheese whey; (B) Test 13: 17.5% w/w oil, 2% w/w cheese whey; (C) Test 8: 25% w/w oil, 24% w/w cheese whey; (D) Test 14: 17.5% w/w oil, 30% w/w cheese whey.

The results obtained by the turbidity measurement showed a nonlinear relationship, presumably because of the complex interaction between the independent variables present in the study. The turbidity of an emulsion containing polydispersed particles is a complex function related to the variation of droplet size, concentration and dispersity. This method has been used to determine the stability of emulsions, being its optimum value its highest. Taking into consideration this statement, the highest turbidity value obtained was 123.27%, from Test 8, a stable emulsion. However, this highest value-stability pattern cannot be observed throughout the measurements. Test 14, for example, another stable one, presents turbidity in the range of 91.17%, the lowest value of all.

4. CONCLUSIONS

The character of an emulsion varies depending on the manufacturing process and the oil-cheese concentrations. The application of an experimental design to predict optimal formulation of emulsions provided good parameters for time of agitation and oil and cheese whey concentrations. These conditions allowed the production of stable emulsions and the observation of some specific characteristics of their formation. The creaming indexes indicate that the most stable emulsions were the ones kept at 4 °C and that contained the highest oil and cheese whey concentrations: 25% w/w oil, 24% w/w cheese whey; and 17.5% w/w oil, 30% w/w cheese whey. The instability of the system observed in some samples, especially the ones with higher oil concentrations, was mainly due to the formation of large particle size which in turn facilitates coalescence. The method applied in the study proved to be efficient, resulting in stable emulsions, with oil and cheese whey at its highest concentrations, proving to be an excellent solution both to the environment and to the human health.

5. REFERENCES

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