Metadata of the article that will be visualized in OnlineFirst

Please note: Images will appear in color online but will be printed in black and white.

1	Article Title	**Development and Characterization of Phytosterol-Enriched Oil Microcapsules for Foodstuff Application**	
2	Article Sub- Title		
3	Article Copyright - Year	**Springer Science+Business Media, LLC 2017 (This will be the copyright line in the final PDF)**	
4	Journal Name	Food and Bioprocess Technology	
5	Corresponding Author	Family Name	**Tolve**
6		Particle	
7		Given Name	**Roberta**
8		Suffix	
9		Organization	University of Basilicata
10		Division	School of Agricultural, Forestry, Food and Environmental Sciences
11		Address	Viale dell'Ateneo Lucano 10, Potenza 85100
12		e-mail	roberta.tolve@unibas.i
13	Author	Family Name	**Condelli**
14		Particle	
15		Given Name	**Nicola**
16		Suffix	
17		Organization	University of Basilicata
18		Division	School of Agricultural, Forestry, Food and Environmental Sciences
19		Address	Viale dell'Ateneo Lucano 10, Potenza 85100
20		e-mail	
21	Author	Family Name	**Can**
22		Particle	
23		Given Name	**Aygül**
24		Suffix	
25		Organization	Çanakkale Onsekiz Mart University
26		Division	Graduate School of Natural and Applied Sciences
27		Address	Terzioğlu Kampüsü, Çanakkale 17020
28		e-mail	
29	Author	Family Name	**Tchuenbou-Magaia**

30		Particle	
31		Given Name	**Fideline Laure**
32		Suffix	
33		Organization	University of Wolverhampton
34		Division	Chemical Engineering, Faculty of Science and Engineering
35		Address	Wulfruna Street, Wolverhampton WV1 1LY
36		e-mail	

37		Received	15 May 2017
38	Schedule	Revised	
39		Accepted	28 August 2017

40	Abstract	Phytosterols are lipophilic compounds contained in plants and have several biological activities. The use of phytosterols in food fortification is hampered due to their high melting temperature, chalky taste, and low solubility in an aqueous system. Also, phytosterols are easily oxidized and are poorly absorbed by the human body. Formulation engineering coupled with microencapsulation could be used to overcome these problems. The aim of this study was to investigate the feasibility of encapsulating soybean oil enriched with phytosterols by spray-drying using ternary mixtures of health-promoting ingredients, whey protein isolate (WPI), inulin, and chitosan as carrier agents. The effect of different formulations and spray-drying conditions on the microencapsules properties, encapsulation efficiency, surface oil content, and oxidation stability were studied. It was found that spherical WPI-inulin-chitosan phytosterol-enriched soybean oil microcapsules with an average size below 50 μm could be produced with good encapsulation efficiency (85%), acceptable level of surface oil (11%), and water activity (0.2–0.4) that meet industrial requirements. However, the microcapsules showed very low oxidation stability with peroxide values reaching 101.7 meq O_2/kg of oil just after production, and further investigations and optimization are required before any industrial application of this encapsulated system.

41	Keywords separated by ' - '	Phytosterols - Microencapsulation - Spray drying - Inulin - Chitosan - Whey protein isolate - Emulsion

42	Foot note information	

Food Bioprocess Technol
DOI 10.1007/s11947-017-1990-4

ORIGINAL PAPER

Development and Characterization of Phytosterol-Enriched Oil Microcapsules for Foodstuff Application

Roberta Tolve[1] · **Nicola Condelli**[1] · **Aygül Can**[2] · **Fideline Laure Tchuenbou-Magaia**[3]

Received: 15 May 2017 / Accepted: 28 August 2017

Abstract Phytosterols are lipophilic compounds contained in plants and have several biological activities. The use of phytosterols in food fortification is hampered due to their high melting temperature, chalky taste, and low solubility in an aqueous system. Also, phytosterols are easily oxidized and are poorly absorbed by the human body. Formulation engineering coupled with microencapsulation could be used to overcome these problems. The aim of this study was to investigate the feasibility of encapsulating soybean oil enriched with phytosterols by spray-drying using ternary mixtures of health-promoting ingredients, whey protein isolate (WPI), inulin, and chitosan as carrier agents. The effect of different formulations and spray-drying conditions on the microencapsules properties, encapsulation efficiency, surface oil content, and oxidation stability were studied. It was found that spherical WPI-inulin-chitosan phytosterol-enriched soybean oil microcapsules with an average size below 50 μm could be produced with good encapsulation efficiency (85%), acceptable level of surface oil (11%), and water activity (0.2–0.4) that meet industrial requirements. However, the microcapsules showed very low oxidation stability with peroxide values reaching 101.7 meq O_2/kg of oil just after production, and further investigations and optimization are required before any industrial application of this encapsulated system.

Keywords Phytosterols · Microencapsulation · Spray drying · Inulin · Chitosan · Whey protein isolate · Emulsion

Introduction

Phytosterols are members of the triterpene family, natural occurring bioactive compounds found in plants and vegetables. Rice bran oil, soybean oil, corn oil, sesame seeds, wheat germ oil, nuts, and pistachios are some natural sources of plant sterols (Moreau et al. 2002; Bacchetti et al. 2011; Gupta et al. 2011; Alemany et al. 2014). The most common plant sterols, campesterols, β-sitosterols, stigmasterols, and ergosterol, are presented in Fig. 1 (Fernandes and Cabral 2007). The chemical structure of phytosterols is similar to cholesterol, with minor differences in relative position of ethyl and methyl groups at C-24 or a double bond at C-22. This similarity explained their interfering with the uptake of both dietary and biliary cholesterol from the intestinal tract. It is well established that high intakes of plant sterols can lower serum total and LDL cholesterol concentrations in humans. Moreover, phytosterols present other benefits, such as a strong anti-inflammatory and anti-diabetic activity and the involvement in the prevention of colon, breast, and prostate cancer (Gabay et al. 2010; Grattan 2013; Panda et al. 2009). For example, Botelho et al. (2014) reported that a daily consumption of 2 g of plant sterols results in a reduction of up to 8.8% of the plasma level of LDL cholesterol. In the wake of these results, in recent years, the production of foods fortified with plant sterols has increased (García-Llatas and Rodríguez-Estrada 2011). These include margarines and dairy products.

✉ Roberta Tolve
roberta.tolve@unibas.i

[1] School of Agricultural, Forestry, Food and Environmental Sciences, University of Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy

[2] Graduate School of Natural and Applied Sciences, Çanakkale Onsekiz Mart University, Terzioğlu Kampüsü, 17020 Çanakkale, Turkey

[3] Chemical Engineering, Faculty of Science and Engineering, University of Wolverhampton, Wulfruna Street, Wolverhampton WV1 1LY, UK

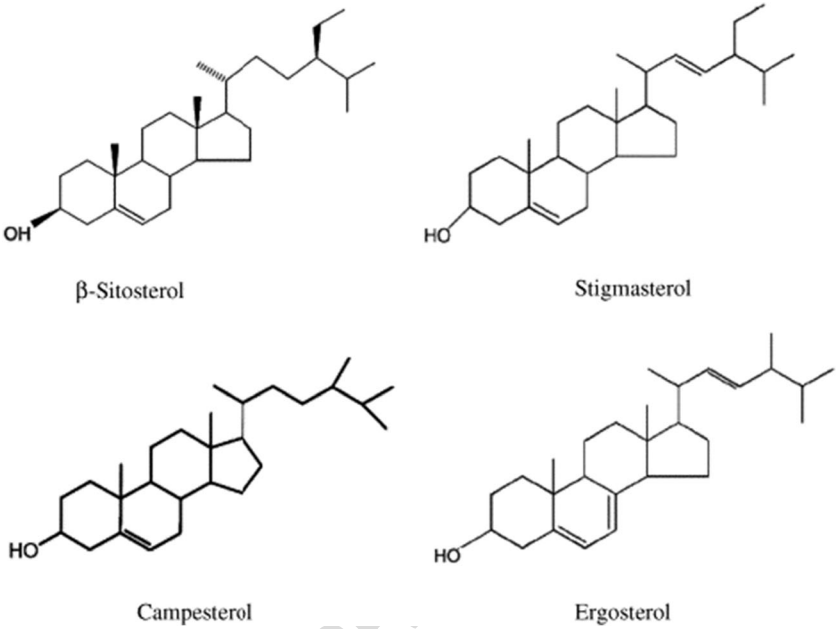

Fig. 1 Molecular structure of some representative phytosterols (Fernandes and Cabral 2007)

