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INFLUENCE OF PUMPKIN SEED OIL IN CONTINUOUS PHASE ON DROPLET SIZE AND STABILITY OF WATER-IN-OIL EMULSIONS

Branislava G. Nikolovski, Jelena D. Ilić, Milan N. Sovilj, Milan P. Nikolić and Jadranka L. Milanović*

Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

The aim of this work was to contribute to the optimized production of water-in-oil emulsions with pumpkin seed oil in the oil phase using a high-speed homogenizer. Pumpkin seed oil is a valuable natural source of essential fatty acids and biologically active micronutrients that contribute to its nutritive value and medical uses, and reduce interfacial tension between water and the oil phases. Therefore, pumpkin seed oil can be considered as a prosperous oil phase whose use can possibly decrease the amount of some emulsifier that is normally involved in every emulsification process.

A central composite rotatable experimental design was implemented to analyze the impact of the contents of polyglycerol polyricinoleate and pumpkin seed oil in the continuous phase, as well as water phase content in the emulsion on droplet size distribution and the response surface methodology was used to obtain optimal conditions for water-in-oil emulsion preparation. Mean size diameter of water droplets was in a range from 400 to 850 nm, with mean peak width of 100 to 220 nm, respectively. The influence of all three investigated factors on the emulsification was determined. Additionally, the emulsions prepared with pumpkin seed oil showed a higher stability during the storage time compared to the emulsions with sunflower oil.

KEY WORDS: emulsification, water-in-oil emulsions, pumpkin seed oil, sunflower oil, PGPR

INTRODUCTION

Tasty, healthy and nutritious, as well as more convenient food products with enhanced stability and shelf life are imperative for today's increasingly demanding markets. Production of water-in-oil (W/O) and water-in-oil-in-water (W/O/W) emulsions is one possible step towards employing the novel idea that biologically active substances and active ingredients in the food industry should be entrapped into some carrier material to form microcapsules or nanoparticles in order to achieve controlled release of active ingredients, flavour retention, mask bad tasting or bad smelling components, stabilize food

* Corresponding author: Branislava G. Nikolovski, University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia, e-mail: barjakb@uns.ac.rs

ingredients, prevent their oxidation or hydrolysis, and adjust their properties and/or increase their bioavailability (1).

Water-in-oil emulsions prepared under conditions of high shear stress in the rotor-stator systems, such as high-speed homogenizers, are thermodynamically unstable due to the relatively high interfacial tension. Therefore, water droplets tend to reduce the total amount of interface by coalescence. In order to reduce interfacial tension between two phases as well as to prevent the occurrence of aggregation and increase emulsion stability, a small amount of surfactant is added to emulsions. A surfactant with a low hydrophilic-lipophilic balance (HLB) is better soluble in oil and normally forms W/O emulsions, contrary to a surfactant with a high-HLB that dissolves in water and forms O/W emulsions (2). Surh et al. (3) investigated which surfactant shows the optimal characteristics for obtaining emulsion W/O. Polyglycerol polyricinoleate PGPR (HLB $\approx$ 3) was chosen among a few low HLB value surfactants, as the surfactant which is well dissolved in corn oil, and enables obtaining emulsion W/O with droplets of small diameter and narrow droplet size distribution. The temperature of emulsification process did not show a meaningful effect on the droplet size and droplet size distribution. (3)

Size of dispersed droplets depends on the concentration of surfactant, meaning that at the same content of dispersed phase, droplet size decreases with increasing concentration of surfactant (4). Although, it was found that high concentrations of surfactant did not have a big influence on the mean droplet size and droplet size distribution, increasing the concentration of PGPR to 8% (w/w) decreased the aggregation of droplets during storage, so these emulsions were more stable (5). However, since the estimated maximum *per capita* mean daily intake of PGPR is 2.64 mg/kg body weight/day (6), it is always recommendable to keep the level of emulsifiers in a product as low as possible.

Previous studies have shown that the mean droplet size and droplet size distribution depend on the dispersed phase content in the emulsion in a way that increasing the water phase content the mean droplet size was increased (7).

