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## Comparison of the Antioxidant Effect of Microencapsulated and Un-microencapsulated Sodium Selenite with Butyl Hydroxyanisole in Soybean Oil

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### Abstract

Microencapsulation of sodium selenite (15000 ppm) was carried out, comprising the following combination: Arabic gum (25, 26, 27, 28 and 29%) and a proportionate amount of Farsi gum (1, 2, 3, 4 and 5%) and using the solvent evaporation method, where the Ethanol purity percentages (80, 85, 90, 95 and 100%) and the ratio of solvent to the mixture [gum+sodium selenite] was 10:1. Subsequently, utilizing the response surface methodology (RSM) software, the optimization was carried out based on the highest efficiency of encapsulation and the smallest size of particles. Two formulas of the optimization were chosen for this study comprised the following: the optimization formula 1 (efficiency 89.5%, particle-size 44.6 $\mu$ m, Arabic gum 27%, Farsi gum 3%, and the percentage of ethanol purity 94%) and the optimization formula 2 (efficiency 86%, particle-size 48.5  $\mu$ m, Arabic gum 29%, Farsi gum 1%, and the percentage of ethanol purity 89%). Ultimately, the two selected optimal (180.6 ppm), the synthetic antioxidants butylated hydroxyanisole (BHA) (200 ppm), and the un-microencapsulated sodium-selenite(8.6 ppm) were added to antioxidant-free soybean oil and then were placed at the 55 °C for (0, 23 and 46 days) equal to 20 °C (0, 180 and 360 days). The oxidation indices were compared with soybean oil that did not contain anti-oxidant. A direct relation was detected between the concentration of Arabic gum and alcohol purity percentage with the efficiency of the encapsulation (94.63%). A reverse relation was detected between the concentration of Arabic gum and alcohol purity percentage with the particle-size (37.5  $\mu$ m). The effective and propositional treatments are presented including, Opt1, Opt2, BHA, un-microencapsulated sodium-selenite and blank (antioxidant-free), respectively.

**Keywords:** Antioxidant activity, Arabic-Farsi gum, Microencapsulation, Sodium-selenite, Soybean oil

### Introduction

Oils and fats provide a significant portion of the body's energy needs, essential fatty acids, and fat-soluble vitamins (Hoseini Mazhari, Kebriti, Gerami, Ghiassi, & Esfandyari, 2011). Soybean-oil consume is limited due to its 5-9% linolenic acid content and being susceptible to auto-oxidation, beta-oxidation and taste reversion in crude oil without antioxidant (Iranian National Standardization Organization [ISIRI], 2017). In this study, selenium sodium was

used as an antioxidant. Selenium is one of the rare and essential minerals in the body. Selenium has various organic and inorganic constituents (Stewart *et al.*, 2012). The most important inorganic forms of selenium include sodium selenate, sodium selenite, sodium selenide and sodium chloride (Combs & Combs, 1984). Selenium is known to be an important component of the glutathione peroxidase enzyme and protects the body against damage caused by free radical oxidation (Rotruck *et al.*, 1973). Selenium binds to proteins in the body and gives rise to selenoproteins that have antioxidant properties. The most important property of selenium is its antioxidant activity and has other properties such as anti-inflammatory and antiviral properties (Yarnazai *et al.*, 2017). The tolerable amount, the recommended limit, and the average estimated human requirement are 400, 55 and 45  $\mu\text{g}$  of selenium per day, respectively (Fraga, 2005). Selenium is a pseudo-metal, so excessive use of sodium selenite is toxic and symptomatic. As the odor of garlic respiration, hair and nail growth decline, nervous system disorders and tooth decay also lead to nausea and vomiting (Meltzer *et al.*, 1993).

## Materials and methods

### Microencapsulation of Na-selenite

Microencapsulation of Na-selenite (15000 ppm) was carried out, Arabic gum (25, 26, 27, 28 and 29%) and a proportionate amount of Farsi gum (1, 2, 3, 4 and 5%) and using the solvent evaporation method, where the Ethanol purity percentages (80, 85, 90, 95 and 100%) and the ratio of solvent to the mixture [gum+Na-selenite] was 10:1. Subsequently, utilizing the response surface methodology (RSM), the optimization was carried out based on the highest efficiency encapsulation (EE) and the smallest particle size.

### Chemical analysis of soybean-oil

The peroxide value by the method of Firestone (1994) and Egan, Kirk, & Sawyer (1997), the Thiobarbituric acid test according to the method of Egan *et al.* (1997), the Anisidine index according to Tompkins & Perkins (1999) in a  $\lambda_{\text{max}}$  350 nm by a spectrophotometer, the Totox index according to the relationship presented by Wanasundara & Shahidi (2005), acidity according to Iranian National Standardization Organization [ISIRI, No. 4179] (2018), and antioxidant activity in all samples according to the relationship presented by Ghanbari, Ghavami, & Safafar (2006) was measured.

### Statistical analysis

Response surface method and central composite design (CCD) with  $\alpha=2$  with 6 central points were used to analyze the results. For oil chemical analysis, 2 optimal formulas with higher desirability percentages, i.e., the highest efficiency and the smallest particle-size were selected and added to soybean oil and compared with some treatments, i.e., un-encapsulated Na-selenite, antioxidant-free and 200 ppm butylated hydroxyanisole (BHA) samples at a temperature of 55 °C for 46 days. The temperature and storage time of soybean oil in the incubator were equilibrated with the shelf-life accelerator program (Version 10) (55 °C and 0, 23 and 46 days) equivalent to 20 °C and 0, 180 and 360 days. According to this accelerated method, in practice, the food was affected by high temperature and the results were generalized to Arrhenius equation and  $Q_{10}$  equal to 2 (Labuza & Schmidl, 1985; Robertson, 2012). The differences between the samples were analyzed using ANOVA and Duncan's test at the probability level of 95% ( $P<0.05$ ). Analysis of variance was performed using SPSS software (Version 17) and Microsoft Excel (Version 2012) was used to plan the graphs.