However, the incorporation of phytosterols into food products is still limited due to their susceptibility to oxidation, especially during high-temperature processing and storage (Fujiwara et al. 2013; Botelho et al. 2014). The oxidation rate also increases with exposure to air, light, chemical agents, and enzymes (Ryan et al. 2009) and leads to the formation of compounds, known oxidation products of phytosterols (POPs). POPs have no beneficial effect and also can cancel out the cholesterol-lowering action of phytosterols (García-Llatas and Rodríguez-Estrada 2011; Liang et al. 2011). For example, cholesterol oxidation products (COPs) have harmful effects such as atherogenic, cytotoxic, mutagenic, and carcinogenic (Valenzuela et al. 2003; Hur et al. 2007). There are few studies that focused on POPs but as a consequence of the structural similarity between plant sterols and cholesterol, the unfavorable effects of COPs to health can be also expected from POPs (Alemany et al. 2014). Other challenges related to the incorporation of phytosterols in food include their chalky taste and water insolubility (Izadi et al. 2012). These challenges could be overcome through formulation and microencapsulation ("micro packaging") in protective matrices. Spray drying is the most common method used for microencapsulation in food industry (Ghosh 2006, Tolve et al. 2016). Before the spray-drying process, the stability of feed emulsion has to be taken into account owing to hydrophobic nature of core material (Gharsallaoui et al. 2007). The first step of the microencapsulation process is the selection of the appropriate wall materials for the core material. Carbohydrates with shorter chains act as matrix formers. Among this, inulin is an interesting polymer. Inulin acts in the body like a dietary fiber, contributing to the improvement of the gastrointestinal system conditions and positively modulating cholesterol metabolism (Costa et al. 2015). Unfortunately, inulin, like most carbohydrates, lacks any emulsifying properties and is being increasingly studied for use in polymeric mixtures or in combination with other encapsulant polymers, like proteins (Botrel et al. 2014; Fernandes et al. 2014). Proteins are often used as oil encapsulants due to their excellent emulsifying and film-forming properties (Gharsallaoui et al. 2007). Whey protein isolate (WPI), an important by-product of cheese production, is widely used in the food industry, both for their emulsifying properties and for their nutritional aspects (Pal et al. 2010). WPI has unsurpassed nutritional quality and inherent functional properties that meet the demands of encapsulation (Ezhilarasi et al. 2013). During the emulsification step, these proteins change their conformation and position themselves in the oil-water interface contributing to the production of a significantly more stable emulsions (Fernandes et al. 2017). The microencapsulation of lipophilic molecules with WPI has led to the production of thick coatings with low porosity and excellent gas barrier against oxygen (Lin and Zhao 2007; Mehyar et al. 2014). Chitosan is a polysaccharide known for its excellent film-forming properties, antioxidant activity, and cholesterol-lowering and emulsion properties already used for the microencapsulation of some lipophilic ingredients such as vitamin D_2, astaxanthin, and olive oil (Rodriguez et al. 2002; Kim and Thomas 2007; Klaypradit and Huang 2008). The use of a combination of wall materials for desired properties increases efficiency of microencapsulation process (da Silva et al. 2014). Recently, inulin with arabic gum was used for microencapsulation of lipophilic molecules, and good results have been found both in terms of yield and emulsion stability (Turchiuli et al. 2014). Moreover, the highest oil encapsulation efficiencies were obtained with protein in combination

with carbohydrates, when compared to the formulation containing only protein (Drusch and Mannino 2009). Emulsions made with a mixture of WPI and chitosan had showed higher stability than that made using only WPI (Speiciene et al. 2007). Considering the well-known characteristics of phytosterols and the lack of studies on its microencapsulation, this study evaluated the feasibility of spray-drying microencapsulation process using inulin, WPI, and chitosan as wall materials. Soybean oil rich in PUFA enriched with phytosterol was used as a core material. The oxidative stability of core material and the retention of phytosterols were evaluated after drying for time zero (t_0) and after a refrigerated storage for 5 months (t_1). The effects of different inlet air temperatures as well as the wall material and the phytosterol concentration on the properties of powder were studied.

Material and Methods

Materials

Phytosterols (Phytopin®, DRT, France) and soybean oil (Sigma-Aldrich, UK) were used as microcapsules' core materials. Whey protein isolate (WPI, protein content 90%, Tecnoblend, Italy), inulin (Orafti® HPX, Belgium), and chitosan (Sigma-Aldrich, Italy) were used as coating materials. Ultra pure water (UPW) was used for analysis whereas distilled water was used for the preparation of microcapsules. Asolectin from soybean (Sigma-Aldrich, UK) was used as processing aid to increase the solubility of phytosterols in soybean oil. All the other chemicals were purchased from Sigma-Aldrich (Italy) and all of them were of analytical grade.

Preparation of Emulsions

Stable oil in water emulsions were produced and spray dried to generate microcapsules. Three different formulations (WI, WIC, and WI2C) with two different phytosterol levels were prepared by mixing different concentration of WPI, inulin, chitosan, asolectin, and phytosterols. The aqueous phase was obtained by mixing a stock solution of WPI with inulin solution containing or not containing chitosan to get the concentrations and the total solid concentration reported in Table 1. In detail, WPI stock solution at 9% (*w*/*w*) was prepared by mixing WPI powder with distilled water using a magnetic stirrer, keeping overnight at room temperature to ensure complete protein dissolution. The pH of the solution was then adjusted to 6.7 using NaOH (1 M) or 3 using HCL (1 M). Chitosan 0.125% (*w*/*w*) in the formulation was obtained from a 2% (*w*/*w*) chitosan stock solution made by dissolving chitosan in distilled water containing 1% of acetic acid with the aid of a magnetic stirrer. Inulin solution was prepared as 1% (*w*/*w*) and 2% (*w*/*w*) by dissolving inulin in water or in chitosan solution. Inulin or inulin containing chitosan was added to WPI solution, and the pH adjusted at 3 or 6.7 according to the presence or absence of chitosan. The oil phase was prepared by dissolving the phytosterols in 25 g soybean oil in order to obtain 5 and 10% (*w*/*w*) solution, with or without asolectin as processing aid. It should be noted that phytosterols also showed relatively low solubility in soybean oil, and a homogeneous oil phase with 10% (*w*/*w*) phytosterols could not be achieved without asolectin. The solution was heated at 70 °C until the phytosterols were completely dissolved. Fine emulsions (aqueous/oil phase at 4:1 ratio (*w*/*w*)) were produced by a homogenizer (Silverson, L4R, Silverson Machines Limited, Buckinghamshire, UK) at 8000 rpm for 4 min. As reported in Table 1, each formulation is characterized by a different concentration of total solids which range from 25.76 to 27.32% (*w*/*w*).

Emulsion Characterization

The microstructure of O/W emulsions was examined with an optical upright microscope (Leica Model DM 2500 microscope base, Wetzlar, Germany). The particles' size and size distribution of the emulsions were measured using a laser light diffraction instrument, Mastersizer (Malvern Instruments Ltd., UK). All measurements were carried out at 20 °C, and the results reported are averages of three readings. The stability of emulsions was evaluated over time, by measuring the change in size distribution with time using the Mastersizer as described above. The rheological measurements were carried out on an advanced rheometer AR 2000 (TA Instruments, USA) equipped with a cone and plate geometry. The rotating cone was 60 mm acrylic plate geometry. All the measurements were carried out at 20 °C.

Microencapsulation by Spray Drying

Spray-drying process was performed in a laboratory-scale Mini Spray Dryer Büchi B-290 (Büchi, Swiss) equipped with a 0.7-mm nozzle. The emulsions were co-current fed into the main chamber through a peristaltic pump. The flow rate of the emulsion and the compressor air pressure were kept constant at 2 mL/min and 6 bars respectively for all the experiments. Technical data and a scheme of the drying apparatus can be found elsewhere (Mini spray dryer B-290 technical data sheet). Three different inlet air temperatures were used (125 ± 4, 155 ± 4, and 185 ± 4 °C), and the outlet air temperature results were 67 ± 5, 95 ± 5, and 115 ± 5 °C, respectively. During the spray-drying process, the emulsions were gently magnetically stirred to prevent creaming of the emulsion droplets. The finished microcapsules were stored in containers sealed with screw caps until further used and analysis.

t1.1 **Table 1** Composition of feed emulsions and total solid concentration

Feed suspension	Water phase			Oil phase		Total solid concentration (wall material + oil) [%w/w]
	WPI [%w/w]	Inulin [%w/w]	Chitosan [%w/w]	Asolectin [%w/w]	Phytosterols [%w/w]	
WI-P5	6.75	1	–	–	5	25.76
WI-P10	6.75	1	–	0.25	10	26.6
WIC-P5	6.75	1	0.125	–	5	25.84
WIC-P10	6.75	1	0.125	0.25	10	26.28
WI2C-P5	6.75	2	0.125	–	5	26.48
WI2C-P10	6.75	2	0.125	0.25	10	27.32

Characterization of the Microparticles

The morphology of the microparticles was observed using scanning electron microscopy (SEM) apparatus (Philips-FEI ESEM XL30). A water activity meter (AquaLab PawKit, Decagon Devices, USA) was used to measure a_w of the spray-dried powders. All measurements were carried out at 25 °C. The moisture content was determined based on AOAC method (AOCS 2000). Specifically, 2-g samples were weighed and dried in oven at 105 °C until its weight is constant. Real density (g cm^{-3}) values were measured using a helium pycnometer (Micromeritics AccuPyc II 1340). Approximately 0.20-g samples were weighted and placed in the testing cup, and then, 10 readings for the density were taken over 20 cycles of pumping and evacuating helium on the sample.

