CHEMICAL ENGINEERING TRANSACTIONS
VOL. 93, 2022
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Marco Bravi, Alberto Brucato, Antonio Marzocchella

ISBN 978-88-95608-91-4; **ISSN** 2283-9216
DOI: 10.3303/CET2293024

Freeze Drying Optimization of Canola Oil with Phytosterols using Alginate and Maltodextrin

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African palm oil (*Elaeis guineensis* Jacq.) is an oil that contains phytosterols, which are rich antioxidants that are capable of decreasing the intestinal absorption of cholesterol in the human body. Freeze-drying is a technique commonly used in the drying and powdering of encapsulated essential oils since its benefits allow the preservation of its physical and chemical properties. The phytosterols are of medical interest due to their potential to treat hypercholesterolemia. Therefore, this study attempts to optimize the drying process of canola oil with phytosterols using the lyophilization process. Water/oil solution (85.2%), maltodextrin/sodium alginate mixture (12.8%) and soy lecithin (2%) were used to prepare the emulsion. A surface response methodology was applied to determine the effects of water/oil and maltodextrin/sodium alginate mixture on the phytosterols encapsulation efficiency, water activity, solubility, and oxidative stability index on the produced lyophilized product. For the optimization, these physical-chemical attributes were evaluated as the dependent variables and the proportions of water/oil ratios and maltodextrin/alginate concentrations as the independent variables. An optimal treatment was determined with concentrations of 13.5 grams of maltodextrin, 1.5 grams of alginate, 84.2 ml of water, and 15.7 ml of oil. A predictive phytosterols content of 40 mg was obtained per gram of lyophilized product, an induction time of 33.15 hours in oxidative stability and solubility of 59.96%.
Keywords: *Elaeis guineensis* Jacq, lyophilization process, surface response, encapsulation, optimization.

1. Introduction

Medicinal plants provide many benefits for the human, and one of the most important is their contribution to health benefits due to their phytochemicals that are also known as bioactive compounds (Fuentes et al., 2020; Olivet et al., 2022). Lately, awareness of individuals has been increasing towards beneficial diets and healthy foods, which perspective has been paid close attention to bioactive compounds (Fuentes et al., 2021; Marcía et al., 2021). Because of the content of bioactive molecules, African palm (*E. guineensis*) has been a commodity of medicinal and pharmacological interest. The most important parts of the palm tree are its fruit (especially its oil content), seeds, and roots (da Silveira, 2018). Besides, the African palm contains considerable amounts of phytosterols, which are steroid alcohols that include plant stanols and sterols, and are alike to cholesterol (Zychowski et al., 2019). Traditionally, African palm oil has been used against infections regarding the genitourinary system, skin, respiratory system, and digestive system, and it also has been used as a treatment against pain and injuries (Ghaedi et al., 2020). However, the phytosterols have some limitations since they have a low water solubility, which is commonly around 2% at 25℃, and they also have a high melting point which makes difficult their use in food systems (Uyen et al., 2020). Additionally, phytosterols are susceptible to free

Paper Received: 20 January 2022; Revised: 17 March 2022; Accepted: 2 May 2022
Please cite this article as: Guerrero I., Maldonado L., Marcia J., Aleman R., Moncada M., Fernandez V.M., Reyes J.T., Montero-Fernandez I., 2022, Freeze Drying Optimization of Canola Oil with Phytosterols Using Alginate and Maltodextrin, Chemical Engineering Transactions, 93, 139-144 DOI:10.3303/CET2293024