## Results and discussion

### Encapsulation optimization of optimal formulation

Two formulas of the optimization chosen for this study comprised the following: the optimization formula 1 (efficiency 89.5%, particle-size 44.6  $\mu\text{m}$ , Arabic gum 27%, Farsi gum

3%, and the percentage of ethanol purity 94%) and the optimization formula 2 (efficiency 86%, particle-size 48.5  $\mu\text{m}$ , Arabic gum 29%, Farsi gum 1%, and the percentage of ethanol purity 89%). Our findings indicated a direct correlation between the concentration of Arabic gum and ethanol purity with encapsulation efficiency. However, the correlation between the concentration of Arabic gum and the percentage of ethanol purity with particle size was inverse. That is, the particle size decreased with increasing concentration of Arabic gum and ethanol purity. Considering the coefficients in Eq. (1) and (2), percent ethanol purity, it had more effect on increasing efficiency and decreasing particle size.

$$(Encapsulation\ Efficiency\ (\%))^{-3} = 1.56 \times 10^{-6} - 3.08 \times 10^{-8}(A) - 2.71 \times 10^{-7}(B) + 4.24 \times 10^{-8}(B^2) - 3.51 \times 10^{-8}(A^2B)$$

R-Squared=0.99

$$(Size\ (\mu\text{m}))^{-1.87} = -4.30 \times 10^{-3} + 3.84 \times 10^{-5}(A) + 4.37 \times 10^{-5}(B)$$

R-Squared=0.97

### Interaction of time treatment on qualitative characteristics of soybean oil

#### Peroxide value

All oil samples at day zero showed the lowest peroxide value. Peroxide value from day zero to day 23 of the storage period showed an increasing trend indicating the initiation of the oxidation process and the accumulation of their constituents, such as peroxides, and then until day 46 has been a downward trend. Since malondialdehydes result from the decomposition of hydroperoxides, after a certain period when the number of primary oxidation products increased, the peroxides and hydroxides began to decompose and thus the peroxide number decreased (Kabiri & Sayyed-Alangi, 2015). The samples of oil encapsulated with selenium sodium showed a lower peroxide value compared to the sample of oil containing un-encapsulated selenium, which could be attributed to the maintenance of antioxidant stability within the capsule. Because the capsule controlled antioxidant release during storage, while the antioxidant is more exposed to oxidizing agents, and loses its antioxidant property earlier.

#### Thiobarbituric acid number

The amount of thiobarbituric acid increased from day zero to day forty six of the storage time. When the peroxide value reaches a certain level, various changes occur and aldehyde and ketone volatiles are produced by the decomposition of hydroperoxides, which are effective in producing an unpleasant odor and taste of fatty substances and increase the number of thiobarbituric acid. In addition, antioxidants remain active for a certain period of time, and their effect gradually decreases over time, which may be due to the preservation of the sample under oxidizing and heat conditions. Thus, as the shelf life increases, the amount of thiobarbituric acid increases (Kabiri & Sayyed-Alangi, 2015; Farag, Mahmoud, & Basuny, 2007).

#### Anisidine index

All oil samples at day zero had the lowest anisidine index. Anisidine index increased from day 0 to day 46 of the storage period. As the shelf life increases, the oxidation process intensifies and the amount of secondary oxidation products increases. The highest anisidine index was observed in the control sample (antioxidant-free) on the forty-sixth day. Among the treatments, samples with synthetic antioxidant butyl hydroxyanisole and samples containing encapsulated sodium selenite (the optimal formula 1 and 2) had the lowest anisidine index on twenty-third day.

### Totox Index

As the results show, the trend of changes in the Totox index was approximately similar to the trend of the peroxide number changes. All oil samples at day zero showed the lowest Totox index. The Totox index increased from day zero to day 23 of the storage time, and then declined to day 46.

### Acid number

The acid number indicates the onset of oil oxidation and is one of the important qualitative parameters of oil in storage conditions. All oil samples at day zero showed the lowest acid number. The acid number increased from day zero to day 46 of the storage period. As the storage time increases, the oxidation process intensifies. Inorganic antioxidants, reacted with free radicals from lipid oxidation, disrupted chain reactions and increased oxidation time compared to the control sample without antioxidants. Since organic acids are from the final oxidation products of the unsaturated fats (Batool *et al.*, 2018) so the acidic number in optimal formula 1 and 2 was significantly reduced compared to the control sample without antioxidant and sodium selenite salt and was similar to the sample containing antioxidant butyl hydroxyanisole.

### Antioxidant activity

All samples had no antioxidant activity at day zero. From the 23 to 46 day, an increase in antioxidant activity was observed in all samples except the control. As Ortiz-Vazquez, Shin, Soto-Valdez, & Auras (2011) pointed out, the migration of synthetic butyl hydroxytoluene into coconut oil at 23 °C did not stop even after 100 days. In this regard, Queiroz, Ishimoto, Bastos, Sampaio, & Torres (2009) reported that garlic has 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging activity as well as linoleic acid oxidation inhibition due to its rich selenium and organosulfur compounds.

### Conclusions

A direct relation was detected between the concentration of Arabic gum and alcohol purity percentage with the efficiency of the encapsulation (94.63%). A reverse relation was detected between the concentration of Arabic gum and alcohol purity percentage with the particle-size (37.5  $\mu\text{m}$ ). The effective and propositional treatments are presented in the following order respectively: blank (antioxidant-free)<un-microencapsulated sodium-selenite<BHA=Opt2 <Opt1.

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