Encapsulation Yield

The encapsulation yield (EY%) was determined as the ratio of the amount of powder collected after every spray-drying experiment to the initial amount of solids contained in the feed suspensions (Eq. (1)):

$$EY = \frac{mass\ of\ powder\ collected}{mass\ of\ solid\ \text{fed}} \times 100 \quad (1)$$

Surface Oil and Encapsulation Efficiency

The amount of total oil and surface oil were determined to calculate the encapsulation efficiency (EE). The total oil content of the powder was determined by Soxhlet apparatus. A known weight of microcapsules was put into an extraction thimble which was closed with glass wool and then placed in a Soxhlet extractor. A condenser was installed on top of the Soxhlet and fed with cooling water at a temperature of 60 °C; the round evaporation flask was filled with 300 ml of petroleum ether and connect to the Soxhlet extractor. The petroleum ether was heated to boiling point and run for 5.5 h. Afterwards, the evaporation flask was put into a rotary evaporator and the petroleum ether was evaporated at 60 °C. A water vacuum pump was used to accelerate the process. Then, the oil contained in the evaporation flask was weighed. The amount of surface oil was determined by a modified method described by Carneiro et al. (2013). Hexane (75 ml) was added to 2 g of powder followed by stirring for 10 min at room temperature. After filtration through a filter paper, the solvent was evaporated in a rotary evaporator (at 60 °C) until constant weight. The non-encapsulated oil was determined by mass difference between the initial clean flask and that containing the extracted oil residue. The ratio of the amount of encapsulated oil to the initial oil amount is defined as the encapsulation efficiency (EE) and was expressed as a percentage (%) according to the Eq. (2):

$$EE = \frac{mass\ of\ total\ oil\text{-}mass\ of\ surface\ oil}{mass\ of\ total\ oil} \times 100 \quad (2)$$

Peroxide Value

Peroxide value (PV) of the encapsulated soybean oil enriched with phytosterols, as extracted above, was measured according the method of the American Oil Chemist's Society (AOCS) Official method Cd 8–53 (AOCS 1989) as follows: 2 g of soybean oil was weighed into a 250-mL Erlenmeyer flask, 25 mL acetic acid/chloroform mixture (3:2 *v*/*v*) was added, and the mixture was swirled for the dissolution of soybean oil. One milliliter of fresh saturated aqueous potassium iodide solution was added; the flask was gently mixed for 1 min and left to stand in darkness for 5 min at room temperature. Then, 75 mL distilled water was added and the content was titrated against 0.01 N sodium thiosulphate ($Na_2S_20_3$) (using starch indicator). PV, expressed as milliequivalents of active oxygen per kilogram of oil (meq O_2/kg), was calculated as follows (Eq. (3)):

$$PV = \frac{S + N}{m} \times 1000 \quad (3)$$

where S is titrant volume (mL), m is sample weight (g), and N is normality of $Na_2S_20_3$.

Loading Capacity

The loading capacity (LC) of microcapsules was performed by estimating the amount of phytosterols loaded in dried particles. Hence, the quantification of encapsulated phytosterols was carried out by calibration curve method using the Liebermann-Burchard reagent. A phytosterol standard solution was prepared by dissolving 10 mg of phytosterols in 10 mL of chloroform. 0, 0.25, 0.5, 0.75, 1, or 1.5 ml of this standard solution was pipetted out into 6 test tubes. Then, 2 mL of the Liebermann-Burchard reagent was added to all tubes. The volume was adjusted to 5.5 ml with chloroform. The samples remained at room temperature, protected from light for 15 min. After this period, concentrations were measured in a spectrophotometer (Cary 1E, Varian, Australia) at 640 nm. The procedure was performed in triplicate. The Liebermann-Burchard reagent was prepared in a 500-mL amber glass bottle fitted with a polyseal cap where 220 mL of cold acetic anhydride and 200 mL of glacial acetic acid were mixed by inversion followed by the addition of 30 ml of cold concentrated sulfuric acid (Kim and Goldberg 1969). The encapsulated phytosterols were measured by adding 2 mL of Liebermann-Burchard reagent into 2 mL of oil extracted from the microparticles. The final volume was completed to 5.5 mL with chloroform, and the absorbance of the samples was measured in the same conditions as for the standard (Fujiwara et al. 2013). Phytosterol concentration was determined by reference to the standard curve whereas the loading capacity was evaluated from the following equation (Eq. (4)):

$$LC = \frac{mass\ of\ encapsulated\ phytosterols}{mass\ of\ powder} \times 1000 \qquad (4)$$

Statistical Analyses

The results obtained were analyzed using a three-way analysis of variance (ANOVA) in order to evaluate the effect of three different formulations, two phytosterol concentrations, and three Inlet air-drying temperatures on the obtained microparticles. The post hoc test LSD was performed on the mean value for each factor. All statistical procedures were computed using the statistical package SYSTAT for Windows (ver. 10, 2003) (Systat Software, Chicago, IL).

Results and Discussion

Emulsion Characterization

Different formulations of soybean oil in water emulsions were made. The soybean oil was fortified with 5 or 10% of phytosterols, and the aqueous phase contained 6.75% (*w*/*w*) WPI, 0.125% (*w*/*w*) chitosan, and 1% or 2% (*w*/*w*) inulin. The percentage of solids was within the wall material concentration recommended for oil encapsulation from 20 to 40% and the typical wall to core material ratio of 4:1 (*w*/*w*) (Jafari et al. 2008a). Figure 2 is a typical light micrograph showing the microstructure of these emulsions. Although the micrographs show a droplet size < 10 μm, the Mastersizer analysis revealed larger particle size with a bimodal or trimodal character depending on the phytosterol concentration in the oil phase (Fig. 3). This discrepancy could be explained by a lack of information about the optical properties of the emulsions. Although a refractive index of 1.45 reported in the literature for emulsions stabilized by whey protein isolate (Sun and Gunasekaran 2010) was chosen for the size measurement by the Mastersizer, the emulsions in this study also contain inulin and chitosan. WPI is known to form aggregates when treated at high temperature such as 70 °C used for the preparation of emulsions. Particle size below 2 μm could be attributed to these protein aggregates. Moreover, the formation of WPI aggregates and network around the droplet surface at high temperature may have also accounted for the apparent larger droplet size revealed by the Mastersizer. Figure 3 also shows a reduction in the percentage of protein aggregates with the increase in inulin concentration. A shift from a trimodal to a bimodal particle size distribution could be seen when the concentration of phytosterols was increased in the formulation. This was presumably due to the presence of asolectin in formulation with 10% phytosterols.