In previous studies of preparation of water-in-oil emulsions as the continuous phase were used sunflower, corn, soybean oil and rapeseed oils. This work was aimed at giving a contribution to the optimized production of water-in-oil emulsions with pumpkin seed oil in the oil phase. Namely, pumpkin seed oil is a valuable natural source of essential fatty acids and biologically active micronutrients like tocopherols, sterols and squalene, which contribute to its nutritive value and medical uses (8, 9). Presence of micronutrients, especially squalene reduces the interfacial tension between the water and oil phases (10). Therefore, pumpkin seed oil can be considered as a prosperous oil phase whose use can possibly decrease the amount of some emulsifier that is normally involved in every emulsification process. The purpose of this study was to create optimal properties of the continuous phase for the preparation of stable emulsions with small droplets diameters by mixing oils of different physicochemical properties (sunflower and pumpkin seed oil).

Pumpkin seed oil, sunflower oil and different mixtures of these two oils were used as the continuous phase when W/O emulsions were produced using a high-speed homogenizer in the presence of PGPR. A central composite rotatable experimental design was implemented to analyze the impact of the contents of polyglycerol polyricinoleate and pumpkin seed oil in the continuous phase, as well as water phase content in the emulsion on droplet size distribution, and the response surface methodology was used to obtain

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optimal conditions for water-in-oil emulsion preparation. Also, the emulsions stability over time was studied.

EXPERIMENTAL

Materials

For the preparation of the continuous phases sunflower oil (“Vital”, Vrbas, R. Serbia), pumpkin seed oil (“GEA”, Slovenska Bistrica, Slovenia) and polyglycerol polyricinoleate (PGPR 90), kindly provided by “Jaffa” a.d. (Crvenka, R. Serbia), were used. Vegetable oils were used in their original form. Demineralized water was used as the dispersed phase.

Preparation of W/O emulsions

Continuous phase was prepared by dissolving a certain amount of PGPR (1-5%) in an appropriate mixture of sunflower and pumpkin seed oil at 50°C, by mixing on a magnetic stirrer for 1/2 h. The aqueous phase was dispersed drop by drop into the continuous oil phase with a continuous stirring by a high-speed homogenizer Ultra turrax T-25 (IKA, Germany) at 24 000 rpm for 5 min. Emulsification temperature was 25°C. The content of the disperse phase was between 10 and 32% (v/v).

Characterization of W/O emulsions

The mean droplet size and droplet size distribution of the water in W/O emulsions stabilized with PGPR, were measured immediately after the formation using a laser light scattering instrument Zetasizer Nano ZS (Malvern Instruments, U.K.). The measurement was repeated at least three times.

For the stability test, the emulsions were transferred to graduated cylinders and stored at room temperature for 2 months. During storage, the emulsions separated into a cream layer and a transparent serum layer. The total height of the emulsion, H_E, and the height of the serum layer, H_S, were measured during the investigation time. The extent of creaming was characterized by the creaming index, H, given by:

$$H = (H_S/H_E)\cdot 100\ (\%)$$

The higher creaming index, H, the worse is the emulsion stability (11).

Statistical analysis

Experimental design. A centrally composite rotatable experimental design (CCRD) was implemented in order to study the effect of emulsion composition parameters on the droplet size distribution. The three input variables were the content of polyglycerol polyricinoleate in continuous phase (1-5% (w/w), X_1), pumpkin seed oil content in the conti-

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nuous phase (0-100% (w/w), X_2) and dispersed phase content (10-32% (w/w), X_3) and the five levels chosen were −2, −1, 0, +1 and +2 as shown in Table 1. The experimental design consisted of 16 runs ($n = 2^k + 2k + m$, where, n = total experimental points, input variables, $k = 3$ and central point, $m = 2$), which included eight factorial points, six axial points and two replicated central points, as shown in Table 2.

Table 1. Treatments levels and coded values for each of the independent variables used in developing the experimental data to optimize the W/O emulsion content.

