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POMEGRANATE SEED OIL: EXTRACTION, SHELF LIFE PREDICTION, AND MICROENCAPSULATION

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ABSTRACT

Interest in oils rich in polyunsaturated fatty acids has increased in recent years about their beneficial effects on human health. Pomegranate seed is an industrial by-product that has a large variety of nutritional value. Pomegranate seed oil (PSO) has an exceptional fatty acid profile. Lipid oxidation is one of the important parameters that limits its use in food products. Thus, the objectives of this study were to evaluate the effects of extraction methods (Soxhlet and stirring) and solvents (*n*-hexane, petroleum ether, diethyl ether, and ethanol) on oxidation parameters in the extraction of PSO, to predict the shelf life of PSO by Rancimat and to encapsulate PSO by spray drying to extend its shelf life. PSO yield varied between 1.88 and 14.32%. The lowest peroxide (2.88 meq O_2/kg), *p*-Anasidine (6.56 meq *p*-Anisidine/kg), and TOTOX (12.13) values were observed when PSO was extracted by stirring using n-hexane as solvent. The shelf lives of PSO at 0, 4, and 20 ºC using Rancimat were predicted as 11593, 8088, and 1916 h, respectively. The main fatty acid in PSO was punicic acid and derivatization had a significant effect on the determination of punicic acid by GC (sodium methoxide, 84.75% and potassium hydroxide, 81.79%). Inlet air temperature of 140 °C, oil: wall material ratio of 1:2 (w/w), and maltodextrin: gum Arabic ratio of 1:1 (w/w) were selected as favorable conditions in microencapsulation of PSO. The addition of antioxidants into feed solutions had no positive effects on oxidation during spray drying.

1.Introduction

The food industry is one of the sectors generating valuable wastes and by-products. Generally, food wastes are considered an environmental problem whereas some by-products and bioactive compounds derived from these materials can be ingredients or raw materials of products with high-added value (Puértolas & Barba, 2016).

Fresh pomegranate (*Punica granatum L.*) and pomegranate products such as jam, jelly, and fruit juice are commonly consumed worldwide. Pomegranate seed remains as an industrial by-product after the production of pomegranate juice whereas it is a potential source of functional lipids. Pomegranate seed oil (PSO) contains an exceptional conjugated fatty acid called punicic acid (9*Z*,11*E*,13*Z*-octadecatrienoic acid), which is a ω-5 long-chain polyunsaturated fatty acid that makes up approximately 65-80% of PSO (Lansky & Newman, 2007). Punicic acid is structurally very similar to conjugated linoleic acid and α-

linolenic acid which have been found to have various health benefits including cancer prevention (Grossmann, Mizuno, Schuster, & Cleary, 2010). Polyunsaturated fatty acids are prone to oxidation; however, conjugated counterparts are more susceptible to oxidative deterioration than the former ones. Oxidative degradation of lipids limits their use in food products due to the development of undesired flavor and odor (Çam, Erdoğan, Aslan, & Dinç, 2013; Loughrill et al., 2019). To date, both traditional and advanced extraction techniques have been used for the recovery of PSO (Abbasi, Rezaei, Emamdjomeh, & Mousavi, 2008; Đurđević et al., 2017; Eikani, Golmohammad, & Homami, 2012; Athanasia M. Goula, 2013; Liu, Xu, Hao, & Gao, 2009). Among these techniques, traditional extraction techniques such as Soxhlet and stirring extraction are still the most commonly used due to simple application processes and low investment costs. However, it is necessary to select suitable extraction techniques and solvents to obtain oil with desirable characteristics such as high yield and good oxidative stability. Besides, even if appropriate extraction conditions are selected, oils rich in conjugated fatty acids have a shorter shelf life compared to other unsaturated fatty acids because of the faster formation of unstable free radicals from conjugated fatty acids (Zhang & Chen, 1997). Thus, it is critical to examine their oxidative stability. Nowadays, different accelerated methods such as Differential Scanning Calorimetry (DSC), Thermal Gravimetric Analysis (TGA), and Rancimat are used to rapidly predict the shelf life of oils. DSC curves help to estimate the oxidation of oils exposing different isothermal temperatures under oxygen flow. A sharp exothermic curve as a function of heat provides information about an oxidation reaction. When the curve is seen, it could be interpreted that a rapid exothermic reaction occurs between oil and oxygen (Pardauil et al., 2011). TGA is also used to estimate the oxidation stability of oils by determining their mass gain or mass loss through oxygen uptake or thermal degradation (Tengku-Rozaina & Birch, 2015). Shelf-life prediction of oils by Rancimat is conducted by using different temperatures in presence of O_2 (Ghosh, Upadhyay, Mahato, & Mishra, 2019). High correlation between thermal analysis and Rancimat results was reported by previous work (Arain, Sherazi, Bhanger, Talpur, & Mahesar, 2009; Symoniuk, Ratusz, Ostrowska-Ligęza, & Krygier, 2017; Tengku-Rozaina & Birch, 2015). In the literature, there is no report regarding the shelf life prediction of PSO by any accelerated methods.