The emulsion stability has been reported to have an influence on microencapsulation efficiency and on properties of spray-dried oil powders (Tan et al. 2005). For this reason, the emulsions must remain stable during the spray-drying process. In the present study, the time taken to complete a spray-drying process for one batch was approximately of 60–90 min during which no coalescence and change in oil droplet size or in the emulsion particle size distribution were observed (Table 2). The viscosity of the emulsions was measured to find out the effect of the different formulations on the viscosity which is known to affect the particle size of the spray droplets and the properties of the resultant powders (Fig. 4). Apparent viscosities of the emulsions, at 145 s^{-1} shear rate, are presented in Table 2. Table 2 also contains the values for power law model and shows the empirical consistency and flow behavior indices. This model suitably explains the experimental data where r^2 values ranged from 0.924 to 0.976. The consistency index provides an indication of the flow properties of the feed suspension, and the flow behavior index (*n*) indicates how close the feed suspension is to Newtonian. The flow behavior index of the feed

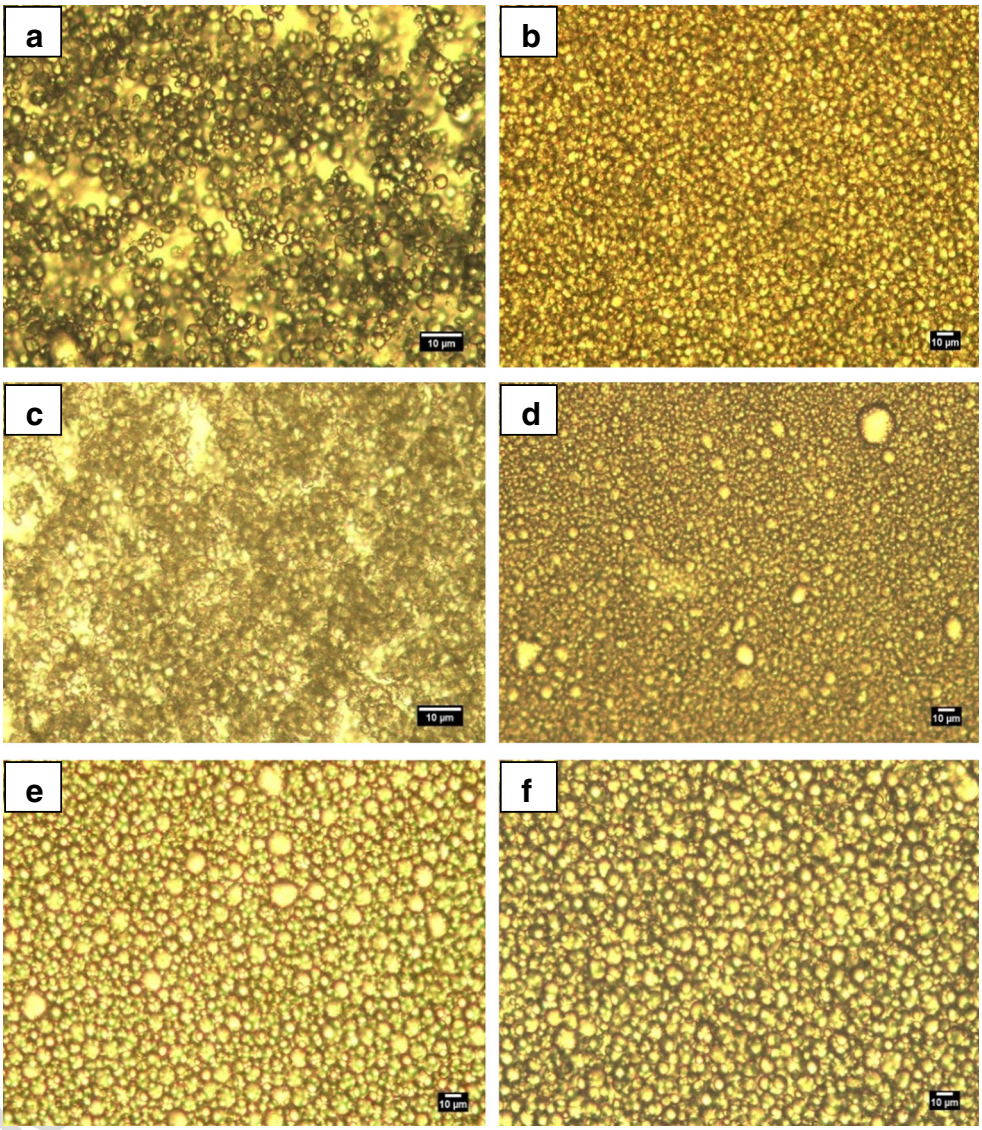

Fig. 2 Optical micrograph of different emulsions obtained by mixing soybean oil, fortified with 5 or 10% of phytosterols, with different wall materials: WI-P5 (**a**), WI-P10 (**b**), WIC-P5 (**c**), WIC-P10 (**d**), WI2C-P5 (**e**), WI2C-P10 (**f**)

suspensions was ranging from 0.1916 and 0.8072, which was considered to be pseudoplastic or shear-thinning fluids ($n < 1$). The consistency index increased with increasing the phytosterol concentration whereas formulation WI2C-P5 exhibited the lower consistency index (0.003 mPa.s^n) while WIC-P10 showed the highest one (2.578 mPa.s^n). Moreover, the increase in viscosity could not be explained only by the increase in the total solid mass as sample formulated with 10% phytosterols and 1% inulin showed higher viscosity (WIC-P10) than that with the same phytosterol concentration but formulated with 2% inulin. Inulin is known to form complex with whey proteins (Schaller-Povolny and Smith 2002). Therefore, it may be postulated that more interaction between inulin and whey proteins occurred at 2% inulin via hydrophobic interaction or Maillard reactions between amino groups and the reducing groups of inulin during the emulsion production at 70 °C. This hampered protein-protein interaction in the aqueous phase resulting in the observed lower viscosity.

Characterization of the Microparticles

Figure 5 is a typical SEM microphotograph of the produced microparticles. The particles are mostly spherical with smooth surface and no visible large pores or fissures. These results indicate that the microparticles could have lower permeability to gases. However, the particle size distribution was very broad which is one of the drawbacks of the spray-drying technology (Carneiro et al. 2013). Water activity (a_w) and moisture content are important indices for spray-dried powder since they can affect the powder shelf life. Generally, food with $a_w < 0.6$ is considered as microbiologically stable and if there is any spoilage occur, it is induced by chemical reactions rather than by microorganism. From the results (Table 3), the a_w

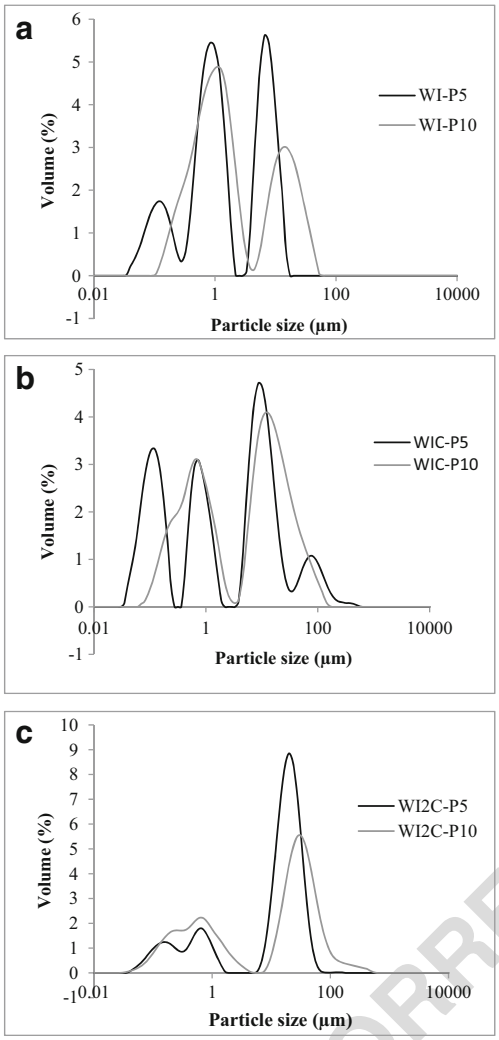

Fig. 3 Particle size distribution of emulsion WI-P5 and WI-P10 (**a**), WIC-P5 and WIC-P10 (**b**, **d**), and WI2C-P5 and WI2C-P10 (**c**)

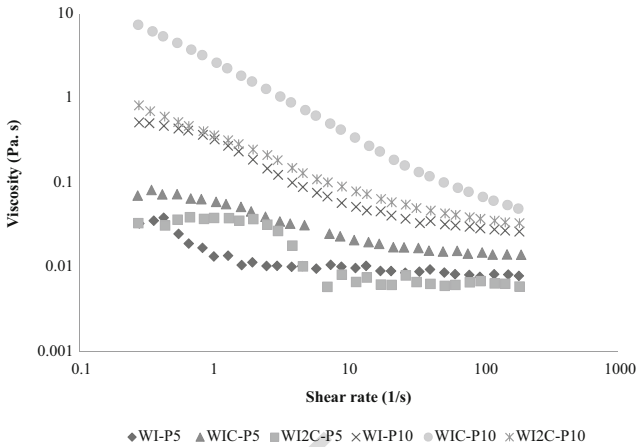

Fig. 4 Log-log plot of viscosity as function of share rate of the emulsion obtained from soybean oil, enriched with different phytosterol concentrations, inulin, and WPI in different concentrations, with or without chitosan

of the samples was in the range of 0.24–0.44. This means that the spray-dried powders produced were relatively microbiologically stable. As shown in Table 4, the a_w of the powders significantly changed with spray-drying inlet air temperature ($p < 0.05$). Also, the coating formulation which is related to the total solid concentration significantly affect the a_w value ($p < 0.05$). The lowest a_w value was found in powder produced by feeding spray dryer at 185 °C with the emulsion with the lowest total solid concentration, made by using from 6.75% WPI and 1% inulin as core material and fortified soybean oil with 5% of phytosterols. The highest a_w was found in powder obtained by feeding spray dryer at 125 °C with the emulsion characterized by the highest total solid concentration, produced with 6.75% WPI, 2% inulin, 0.125% chitosan as core material, and fortified soybean oil with 10% of phytosterols. Also, the phytosterol concentration affected the a_w and significantly lower value has been obtained for the formulation characterized by a higher phytosterol concentration ($p < 0.05$). The moisture of the microcapsules ranged from 2.6 to 5% and was affected by the inlet air-drying temperatures, the formulation, and the phytosterol concentration ($p < 0.05$). Lower humidity values were found in powders produced by feeding spray dryer at 185 °C. The lowest moisture content value was found in powder produced by feeding spray dryer with the emulsion made by using from 6.75% WPI and 1% inulin as core material and fortified soybean oil with 5% of phytosterols. The highest moisture content value