radicals and oxidation during thermal processes (Almeida et al., 2020). As a result, the freeze-drying technique can assist to improve its stability from oxidation, contamination, or undesirable reactions. There are different types of polymers and chemicals which can act as encapsulation agents among few alginates have gained satisfactory attention due to its great results in oil systems (Guo et al., 2018). Alginates are natural polysaccharides, which are soluble in water, economically accessible, non-toxic, and compatible with oil systems (Ghaedi et al., 2020). The alginates have 3 different linear arrangements of blocks. Firstly, the G blocks are composed of L-guluronic acid which causes reactions with cations to provide greater gel strength. Secondly, the M blocks are composed of D-mannuronic acid, and the MG blocks are known for their solubility in lower pH (Tavassoli et al., 2016). Therefore, alginate can crosslink in M and G blocks leading to the formation of the egg-box model which is an important structure to form capsules under specific conditions and under the presence of divalent cations such as Ca, Ba, Mn, Cu, and Zn (Priyadarshi et al., 2021). In addition to alginates, maltodextrins are polysaccharide compounds that have been used in food systems because of their high solubility, low viscosity, and colorless nature. As an encapsulation agent, maltodextrins are known on spray-dried process applications and it is highly compatible with alginate to act as a wall and outer shell of capsules (Maqsoudlou et al., 2020). Furthermore, maltodextrin is highly digestible releasing inner compounds in different matrices and gastrointestinal conditions (González et al., 2019). Its uses can be seen in the encapsulation of shark liver oil and different oil-soluble vitamins (A, E, K, and D) leading to a decrease in oxidation and extended shelf life (Ribeiro et al., 2020). Consequently, alginate/maltodextrin (AM) combination can be promising to successfully provide capsules walls in oil systems. The shelf life of lipid-based compounds is a major problem and the lyophilization process has been proposed to extend shelf-life specifically for products that are heat sensitive (Lopez et al., 2020). Lyophilization is a food preservation method in which the product is dried by a sublimation process. This process can have an impact on the product's sensory and physical-chemical characteristics (Harguindeguy & Fissore, 2020). Therefore, this study aims to develop from alginate/maltodextrins and optimization process to determine its effects on phytosterols content, oxidation stability, solubility, and water activity of palm oil in a freeze-drying process.

2. Materials and method

2.1 Emulsion ingredients and preparation of phytosterols lyophilized product

The following ingredients were used for the development of the research: Sodium alginate (TICA-algin® 400 Powder, TIC Gums, Westchester, Illinois, USA), maltodextrin (DE 10-15, Shandong Bangye Co Ltd, Shandong, China), canola oil (Wesson, Chicago, Illinois, USA), soy lecithin (Landor trading, Williamsport, PA, USA), phytosterols standard (BIOSA, Puerto Cortes, Honduras), deionized water (Barnstead ™ Nanopure ™ Thermoscientific, MA, USA).

The research was carried out at the Food Analysis Laboratory facilities of the Zamorano University, Valle del Yeguare, San Antonio de Oriente Municipality, Francisco Morazán Province, Honduras. The phytosterols and canola oil were mixed using 0.15 g of phytosterols per gram of canola oil (slight modification) (Comunian et al., 2017). The formulation of the emulsion is described as follows: water/oil (85.2%) solution, maltodextrin/sodium alginate (12.8%) mixture and soy lecithin (2%). Mixing was done in a 250 mL beaker under constant stirring for 1 hour at 400 rpm. The lyophilization process was carried out in a freeze-dryer (Virtis Advantage Pro, SP Scientific, PA, USA) and it was freezing at −50 ºC for 48 h in an ultra-low temperature freezer. The chamber pressure was kept below 20 µBar during the drying process. When the freeze-drying process was complete, the vials were immediately filled with nitrogen gas, sealed, and stored protected from light at 4ºC.

2.2. Experimental design & statistical analysis

A 2k factorial (k=2) with 2k axial points (k=2) and 6 central points, were used and coupled with the surface response methodology (SRM), and the SRM consisted on the levels of maltodextrin/sodium alginate ratios (85:15, 90:10, and 95:5) and water/oil ratios (80:20, 85:15, and 90:10). The response variables were the solubility, oxidative stability index, water activity, and phytosterols content and were adjusted to the model (Equation 1) at P≥0.05. The optimization was based on desirability optimization methodology, and it was considered to maximize the water solubility, oxidative stability index and phytosterol content. Besides, an ANOVA (Analysis of the Variance) and Tukey test (P≥0.05) was applied in the phytosterols composition and particle size of the treatments. On the surface response, the STATISTICA 7 ™ program was used and the analysis of the variance was analyzed through the Statistical Analysis System program (SAS®, Version 9.4).