Independent variable	Symbol		Level	
	Uncoded	Coded	Uncoded	Coded
PGPR content, % (w/w)	E	X_1	1 2 3 4 5	-2 -1 0 1 2
Pumpkin seed oil content in continuous phase, % (w/w)	PO	X_2	0 25 50 75 100	-2 -1 0 1 2
Water phase content, % (w/w)	W	X_3	10 15.5 21 26.5 32	-2 -1 0 1 2

$X_1 = (E-3)/1$, $X_2=(PO-50)/25$, $X_3=(W-21)/5.5$,

Table 2. Experimental design and results of production of W/O emulsions.

Treatment	**PGPR, E, % (w/w)**	**Pumpkin seed oil, PO, % (w/w)**	**Water phase, W, % (w/w)**	**Mean diameter, d, nm**	**Peak width, σ, nm**
	X1	X2	X3		
1	2 (−1)	25 (−1)	15.5 (−1)	652	185
2	2 (−1)	25 (−1)	26.5 (1)	808	207
3	2 (−1)	75 (1)	15.5 (−1)	569	152
4	2 (−1)	75 (1)	26.5 (1)	728	184
5	4 (1)	25 (−1)	15.5 (−1)	459	144
6	4 (1)	25 (−1)	26.5 (1)	711	193
7	4 (1)	75 (1)	15.5 (−1)	502	203
8	4 (1)	75 (1)	26.5 (1)	618	184
9	3 (0)	50 (0)	21 (0)	637	207
10	3 (0)	50 (0)	21 (0)	634	176
11	1 (−2)	50 (0)	21 (0)	839	220
12	5 (2)	50 (0)	21 (0)	506	149
13	3 (0)	0 (−2)	21 (0)	746	140
14	3 (0)	100 (2)	21 (0)	619	124
15	3 (0)	50 (0)	10 (−2)	418	100
16	3 (0)	50 (0)	32 (2)	827	177

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Response surface modeling (RSM)

A second order polynomial equation was used to fit the coded variables:

$$Y = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_{11}X_1^2 + A_{22}X_2^2 +$$
$$+ A_{33}X_3^2 + A_{12}X_1X_2 + A_{23}X_2X_3 + A_{13}X_1X_3$$

where, Y represents the experimental response (peak diameter), A_0, A_1, A_2, A_3, A_{11}, A_{22}, A_{33}, A_{12}, A_{23} and A_{13} are constants and regression coefficients, respectively of the model and X_1, X_2, and X_3 are independent variables.

The CCRD experimental design was combined with RSM to solve the regression equation and investigate the effect of three independent input variables of PGPR content, pumpkin seed oil content and water phase content to arrive at the optimal conditions giving the minimum of the mean droplet sizes. Statistical analyses were done by a computer program Statistica 10.

RESULTS AND DISCUSSION

Results obtained under the different testing conditions are shown in Table 2. Mean size diameter of water droplets was in a range from 400 to 850 nm, with mean peak width of 100 to 220 nm, respectively. An overall observation of the results showed that the mean droplet size generally decreased with PGPR content increase, water phase content decrease and there was an optimal value of the pumpkin seed oil content.

Regression model

The developed regression model for the relationship between the mean droplet size (Y) and the coded values of independent variables of PGPR content (X_1), pumpkin seed oil content in the continuous phase (X_2), and water content (X_3) and their interactions is shown in the following equation:

$$Y = 624.4 - 70.8X_1 - 29.1X_2 + 93.9X_3 + 9.2X_1^2 + 11.7X_2^2 -$$
$$-3.2X_3^2 + 14.1X_1X_2 - 16.5X_2X_3 + 6.4X_1X_3$$

The significance of each coefficient and its interactions was determined using the t-test (t-value). The linear terms of X_1 (absolute t value =7.72), X_2 (absolute t value =3.2) and X_3 (absolute t value =10.2) gave a highly significant effect on the mean droplet size having an absolute t value higher than the critical t value of 2.26 (at p value <0.05 and degree of freedom =9). All other terms did not show a significant effect on the mean droplet size.

Analysis of variance (ANOVA) of the results of the model indicate a good model performance with an R^2 value of 0.97, which implies that 97% of the variations associated with the mean droplet size can be attributed to the three independent variables. The peak width values cannot be modeled with a second order polynomial equation.