Q4 **Table 2** Rheological parameters, apparent viscosity, and particle size distribution for the feed suspensions produced with the different wall materials

Feed suspension	Rheological parameters			Apparent viscosity at 145 s^{-1} (Pa.s)	Particle size distribution parameters			
	Consistency index, k ($Pa.s^n$)	Flow behavior index, *n*	r^2		D[4.3] (μm) (mean ± SD)	d50 (μm) (mean ± SD)	d90 (μm) (mean ± SD)	Span (mean ± SD)
WI-P5	0.017	0.807	0.976	$8.144 * 10^{-3}$	3.554 ± 0.001	1.205 ± 0.001	9.666 ± 0.001	7.897 ± 0.004
WI-P10	0.474	0.255	0.940	$26.98* 10^{-3}$	5.779 ± 0.039	1.254 ± 0.005	18.498 ± 0.120	14.493 ± 0.044
WIC-P5	0.027	0.638	0.924	$14.09* 10^{-3}$	15.160 ± 0.508	2.606 ± 1.476	30.326 ± 5.084	13.167 ± 4.151
WIC-P10	2.578	0.192	0.945	$54.26* 10^{-3}$	30.913 ± 4.271	13.822 ± 2.365	80.073 ± 5.219	5.835 ± 0.600
WI2C-P5	0.003	0.641	0.925	$6.332* 10^{-3}$	15.290 ± 0.170	15.104 ± 0.142	30.364 ± 0.179	1.996 ± 0.008
WI2C-P10	0.340	0.481	0.971	$33.87* 10^{-3}$	26.281 ± 1.301	17.515 ± 0.328	56.662 ± 1.426	3.227 ± 0.028

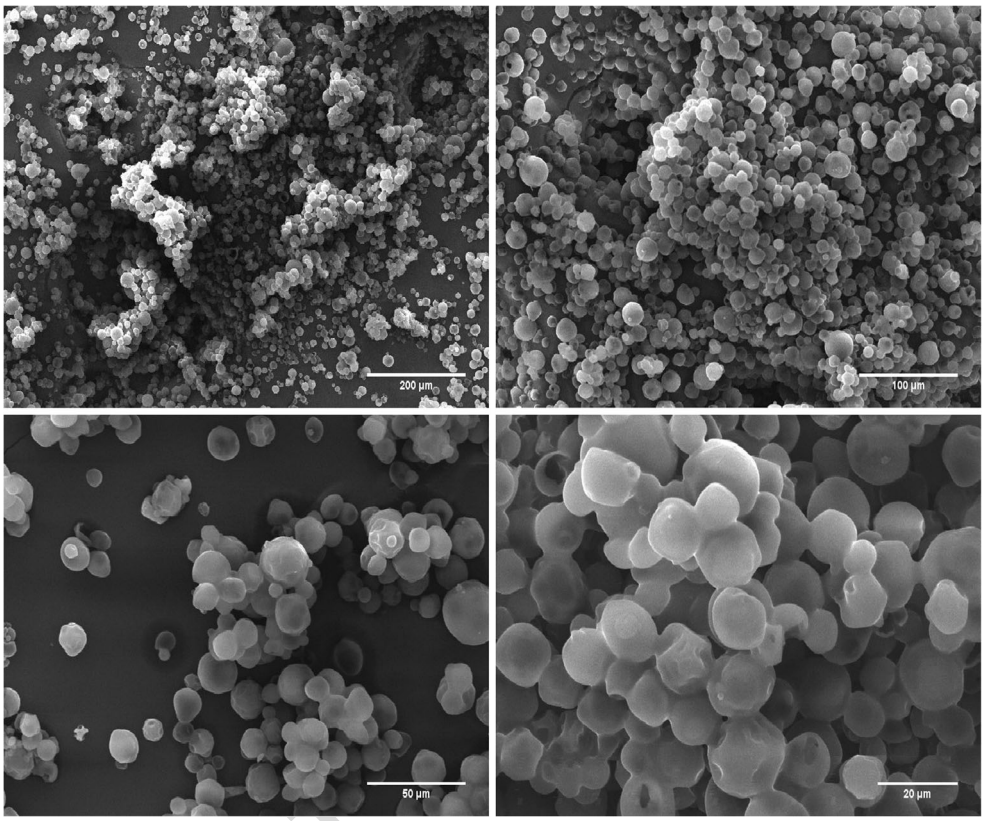

Fig. 5 Microphotographs of particles produced at 185 °C utilizing formulation WI-P5 taken at ×250, ×500, ×1000, and ×2000

was found in powder obtained by feeding spray dryer with an emulsion produced with 6.75% WPI, 2% inulin, 0.125% chitosan as core material, and fortified soybean oil with 10% of phytosterols. Also, the phytosterol concentration affected

t3.1 **Table 3** Characteristics of the generated microcapsules using different wall materials at different drying air temperatures

t3.2	Sample	Inlet drying temperatures (°C)	a_w	Moisture content (%)	Encapsulation efficiency of oil (%)	Surface oil (%)	Loading capacity (%)	Peroxide value (meq O_2/kg)	Real density (g/cm^3)
t3.3	WI-P5	125	0.26 ± 0.00	4.3 ± 0.1	68 ± 5	17.33 ± 0.15	0.42 ± 0.01	29.66 ± 0.67	1.10 ± 0.00
t3.4		155	0.26 ± 0.02	3.3 ± 0.2	78 ± 7	14.70 ± 1.10	0.39 ± 0.01	34.27 ± 0.62	1.08 ± 0.00
t3.5		185	0.24 ± 0.02	2.6 ± 0.2	79 ± 2	12.46 ± 0.75	0.55 ± 0.01	41.16 ± 0.81	1.08 ± 0.00
t3.6	WI-P10	125	0.35 ± 0.02	4.8 ± 0.1	69 ± 4	18.39 ± 0.64	0.49 ± 0.01	41.49 ± 0.05	1.07 ± 0.00
t3.7		155	0.32 ± 0.01	4.2 ± 0.0	61 ± 3	16.43 ± 0.70	0.40 ± 0.01	43.94 ± 0.90	1.07 ± 0.00
t3.8		185	0.31 ± 0.02	4.0 ± 0.0	65 ± 1	13.74 ± 0.34	0.78 ± 0.02	45.51 ± 0.40	1.08 ± 0.00
t3.9	WIC-P5	125	0.38 ± 0.01	4.6 ± 0.0	71 ± 0	18.87 ± 0.09	0.51 ± 0.01	56.89 ± 1.43	1.06 ± 0.00
t3.10		155	0.38 ± 0.01	4.1 ± 0.0	76 ± 2	15.69 ± 0.33	0.48 ± 0.01	58.01 ± 0.74	1.03 ± 0.00
t3.11		185	0.28 ± 0.01	3.7 ± 0.0	79 ± 0	13.97 ± 0.69	0.50 ± 0.00	62.51 ± 3.99	1.07 ± 0.00
t3.12	WIC-P5	125	0.40 ± 0.01	4.8 ± 0.0	73 ± 2	18.43 ± 0.62	0.64 ± 0.00	62.42 ± 0.35	1.10 ± 0.00
t3.13		155	0.38 ± 0.02	4.4 ± 0.0	66 ± 1	17.66 ± 0.31	0.85 ± 0.01	81.97 ± 2.63	1.08 ± 0.00
t3.14		185	0.32 ± 0.02	4.2 ± 0.0	74 ± 4	15.42 ± 0.68	0.95 ± 0.01	101.74 ± 4.18	1.07 ± 0.00
t3.15	WI2C-P5	125	0.41 ± 0.01	5.0 ± 0.0	69 ± 0	16.92 ± 0.71	0.54 ± 0.03	44.50 ± 0.77	1.10 ± 0.00
t3.16		155	0.42 ± 0.01	4.5 ± 0.0	72 ± 2	15.20 ± 0.33	0.53 ± 0.01	49.68 ± 0.47	1.08 ± 0.00
t3.17		185	0.40 ± 0.01	4.3 ± 0.0	76 ± 1	13.96 ± 0.01	0.54 ± 0.04	60.89 ± 4.07	1.09 ± 0.00
t3.18	WI2C-P10	125	0.44 ± 0.01	5.2 ± 0.0	80 ± 0	13.43 ± 0.72	0.94 ± 0.01	60.07 ± 1.23	1.10 ± 0.00
t3.19		155	0.37 ± 0.02	4.6 ± 0.0	83 ± 0	11.49 ± 0.71	0.86 ± 0.01	67.72 ± 5.59	1.09 ± 0.00
t3.20		185	0.37 ± 0.01	4.5 ± 0.0	85 ± 1	9.68 ± 0.34	0.85 ± 0.06	69.41 ± 0.82	1.09 ± 0.04