$$Y = \beta 0 + \beta 1x1 + \beta 2x2 + \beta 12x1x2 + \beta 11x12 + \beta 22x1x22) \quad (1)$$

2.3 Extraction of total phytosterols

For the extraction and quantification, 500 mg of the lyophilized product were weighed in a 50 mL flask, followed by the addition of 5 mL of internal standard Epicoprostanol, 5β-cholestan-3α-ol (ID: C5050-000, Steraloids, Inc., Newport, RI) in toluene (P/N T291-4, Optima, Fisher Scientific, Inc., Pittsburgh, PA) at a concentration of 5 mg/mL, and 5 mL of NaOH/MeOH (2.3 N). Subsequently, the solution was transferred to a water bath at 100ºC for 45 min. After cooling, the solution was transferred to a 50 mL Erlenmeyer flask where 7 mL of 3.0 N HCL and 40 mL of saturated NaCl solution were added. A 2 mL vial was prepared with 0.3 mL of sample (organic phase), 0.25 mL of pyridine, 0.5 of BSTFA (N, O-Bistrifluoroacetamide) to be injected into the gas Chromatograph as the AOCS Official Method Ce 12-16 suggested.

2.4. Phytosterols profile of the lyophilized product in GC-FID

The phytosterols were determined by GC-FID using (5%-phenyl)-methylpolysiloxane HP-5 column (30 m × 0.32 mm i.d., 0.25 µm film; Agilent J&W Santa Clara, CA, USA). For 60 min, the oven was kept at 250ºC, and later, it was ramping up to 265 ºC at 15 ºC min^{-1} and maintained for 7 min. At post-run, the oven was kept for 3 min at 250ºC. The flow rate of hydrogen carrier gas was 1 mL min^{-1}, and at the FID, the flow rates for hydrogen, nitrogen, and air gases were 30, 30, and 400 mL min^{-1}, respectively. The injection volume was 1 µL (split ratio of 25:1) and the inlet and detector temperatures were performed at 290ºC. The identification was performed according to the AOCS Official Method Ce 12-16.

2.5. Oxidative Stability Index (OSI), Water Activity (aw) & Solubility (S)

The OSI analysis was performed in triplicates using the AOCS Cd 12b-92 method, through Rancimat device (873 Rancimat Biodicel, Metrohm, Switzerland). For the aw, the analysis was performed using the AOAC 978.18 method through AQUALAB Series 3 device (Decagon Devices, WA, USA). For solubility, the analysis was done with slight modifications (Ahmed et al., 2010), in a centrifuge tube, 2 g of the lyophilized product was dissolved in 24 mL of deionized water, using a vortex at 2500 rpm for 5 min. Subsequently, the solution was transferred to a water bath at 30ºC for 30 min and then centrifuged at 3000 rpm for 15 min. The supernatant was removed and placed in a previously weighed Petri dish and the water was evaporated in an oven at 105 ºC within 24 hours.

3. Results and discussion

3.1 Phytosterols content and profile

The most abundant phytosterols were campesterol, (17.30-21.86 %), stigmasterol (14.02-15.75 %), and β-Sitosterol (47.47-55.79 %), and other 13 phytosterols were found (6.46-6.60 %). The phytosterol composition of palm oil was similar to Hassanien (2013) report, and the three phytosterols founded were recovered in similar proportions. On the other hand, the phytosterol content per gram of lyophilized palm oil decreased when maltodextrin was incorporated, and a negative coefficient with significance was showed towards the maltodextrin/alginate ratio. The loss of phytosterol content can be since the oil droplets are not coated entirely by the maltodextrin/alginate in the lyophilized process. Maltodextrin influences the swelling of oil particles in capsules, and at appropriate concentration levels, this hydrocolloid can influence the swelling capacity of the capsule and may not influence the cross-linkage capacity and gelation capacity of alginate that may be related to the efficient coating of oil droplets (Bonda et al., 2020).