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The 3-dimensional desirability surface plots obtained by response surface regression of the mean droplet size dependence on independent variables of PGPR content (X_1), pumpkin seed oil content in the continuous phase (X_2), and water content (X_3) are shown in Figure 1. The independent variable values are set to the optimal values while the desirability was set to 0 values for the droplet diameters of 900 nm, and to 1 for the droplet diameter of 200 nm.

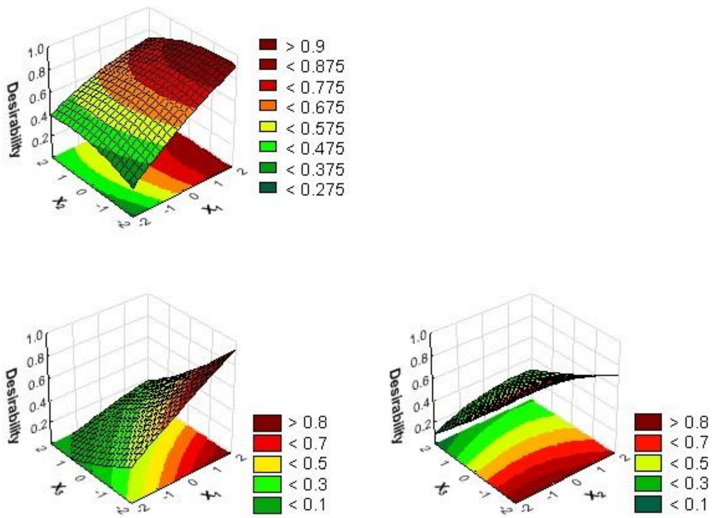

Figure 1. The desirability surface plots obtained by response surface regression of the mean droplet size dependence on the independent variables: PGPR content (X_1), pumpkin seed oil content in continuous phase (X_2), and water content (X_3). Optimal values: PGPR content (X_1= 2), water phase content (X_3= -2), and pumpkin seed oil content (X_2= -1,033). Desirability: 0 (900 nm), 0.5 (550 nm) and 1 (200 nm)

The profiles for the predicted values and desirability are shown in Figure 2. As it was expected, the results confirmed that the smallest water droplets were obtained at the highest value of PGPR content (X_1= 2), the lowest value of water phase content (X_3= -2), and at the optimum content of pumpkin seed oil (X_2= -1,033), the corresponding experimental values were 5% (w/w) PGPR, 10% (w/w) water phase, and 24.2% (w/w) pumpkin seed oil in the continuous phase.

Stability of the obtained emulsions during storage time was analyzed by observing the creaming index. The results of creaming index of the emulsions prepared with different values of independent variables are shown in Figure 3. Water-in-pumpkin seed oil emulsion became more stable than water-in-sunflower oil emulsion after 3 days of storage, and the difference between these two emulsions increased with increasing storage time in a favor of pumpkin seed oil (Figure 3a). The emulsions with 50% (w/w) of pumpkin seed oil were the most stable of all three emulsions for the first 12 days, after which their stability decreased and the most stable became the water-in pumpkin seed oil emulsion (Figure 3a).

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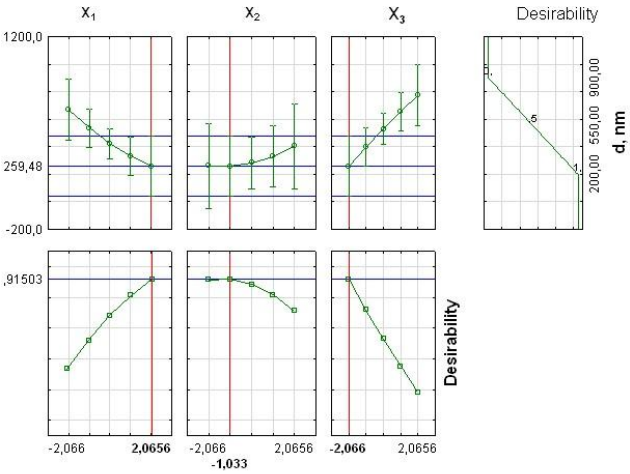
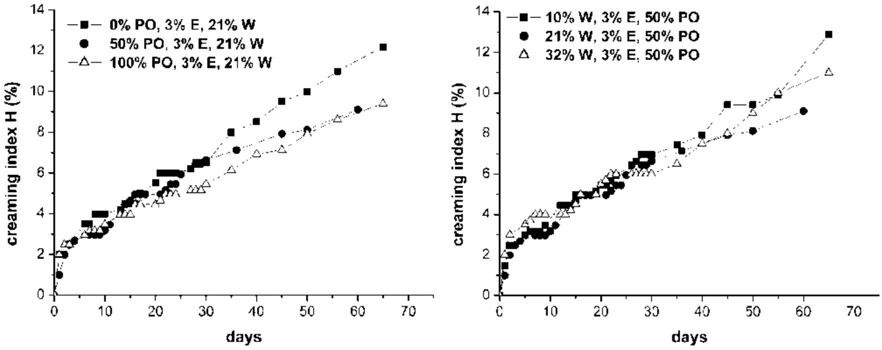