Results are expressed as mean ± SD

t4.1 **Table 4** Characteristics of the generated microcapsules taking into account three different formulations, two phytosterol concentrations, and three inlet drying temperatures

t4.2 Factors		Loading capacity (%)		Encapsulation efficiency (%)		a_w		Moisture content(%)		Peroxide value (meq O_2/kg)	
t4.3		*p*		*p*		*p*		*p*		*p*	
t4.4 Formulation	WI	< 0.005	0.50^a		70^b		0.29^c		3.9^c		39.34^c
t4.5	WIC		0.66^b	< 0.005	73^b	< 0.005	0.36^b	< 0.005	4.3^b	< 0.005	58.71^b
t4.6	WI2C		0.71^c		77^a		0.40^a		4.7^a		70.59^c
t4.7 Phytosterol concentration	P5	< 0.005	0.50^a	n.s.	73^a	< 0.005	0.34^b	< 0.005	4.1^b	< 0.005	48.62^b
t4.8	P10		0.71^b		74^a		0.36^a		4.5^a		63.81^a
t4.9 Inlet air drying temperatures (°C)	125 °C	< 0.005	0.59^a		72^b		0.32^b		3.9^c		49.18^c
t4.10	155 °C		0.59^a	< 0.005	73^b	< 0.005	0.36^a	< 0.005	4.2^b	< 0.005	55.93^b
t4.11	185 °C		0.70^b		76^a		0.37^a		4.8^a		63.53^c

For each column, means with different superscript letters are significantly different (LSD test at $p < 0.05$)

the a_w and significantly lower value has been obtained for the formulation characterized by a higher phytosterol concentration ($p < 0.05$).

Encapsulation Yield and Encapsulation Efficiency

Encapsulation yield and encapsulation efficiency are key aspects that must be considered in microencapsulation process. The yield of microcapsules produced by spray drying depends on the experimental conditions (inlet air temperature, flow rate, and compressed air flow). In the present study, the microcapsules' yield ranged from 38.6 to 67.57% (Fig. 6) and mainly influenced by the inlet air temperature. Increased product yield was obtained with an increase in inlet air temperature from 125 to 185 °C, which can be attributed to the greater efficiency of heat and mass transfer processes and to decrease of the powder moisture and stickiness that resulted in a reduced adhesion on the inner surface of the drying chamber. Q5 This is in agreement with the results published by Cai and Corke (2000) and Tonon et al. (2008). The encapsulation efficiency (EE%) is commonly determined indirectly by extracting the non-encapsulated oil present on the surface of microcapsules through washing powders with an organic solvent (Velasco et al. 2003). The presence of free oil influences adversely the physical properties of spray-dried powders; in particular, it could induce more rapid lipid oxidation (Bae and Lee 2008). The surface oil contents were in the range of 9.68–18.78% whereas the EE % were from 51.28 to 86.03% (Table 2). The highest encapsulation efficiency was found for the formulation WI2C-P10 with the highest total solid content and was significantly related to the inlet air drying temperatures. Also, Jafari et al. (2008b) have found that the total solid concentration had a positive effect on the encapsulation efficiency. This could be explained by the kinetics of crust formation at the surface of the droplet. A higher solid content increases the rate of the crust formation, reducing the diffusion of the oil to the drying particle surface. This is supported by the decrease in the quantity of surface oil with the increased of the inlet air drying temperatures. These findings corroborate the results from the study of inlet air temperature on the microencapsulation of flaxseed oil by spray drying (Tonon et al. 2008). Q6

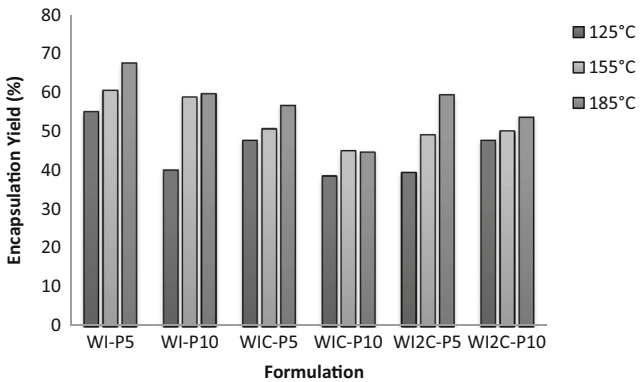

Fig. 6 Encapsulation yield (%) of powder obtained by different formulations at different inlet drying temperatures (°C)

Peroxide Value

The evaluation of the lipid oxidation in microencapsulated oils is important because it results in loss of nutritional value and development of undesirable reactions. The oxidative stability of the encapsulated soybean oil was evaluated by measuring the peroxide value immediately after drying and after 5 months of storage at 4 °C. Figure 7 shows peroxide values between 29.63 and 101.7 meq O_2/kg of oil with higher values obtained from microcapsules formulated with higher phytosterol concentration (10% *w*/*w* when compared to 5% *w*/*w*) or spray dried at higher inlet air temperature. High oxidation values

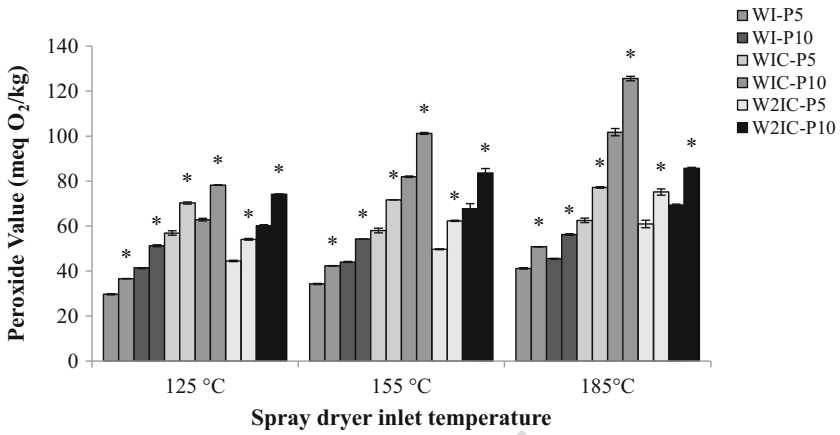

Fig. 7 Peroxide values (meq O_2/kg) of phytosterol microcapsules prepared with different formulations at different inlet drying temperatures, during storage at 4 °C in a refrigerator