The microencapsulation process, on the other hand, is widely used to improve the oxidative stability of polyunsaturated fatty acids against lipid oxidation and to increase their shelf life. The efficiency, yield, and stability of microcapsules depend on the selection of suitable wall materials and operational conditions for the encapsulation process (Carneiro, Tonon, Grosso, & Hubinger, 2013; Tonon, Grosso, & Hubinger, 2011). PSO has been microencapsulated by using skim milk powder (Goula & Adamopoulos, 2012); maltodextrin, skim milk powder, and gum Arabic (Goula & Lazarides, 2015); starch derivates and whey protein concentrate mixes (Sahin-Nadeem & Afşin Özen, 2014); and capsule (modified corn starch) (Bustamante, Hinojosa, Robert, & Escalona, 2017). Nevertheless, there is no information on the evaluation of the oxidative stability of PSO by using synthetic or natural antioxidants during spray drying.

The objectives of this study were: (i) to evaluate effects of extraction methods and solvents on oxidation of PSO, (ii) to predict the shelf life of PSO under accelerated oxidation conditions, (iii) to determine effects of spray drying temperature and coating materials on microencapsulation of PSO (iv) to observe effects of natural and synthetic antioxidants on oxidation of PSO during spray drying.

2. Materials and methods

2.1. Materials

The seeds from mixed pomegranate cultivars as a by-product of the fruit juice industry were kindly provided by a local producer (Göknur, Niğde, Turkey). The seeds were dried in the dark at room temperature for 7

days and, then stored at 4 ºC until subsequent analyses. Pomegranate peel phenolics were extracted by a method described by Çam, İçyer, and Erdoğan (2014). The chemicals, standards, and wall materials were obtained from Sigma Aldrich Co. (St. Louis, MO, USA) or Merck (Darmstadt, Germany) unless otherwise stated.

2.2. Methods

2.2.1. PSO extraction

Before extraction, pomegranate seeds were immediately ground by a laboratory mill to increase the mass transfer. PSO from the seeds was extracted using two extraction methods (Soxhlet and stirring extractions) and four solvents (*n*-hexane, petroleum ether, diethyl ether, and ethanol).

In Soxhlet extraction, 50 g of the milled seeds were placed into a cellulose thimble which is then put into the main chamber of the Soxhlet extractor. The solvent (250 mL) was poured into the main chamber where it was heated under reflux at the boiling temperature of the solvent, and extraction was maintained for 120 min. After extraction, the solvent was removed at 40 ºC by a vacuum evaporator (Buchi, Flawil, Switzerland) and the oil was dried to constant weight in an oven at 105 ºC.

In stirring extraction, 50 g of milled pomegranate seeds were weighed into a glass beaker. After the addition of the solvent (250 mL), it was stirred at room temperature for 120 min. The mixture was centrifuged at 3,000 g for 5 min, the resulting supernatant was collected and then the solvent was evaporated at 40 ºC under vacuum (Buchi, Flawil, Switzerland). Subsequently, the oil was dried to constant weight in an oven at 105 ºC.

2.2.2. Lipid oxidation measurements

Peroxide value (PV), p-Anisidine value (*p*-AV), and total oxidation (TOTOX) value were determined to assess the oxidative stability of PSOs. PSO extraction was performed as described in the PSO extraction section except that the solvent was removed under nitrogen flow.

Peroxide value was determined by employing a method given in the literature (Wrolstad et al., 2004). Briefly, 5 g of PSO was dissolved in 30 mL of acetic acid-chloroform (3:2, v/v) solution. A 0.5 mL of saturated KI solution and 30 mL of distilled water were added to this mixture. This mixture was slowly titrated with $Na_2S_2O_3$ (0.01N) until the yellow color almost disappeared. After the addition of 0.5 mL of starch solution (1%), the titration was slowly maintained with Na_2S2O_3 until the violet color fully disappeared. The PV was calculated by following Eq. (1):

$$\mathrm{PV} = \frac{(\mathrm{S}-\mathrm{B})*\mathrm{N}*1000}{\mathrm{w}} \quad (1)$$

where S: $Na_2S_2O_3$ volume (Sample), B: $Na_2S_2O_3$ volume (Blank), N: normality of Na_2S2O_3, W: mass of sample.

p-AV was also measured spectrophotometrically according to AOCS Official Method Cd-18-90 (AOCS, 1998). PSO was dissolved in isooctane and then 1mL of *p*-Anisidine solution in acetic acid (0.25 g/mL) was added to this mixture. After 10 min, the absorbance was measured at 350 nm. The *p*-AV was calculated by following Eq. (2):

$$p-\mathrm{AV} = \frac{25*(1.2*\mathrm{A2}-\mathrm{A1})}{\mathrm{W}} \quad (2)$$

where A1: absorbance of fat solution, A2: absorbance of fat-anisidine solution, W: mass of sample.