Figure 2. Profiles for predicted values and desirability

The water phase content showed an effect on the emulsions stability in the first ten days after the preparation, when the most unstable was the emulsion with the highest water phase content. After that, the creaming index curves alternatingly crossed each other (Figure 3b).



a) b)

Figure 3. Creaming index of emulsions prepared based on different values of independent variables: a) influence of pumpkin seed oil; b) influence of water phase content

CONCLUSION

A central composite rotatable experimental design was implemented to analyze the impact of the contents of polyglycerol polyricinoleate, pumpkin seed oil in the continuous phase and water phase content on droplet size distribution, and the response surface methodology was used to obtain optimal emulsification conditions. Mean size diameter of water droplets was in a range from 400 to 850 nm, with mean peak width of 100 to 220 nm, respectively. The influence of all three investigated factors on emulsification was determined. An overall observation of the results showed that the mean particle size generally decreased with PGPR content increase, water phase content decrease, and there was an optimal value of pumpkin seed oil content. Optimal independent parameters values were 5% (w/w) PGPR, 10% (w/w) water phase, and 24.2% (w/w) pumpkin seed oil in the continuous phase. The emulsion prepared with some smaller amounts of emulsifier and higher water contents had smaller water droplets and were more stable when pumpkin seed oil was present in the oil phase. Additionally, emulsions prepared with pumpkin seed oil showed a higher stability during the storage time compared to the emulsions with sunflower oil.

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УТИЦАЈ ПРИСУСТВА ТИКВИНОГ УЉА У КОНТИНУАЛНОЈ ФАЗИ НА ВЕЛИЧИНУ КАПИ И СТАБИЛНОСТ ЕМУЛЗИЈА ВОДА У УЉУ

Бранислава Г. Николовски, Јелена Д. Илић, Милан Н. Совиљ, Милан П. Николић и Јадранка Л. Милановић

Универзитет у Новом Саду, Технолошки факултет, Булевар цара Лазара 1, 21000 Нови Сад, Србија

Циљ овога рада је оптимизација процеса добијања емулзија вода у уљу са тиквиним уљем као компонентом континуалне фазе. Тиквино уље је драгоцен природан извор есенцијалних масних киселина и биолошки активних микронутријената, који доприносе нутритивној вредности тиквиног уља и постизању ефеката у лечењу одређених болести, а чије присуство доприноси смањењу међуповршинског напона између воде и уља. Стога, ако би се тиквино уље користило као континуална фаза или компонента континуалне фазе могуће је очекивати да би се систему могла додавати мања количина емулгатора.

При планирању експеримената примењен је централно композитни план који укључује варирање садржаја емулгатора полиглицеролполирицинолеата (ПГПР), садржаја тиквиног уља у континуалној фази и садржаја водене фазе у емулзији у циљу испитивања њиховог утицаја на расподелу величина капи воде у уљу. Добијене вредности средњег пречника капи варирају у опсегу од 400 до 850 nm, са одговарајућим вредостима ширине пика од 100 до 220 nm. Методом одзивне површине процењен је и потврђен утицај сва три испитивана фактора на величину пречника капи. Такође, емулзије припремљене са тиквиним уљем у континуалној фази показују већу стабилност током стајања.

Кључне речи: Емулговање, емулзије вода у уљу, уље семена тикве, сунцокретово уље, ПГПР

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