3.2 Water Activity

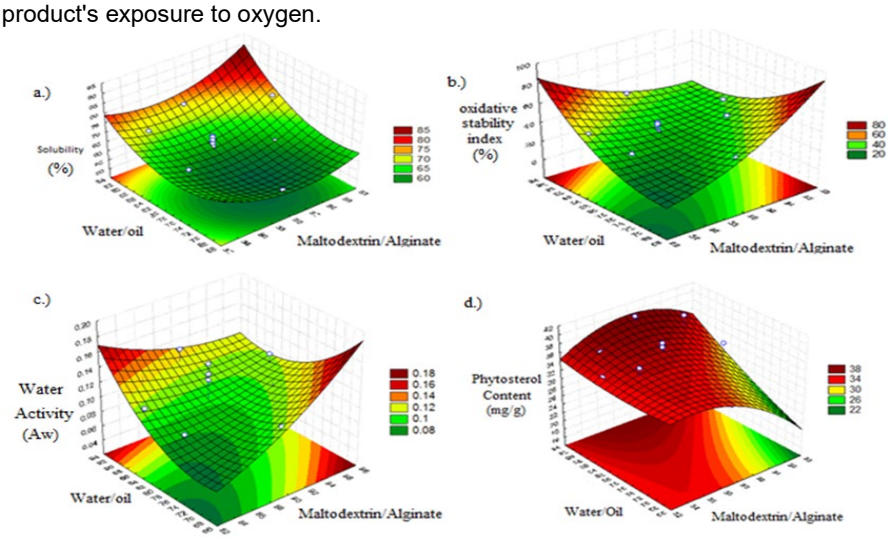
The water activity is critical when determining microbial activity, and food-related deteriorated chemical reactions such as lipid oxidation and non-enzymatic and enzymatic reactions. In Figure 1c, it can be observed that the aw increased when water and maltodextrin were added. These results are not surprising since when the oil concentrations are higher in emulsion preparations it decreases the moisture content and eventually the aw (Charles et al., 2021). Similarly, maltodextrin has increased the water activity in fish oils when it has been applied with wall coating material such as starch (Quispe et al., 2011). High water activity levels may not only promote microbial activity, lipid oxidation, and non-enzymatic and enzymatic reactions but also favor the free water to affect the coated material to have high molecular mobility, driving to the release of the coating oil droplets as the product is stored (Velasco et al., 2000).

3.3 Solubility and oxidative stability index

In this study, it was observed that when the emulsion mixture was prepared with higher amounts of water and maltodextrin, solubility increased (Figure 1a). When the oil concentrations were higher in the emulsion preparations, it decreased water solubility. Generally, the water solubility of oil is very low (less than 100 ppm)

(Wang, et al., 2010). Some reports found that the addition of maltodextrins increases the solubility in encapsulated products (Ahmed et al., 2010), but others contrasted these observations (Goula & Adamopoulos, 2008). This variation may be because of the effects of processing parameters and wall coating material composition on residual moisture content, which has an impact on the powder's solubility. Low water-soluble powders often require a high dosage to achieve the desired bioavailability of a specific nutrient. Solubility is one of the most critical parameters to obtain the wanted level of pharmacological response from a specific drug, which is usually encapsulated (Vemula et al., 2010).

Commonly, the oxygen diffusion in freeze-dried powders is high because of its porous nature when compared to other types of dried powders. Besides, the rate of oxygen diffusivity is affected by moisture content and water activity (Anwar & Kunz, 2011). In figure 1b, it can be seen that the OSI value was affected by the concentrations of water and maltodextrin that were added to the emulsion. When water activity and OSI values were compared, it could be observed that a similar trend could be seen (Figure 1b & 1c). The oxygen diffusion not only depends on the porosity of the powder but also on adsorbed water that forms a protective layer against oxidation. These results of the oxidative stability index for canola oil are similar (Merrill et al., 2008), but higher in other studies (Farhoosh et al., 2009). These differences may be associated with the origin of the oil and the use of different antioxidants or processing conditions. Another critical factor that favors the increase of oxidative stability was the presence of phytosterols since they are known to suppress oxidation (Yoshida & Niki, 2003). Oxidative rancidity affects the product's physical stability, nutritional quality, and appearance and it is caused by the product's exposure to oxygen.