PV and *p*-AV were used to measure TOTOX value (Sun-Waterhouse, Zhou, Miskelly, Wibisono, & Wadhwa, 2011). TOTOX value was calculated by following Eq. (3):

$$\text{TOTOX value} = (2 * \text{Peroxide value}) + p-\text{Anisidine value} \quad (3)$$

2.2.3. Oxidative stability and shelf-life prediction by Rancimat

To evaluate the thermal oxidation stability of PSO, the oil (5 g) was exposed to different temperatures (60, 70, 80, 90, 100, 110, 120 and 130 ºC) with an airflow rate of 20 L/h using Rancimat (743 model, Metrohm, Switzerland). The oxidative stability of PSO was expressed as an induction period (IP) in the respective temperature. The IP was the time required to

cause a sudden increase in conductivity. In addition, commercial sunflower and canola oils were used as the references at 110 and 120 ºC. Subsequently, the shelf life prediction of PSO was performed by plotting the temperatures (T, ºC) vs. IPs using Eq. (2). The plot was extrapolated to lower temperatures to predict the shelf life of PSO at 0, 4, and 20 ºC.

$$t = A * e^{(B*T)} \quad (4)$$

where A and B are the coefficients of the equation.

2.2.4. Fatty acid composition by GC-FID

Fatty acid methyl esters (FAMEs) of PSOs were prepared according to two base-catalyzed methods using sodium methoxide (Wrolstad et al., 2004) and methanolic potassium hydroxide (AOCS, 1997) to determine the effects of derivatization methods on the content of punicic acid. Subsequently, the fatty acid composition of PSO was determined by gas chromatography (GC-6890, Agilent, USA) equipped with a flame ionization detector (FID). FAMEs were separated in a capillary column (100 m x 0.25 mm, 0.2 µm film thickness, HP-88) (J&W Scientific, Folsom, Calif., USA). The chromatographic conditions were as follows: column temperature was programmed from 140 ºC (kept for 5 min.) to 240 ºC at 4 ºC/min (kept for 15 min); injector and detector temperatures, 260 and 280 ºC, respectively. The split ratio was 1:30. The flow rate of hydrogen as the carrier gas was 1 mL/min. FAMEs were identified by comparing their retention times with FAME standard mixture (Bellefonte, PA, USA).

2.2.5. Preparation of emulsions and microencapsulation by spray drying

The microencapsulation process was conducted according to McNamee, O'Riorda, and O'Sullivan (2001) with some modifications in the process parameters. Briefly, five different emulsions were prepared to evaluate the effects of three factors, including the inlet temperatures of 140, 160, and 180 ºC, oil: wall material ratios of 1:2 and 1:3, and MD: GA ratios of 1:1 and 2:1. In emulsification, MD was dissolved in 100 mL distilled water under continuous mixing at 24 000 rpm with an Ultra-Turrax homogenizer (IKA-T18 Basic, Staufen, Germany) for 10 min. PSO and GA were slowly added into the solution and the resulting emulsion was homogenized for a further 10 min. In all treatments, the emulsions had a total mass fraction of 30% and a volume of 200 mL.

After emulsification, the emulsions were spray-dried using a laboratory scale spray dryer (Buchi-B290, Flawil, Switzerland) with a chamber diameter of 16.5 cm and a chamber length of 60 cm. The airflow and feed rates were 600 mL/h and 8 mL/min, respectively. PSO microcapsules were collected with the help of a cyclone separator and then stored in a plastic container at 4 ºC until required for use.

2.2.6. Microencapsulation yield

The total amount of solid mass in feed emulsion and mass of microcapsules were used to calculate microencapsulation yield according to Eq.5 based on dry matter content:

$$\text{Microencapsulation yield (\%)} = \frac{\text{Mass of microcapsules (g)}}{\text{Total amount of solid mass (g)}} * 100 \quad (5)$$

2.2.7. Microencapsulation efficiency

Total oil content: An enzymatic digestion method was used to determine the total oil content of PSO microcapsules (Curtis, Berrigan, & Dauphinee, 2008). Briefly, 30 mg of porcine pancreatin and 250 mg of PSO were mixed. Ten mL of sodium phosphate buffer was added to the mixture. The mixture was vortexed and then shaken at 37 °C at 60 rpm for 1 h in a water bath. Ten mL of ethyl acetate was added after the mixture was cooled to room temperature. The mixture was centrifuged at 1000 rpm for 10 min. The top layer (1-2 mL) was removed and its weight was recorded. This layer was dried under continuous nitrogen flow at 40 °C for 1 h. The final weight of extracted oil was recorded.