were also observed in the controls, obtained without adding phytosterols in the oily phase. These values ranged from 32.85 to 51.60 meq O_2/kg of oil at t_0 and from 40.67 to 62.72 meq O_2/kg of oil at t_1. Therefore, it was assumed that this increase could also be attributed to the oxidation of the oil. After 5 months, as expected, there was a sharp increase in the oxidation rate of the microcapsules obtained from all the emulsions with peroxide values in the range of 36.63–125.6 meq O_2/kg of oil. These higher values of peroxide were unexpected and are very different from those presented in the literature. Carneiro et al. (2013) reported a peroxide value ranging from 6.12 to 8.77 meq O_2/kg oil for flaxseed oil microencapsulated using different wall materials. Significantly lower values were also reported by Bae and Lee (2008). These researchers evaluated the oxidative stability of avocado oil microencapsulated by spray-drying technique, using maltodextrin and WPI as wall material. Similarly, Partanen et al. (2008), evaluating the effect of storage conditions on the oxidative stability of flaxseed oil encapsulated by spray drying using WPI as wall material, have reported a very low lipidic oxidation in the encapsulated samples. Given that there was no significant difference between the density of all that samples and that the microcapsules were spherical and smooth with no apparent pores, the high level of oxidation observed in this study could be attributed to the following conditions: (1) the high temperature (70 °C) in combination with a high shear mixing involved in the production of the emulsions, which may have initiated and accelerated the oxidation; (2) the oil extraction made with Soxhlet apparatus with exposure of the microcapsules at 60 °C for 5.5 h; (3) the presence of phytosterols which might have acted as a pro-oxidant, resulting in the increase in the oil oxidation rate as demonstrated by Winkler and Warner (2008) with 1 and 2.5% phytosterols added to heated stripped soybean oil; and (4) the combination of (1), (2), and (3). These conditions will be investigated in further studies. The hypothesis (3) could also confirm that of Yoshida and Niki (2003) who suggested a possible pro-oxidant effect of some hytosterols, such as stigmasterol. Based on the abovementioned literature, it could be speculated that the combination of high emulsification temperature with the pro-oxidant effect of phytosterols at relatively low concentration might have significantly contributed to the observed high oxidation of the encapsulated samples. Moreover, it is to be excluded that high oxidation value observed in this study could be correlated with a high value of unencapsulated oil as reported by other researchers (Bae and Lee 2008; Tonon et al. 2011). The surface oil values observed in this study ranged between 9.68 and 18.78% and were comparable with those presented in the literature. In fact, the surface oil reported by Bae and Lee (2008) was in the range of 11.39–15.75%, with a level of peroxide value always lower than 5 meq O_2/kg of oil both at t_0 and after 8 weeks of storage at 4 or 25 °C.

Loading Capacity

The amount of loaded phytosterols was determined using the Liebermann-Burchard reaction, often used for the steroid determination. Sterols react with strong acids to give colored products. The linearity of the method was established using phytosterol standard solution. The analytical curve showed a Pearson regression coefficient (R^2) of 0.9847. Loading capacity, obtained by the ratio between the phytosterols concentration and the mass of the powder collected, ranged from 0.39 to 0.95%. The results showed a significant effect of the formulation and the air-drying temperatures. As expected, the LC% increased as a function of initial phytosterol content in the emulsion. The presence of chitosan and higher inulin concentration in the emulsions formulation resulted in the increase in the loading capacity. Again, this could be explained by the relatively high viscosity of this formulation, which resulted in a reduced oil migration to the surface at early stages of the drying, thus improving the encapsulation and loading efficiency.

Conclusion

WPI-inulin-chitosan microcapsules containing phytosterols solubilized in soybean oil were successfully produced. The resultant microparticles were spherical and uniform, with an average size lower than 50 μm. A significant effect of the formulation, the phytosterol concentration, and of the inlet air-drying temperature on the microcapsules properties was found. An oil encapsulation efficiency of 85% with phytosterol loading of 0.95 g/g of powder was achieved. However, although a lower level of surface oil was obtained, the peroxide values of the microcapsules were unexpectedly relatively high even just after the production. It was hypothesized that a combination of high temperature emulsification and the prooxidant capacity of phytoestorols could be the main contributor to this high oil oxidation. This hypothesis will be investigated in further studies.

Acknowledgements The authors would like to thank Tecnoblend srl (Italy), Beneo™ (Belgium), and DRT Group (France) for having provided WPI, inulin, and phytosterol powder, respectively. The authors also thank Prof. Zhibing Zhang of the University of Birmingham for his support.

References

Alemany, L., Barbera, R., Alegría, A., & Laparra, J. M. (2014). Plant sterols from foods in inflammation and risk of cardiovascular disease: a real threat? *Food and Chemical Toxicology, 69*, 140–149.

Alvim, I. D., Souza, F. D. S. D., Koury, I. P., Jurt, T., & Dantas, F. B. H. (2013). Use of the spray chilling method to deliver hydrophobic components: physical characterization of microparticles. *Food Science and Technology (Campinas), 33*, 34–39.

AOCS. (1989). Method cd. In D. Firestone (Ed.), *Official methods and recommended practices of the American Oil Chemists's Society, 4th edn. AOCS press* (pp. 8–53). Champaign, IL: AOCS.

AOCS. (2000). Official methods and recommended practices of the American Oil Chemists's Society, 14th edn. In *AOCS press*. Washington, DC: AOCS.

Bacchetti, T., Masciangelo, S., Bicchiega, V., Bertoli, E., & Ferretti, G. (2011). Phytosterols, phytostanols and their esters: from natural to functional foods. *Mediterranean Journal of Nutrition and Metabolism, 4*(3), 165–172.

Bae, E. K., & Lee, S. J. (2008). Microencapsulation of avocado oil by spray drying using whey protein and maltodextrin. *Journal of Microencapsulation, 25*(8), 549–560.

Botelho, P. B., Galasso, M., Dias, V., Mandrioli, M., Lobato, L. P., Rodriguez-Estrada, M. T., & Castro, I. A. (2014). Oxidative stability of functional phytosterol-enriched dark chocolate. *LWT-Food Science and Technology, 55*(2), 444–451.

Botrel, D. A., de Barros Fernandes, R. V., Borges, S. V., & Yoshida, M. I. (2014). Influence of wall matrix systems on the properties of spray-dried microparticles containing fish oil. *Food Research International, 62*, 344–352.

Carneiro, H. C., Tonon, R. V., Grosso, C. R., & Hubinger, M. D. (2013). Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials. *Journal of Food Engineering, 115*(4), 443–451.

Costa, G. T., Abreu, G. C. D., Guimarães, A. B. B., Vasconcelos, P. R. L. D., & Guimarães, S. B. (2015). Fructo-oligosaccharide effects on serum cholesterol levels. An overview. *Acta Cirurgica Brasileira, 30*(5), 366–370.

da Silva, P. T., Fries, L. L. M., de Menezes, C. R., Holkem, A. T., Schwan, C. L., Wigmann, É. F., et al. (2014). Microencapsulation: concepts, mechanisms, methods and some applications in food technology. *Ciência Rural, 44*(7), 1304–1311.

Drusch, S., & Mannino, S. (2009). Patent-based review on industrial approaches for the microencapsulation of oils rich in polyunsaturated fatty acids. *Trends in Food Science & Technology, 20*(6), 237–244.9.

Ezhilarasi, P. N., Indrani, D., Jena, B. S., & Anandharamakrishnan, C. (2013). Freeze drying technique for microencapsulation of Garcinia fruit extract and its effect on bread quality. *Journal of Food Engeneering, 117*, 513–520.

Fernandes, P., & Cabral, J. M. S. (2007). Phytosterols: applications and recovery methods. *Bioresource Technology, 98*(12), 2335–2350.

Fernandes, R. V. B., Borges, S. V., & Botrel, D. A. (2014). Gum arabic/starch/maltodextrin/inulin as wall materials on the microencapsulation of rosemary essential oil. *Carbohydrate Polymers, 101*, 524–532.

Fernandes, R. V. B., Botrel, D. A., Silva, E. K., Pereira, C. G., Carmo, E. L. D., Dessimoni, A. L. D. A., & Borges, S. V. (2017). Microencapsulated ginger oil properties: influence of operating parameters. *Drying Technology*, 1–10.

Frascareli, E. C., Silva, V. M., Tonon, R. V., & Hubinger, M. D. (2012). Effect of process conditions on the microencapsulation of coffee oil by spray drying. *Food and Bioproducts Processing, 90*(3), 413–424.

Fujiwara, G. M., Campos, R., Costa, C. K., Dias, J. D. F. G., Miguel, O. G., Miguel, M. D., et al. (2013). Production and characterization of alginate-starch-chitosan microparticles containing stigmasterol through the external ionic gelation technique. *Brazilian Journal of Pharmaceutical Sciences, 49*(3), 537–547.

Gabay, O., Sanchez, C., Salvat, C., Chevy, F., Breton, M., Nourissat, G., et al. (2010). Stigmasterol: a phytosterol with potential anti-osteoarthritic properties. *Osteoarthritis and Cartilage, 18*(1), 106–116.

García-Llatas, G., & Rodríguez-Estrada, M. T. (2011). Current and new insights on phytosterol oxides in plant sterol-enriched food. *Chemistry and Physics of Lipids, 164*(6), 607–624.

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., & Saurel, R. (2007). Applications of spray-drying in microencapsulation of food ingredients: an overview. *Food Research International, 40*(9), 1107–1121.

Ghosh, S. K. (2006). Functional coatings and microencapsulation: a general perspective. In *Functional coating* (1st ed., pp. 1–26). Weinheim: WILEY-VCH Verlag GmbH {&} Co. KGaA.