*a= solubility, b= oxidative stability index, c= water activity, d= phytosterol content.

Figure 1 : Surface response plot of solubility, oxidative stability index, water activity & phytosterol content.

3.4 Optimization and particle size

For the optimization, solubility, oxidative stability index, and phytosterols content were considered. The optimal points found were 90:10 for the maltodextrin/alginate mixture and 84.30:15.70 for the ratio of water/oil. The optimal experimental treatment was carried out, according to the values established by the desirability function, and the result was a phytosterols content of 40 ± 1.4 mg for each gram of lyophilized products, an induction time of 33.15 ± 1.59 h in the oxidative stability analysis and solubility of 59.96 ± 0.88 %. Successfully, maltodextrin & alginate (80:1 ratio) mixture with water in oil emulsion (80:20 ratio) was applied in flaxseed oil using whey protein isolate as emulsifier agent (Fioramonti et al., 2017). In olive oil, maltodextrin & alginate mixture (12:2.5 ratio) with lemon juice/oil emulsion (1:1 ratio) showed the least suitability for microencapsulating emulsion when compared to the effects of Arabic gum and carboxymethyl cellulose (Silva et al., 2013). In the future is suggested to study more in detail not only the impact of encapsulated agents or oil in water levels but also the effects of emulsifying agents and homogenization conditions towards the microencapsulation of African palm oils to comprehend in more detail the efficiency of wall coating material in a lyophilized process. In powders, particle size is directly proportional to the mass transfer rate from the particle surface to the surrounding (Reineccius, 2004). Figure 2, illustrates the droplet size obtained from the treatments that ranged between 496 (T3) and 734 µm (T8) with a significant difference of 0.05. The average particle size of the freeze-dried African palm oil was higher than those showed in the freeze-dried of olive oil (Silva et al., 2013). In addition, lower particle sizes were reported when other hydrocolloids were used such as gum arabic and carboxymethyl

cellulose with maltodextrin and/or sodium alginate in olive oil (Silva et al., 2013). These results primarily can be related to the application of homogenization when preparing the emulsion (Silva et al., 2013). Additionally, smaller particle size may be associated with lower emulsion dry matter content (Tontul, & Topuz, 2013). Certainly, fluctuations in particle size of lyophilized powders could be associated to process parameters (Zimmermann et al., 2000).

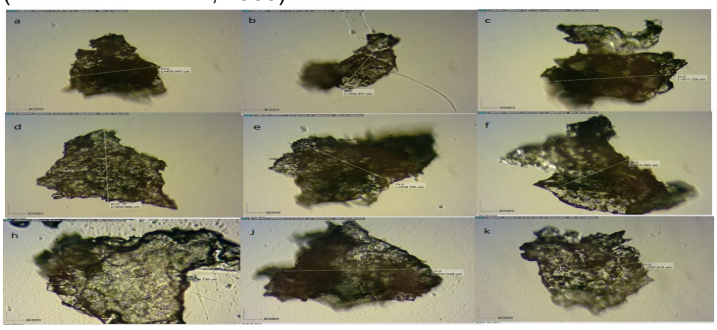


*Figure 2. Particle size and morphology of lyophilized product by inverted microscopy image. a=T1, b=T2, c=T3, d=T4, e=T5 f=T6, h=T7, J=T8, k=T9. * T stands for the treatment number.*

4. Conclusions

The results of this research showed that the surface response methodology is a useful mechanism when determining the effects of maltodextrin and alginate ratios on the oxidation stability, water activity, solubility, and phytosterol content towards the production of lyophilized African oil powder. The results point out that a 90:10 ratio of maltodextrin/alginate and 84.30:15.70 proportion of water/oil levels allowed to maximize the solubility, phytosterol content, and oxidation stability. High concentrations of maltodextrin can lead to higher levels of solubility and oxidation stability, but higher levels of water activity. For future research, it is recommended to examine in more detail not only the influence of wall coating material or emulsion oil composition but also the effects of homogenization processing parameters as well as emulsifiers used.

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