Grattan, B. J. (2013). Plant sterols as anticancer nutrients: evidence for their role in breast cancer. *Nutrients, 5*(2), 359–387.

Gupta, A. K., Savopoulos, C. G., Ahuja, J., & Hatzitolios, A. I. (2011). Role of phytosterols in lipid-lowering: current perspectives. *QJM: An International Journal of Medicine, 104*(4), 301–308.

Hogan, S. A., McNamee, B. F., O'Riordan, E. D., & O'Sullivan, M. (2001). Emulsification and microencapsulation properties of sodium caseinate/carbohydrate blends. *International Dairy Journal, 11*(3), 137–144.

Hur, S. J., Park, G. B., & Joo, S. T. (2007). Formation of cholesterol oxidation products (COPs) in animal products. *Food Control, 18*(8), 939–947.

Izadi, Z., Nasirpour, A., & Garousi, G. (2012). Optimization of phytosterols dispersion in an oil/water emulsion using mixture design approach. *Journal of Dispersion Science and Technology, 33*(12), 1715–1722.

Jafari, S. M., Assadpoor, E., Bhandari, B., & He, Y. (2008a). Nanoparticle encapsulation of fish oil by spray drying. *Food Research International, 41*, 172–183.

Jafari, S. M., Assadpoor, E., He, Y., & Bhandari, B. (2008b). Encapsulation efficiency of food flavours and oils during spray drying. *Drying Technology, 26*(7), 816–835.

Kim, E., & Goldberg, M. (1969). Serum cholesterol assay using a stable Liebermann-Burchard reagent. *Clinical Chemistry, 15*(12), 1171–1179.

Kim, K. W., & Thomas, R. L. (2007). Antioxidative activity of chitosans with varying molecular weights. *Food Chemistry, 101*(1), 308–313.

Klaypradit, W., & Huang, Y. W. (2008). Fish oil encapsulation with chitosan using ultrasonic atomizer. *LWT-Food Science and Technology, 41*(6), 1133–1139.

Liang, Y. T., Wong, W. T., Guan, L., Tian, X. Y., Ma, K. Y., Huang, Y., & Chen, Z. Y. (2011). Effect of phytosterols and their oxidation products on lipoprotein profiles and vascular function in hamster fed a high cholesterol diet. *Atherosclerosis, 219*(1), 124–133.

Lin, D., & Zhao, Y. (2007). Innovations in the development and application of edible coatings for fresh and minimally processed fruits and vegetables. *Comprehensive Reviews in Food Science and Food Safety, 6*(3), 60–75.

Mehyar, G. F., Al-Isamil, K. M., Al-Ghizzawi, H. A. M., & Holley, R. A. (2014). Stability of cardamom (*Elettaria Cardamomum*) essential oil in microcapsules made of whey protein isolate, guar gum, and carrageenan. *Journal of Food Science, 79*(10), C1939–C1949.

Mini Spray Dryer B-290, 3th July 2010 https://www.buchi.com/en/products/spray-drying-and-encapsulation/mini-spray-dryer-b-290.

Moreau, R. A., Whitaker, B. D., & Hicks, K. B. (2002). Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. *Progress in Lipid Research, 41*(6), 457–500.

Pal, S., Ellis, V., & Dhaliwal, S. (2010). Effects of whey protein isolate on body composition, lipids, insulin and glucose in overweight and obese individuals. *British Journal of Nutrition, 104*(5), 716–723.

Panda, S., Jafri, M., Kar, A., & Meheta, B. K. (2009). Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from Butea monosperma. *Fitoterapia, 80*(2), 123–126.

Partanen, R., Raula, J., Seppänen, R., Buchert, J., Kauppinen, E., & Forssell, P. (2008). Effect of relative humidity on oxidation of flaxseed oil in spray dried whey protein emulsions. *Journal of Agricultural and Food Chemistry, 56*(14), 5717–5722.

Pillai, D. S., Prabhasankar, P., Jena, B. S., & Anandharamakrishnan, C. (2012). Microencapsulation of Garcinia cowa fruit extract and effect of its use on pasta process and quality. *International Journal of Food Properties, 15*(3), 590–604.

Rodriguez, M. S., Albertengo, L. A., & Agullo, E. (2002). Emulsification capacity of chitosan. *Carbohydrate Polymers, 48*, 271–276.

Ryan, E., McCarthy, F. O., Maguire, A. R., & O'Brien, N. M. (2009). Phytosterol oxidation products: their formation, occurrence, and biological effects. *Food Reviews International, 25*(2), 157–174.

Schaller-Povolny, L. A., & Smith, D. E. (2002). Interaction of milk proteins with inulin. *Milchwissenschaft, 57*(9–10), 494–496.

Speiciene, V., Guilmineau, F., Kulozik, U., & Leskauskaite, D. (2007). The effect of chitosan on the properties of emulsions stabilized by whey proteins. *Food Chemistry, 102*(4), 1048–1054.

Sun, C., & Gunasekaran, S. (2010). Rheology and oxidative stability of whey protein isolate-stabilized menhaden oil-in-water emulsions as a function of heat treatment. *Journal of Food Science, 75*(1), C1–C8.

Tan, L. H., Chan, L. W., & Heng, P. W. S. (2005). Effect of oil loading on microspheres produced by spray drying. *Journal of Microencapsulation, 22*(3), 253–259.

Thies, C. (2001). Microencapsulation: what it is and purpose. In P. Vilstrup (Ed.), *Microencapsulation of food ingredients* (pp. 1–29). Surrey: England: Leatherhead Publishing.

Tolve, R., Galgano, F., Caruso, M. C., Tchuenbou-Magaia, F. L., Condelli, N., Favati, F., & Zhang, Z. (2016). Encapsulation of health-promoting ingredients: applications in foodstuffs. *International Journal of Food Sciences and Nutrition, 67*(8), 888–918.

Tonon, R. V., Brabet, C., & Hubinger, M. D. (2008). Influence of process conditions on the physicochemical properties of açai *(Euterpe oleraceae* Mart.) powder produced by spray drying. *Journal of Food Engineering, 88*(3), 411–418.

Tonon, R. V., Grosso, C. R., & Hubinger, M. D. (2011). Influence of emulsion composition and inlet air temperature on the microencapsulation of flaxseed oil by spray drying. *Food Research International, 44*(1), 282–289.

Turchiuli, C., Munguia, M. J., Sanchez, M. H., Ferre, H. C., & Dumoulin, E. (2014). Use of different supports for oil encapsulation in powder by spray drying. *Powder Technology, 255*, 103–108.

Valenzuela, A., Sanhueza, J., & Nieto, S. (2003). Cholesterol oxidation: health hazard and the role of antioxidants in prevention. *Biological Research, 36*(3–4), 291–302.

Velasco, J., Dobarganes, C., & Márquez-Ruiz, G. (2003). Variables affecting lipid oxidation in dried microencapsulated oils. *Grasas y Aceites, 54*(3), 304–314.

Winkler, J. K., & Warner, K. (2008). The effect of phytosterol concentration on oxidative stability and thermal polymerization of heated oils. *European Journal of Lipid Science and Technology, 110*(5), 455–464.

Yoshida, Y., & Niki, E. (2003). Antioxidant effects of phytosterol and its components. *Journal of Nutritional Science and Vitaminology, 49*(4), 277–280.

AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES.

Q1. Please check if the affiliations are presented correctly. Also, please check if the corresponding author's telecommunication data are captured correctly.
Q2. The occurrence of "phytostorol" was changed to "phytosterol." Please check if the action taken is appropriate.
Q3. Ref. "ver. 10, 2003" is cited in the body but its bibliographic information is missing. Kindly provide its bibliographic information in the list.
Q4. Please give the significance of asterisks in Table 2 in the form of a table note.
Q5. Ref. "Cai and Corke (2000)" is cited in the body but its bibliographic information is missing. Kindly provide its bibliographic information in the list.
Q6. The citation "Tonon et al. 2013" has been changed to "Tonon et al., 2008" to match the author name/date in the reference list. Please check if the change is fine in this occurrence and modify the subsequent occurrences, if necessary.
Q7. The sentence "Moreover..." has been modified. Please check if the intended meaning of the sentence has been retained.
Q8. References [Alvim et al, 2013, Frascareli et al, 2012, Hogan et al, 2001, Mini Spray Dryer B-290, 3th July, 2010, Pillai et al, 2012, Thies, 2001] were provided in the reference list; however, this was not mentioned or cited in the manuscript. As a rule, all references given in the list of references should be cited in the main body. Please provide its citation in the body text.
Q9. Please provide complete bibliographic details of this reference (Microencapsulated ginger oil properties...).