Surface oil content: To determine surface oil content, 50 mL of isohexane and 5 g of PSO were mixed and shaken for 10 min at 225 rpm. The slurry was filtered and washed three times with 20 mL of isohexane. Isohexane was evaporated at 40 °C for 25 min. Then, the remaining oil was dried at 90 °C for 30 min to completely remove isohexane from the oil. The

final weight of the extracted oil was recorded (Anwar & Kunz, 2011).

Calculation of microencapsulation efficiency: The ratio between the amount of total oil and surface oil of microcapsules were used to determine microencapsulation efficiency according to Eq.6 based on dry matter content:

$$\text{Microencapsulation efficiency (\%)} = \left(1 - \frac{\text{Surface oil (g)}}{\text{Total oil (g)}}\right) * 100 \quad (6)$$

2.3. Statistical analysis

One-way analysis of variance (ANOVA) was performed to evaluate the differences between extraction technique, solvents, and microencapsulation conditions. The significant difference at $p<0.05$ was tested using the Tukey HSD test in 22.0 SPSS (SPSS Inc., Chicago, USA).

3.Results and discussion

3.1. Oil yield

The effects of the extraction methods and solvents on oil yield are shown in Fig. 1. The extraction methods were carried out under similar conditions (50 g pomegranate seeds with 250 mL solvent and extraction time of 120 min) to investigate the effect of methods. The extraction of PSO by the Soxhlet method resulted in a higher oil yield compared to the stirring method regardless of extraction solvents. Oil yields were also influenced by extraction solvents but were not changed significantly ($p>0.05$) when used *n*-hexane, petroleum ether, and diethyl ether.

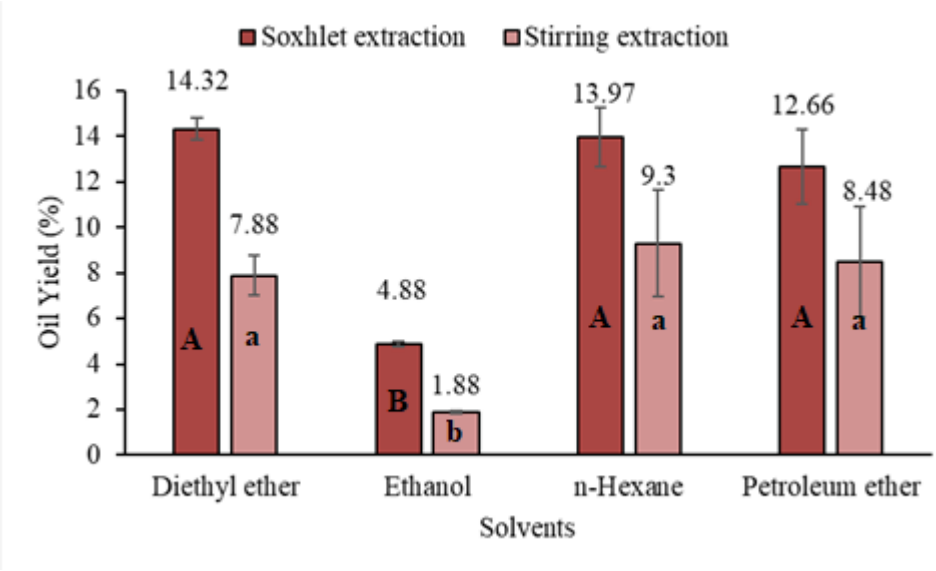

Figure 1. PSO yields for extraction techniques and solvents. (†[a-b] Means on bar different letters differ significantly ($p<0.05$)).

These solvents produced oil yields in the range of 12.66-14.32% for the Soxhlet method and 7.88-9.30% for the stirring method. A similar result (12.74%) was observed in the study of Khoddami, Man, and Roberts (2014) where PSO was extracted by sequential extraction using cold press and Soxhlet with petroleum ether. Besides, the reported results were partially closed to those investigated by Fadavi, Barzegar, and Hossein Azizi (2006) showing total lipid content (66.3-193 g/kg) in seeds of 25 varieties of pomegranates by Soxhlet with petroleum benzene. In a previous study, Abbasi, Rezaei, and Rashidi (2007) reported that PSO yields were 18.6% for Soxhlet with n-hexane (extraction time, 6 h) and 13.0% for stirring with n-hexane (extraction time, 4 h). The lower results in our study could be attributed to shorter extraction time (2 h) and varietal differences among pomegranate cultivars. In the present study, the lowest oil yields were observed when used ethanol as solvent in both

Soxhlet (4.88%) and stirring (1.88%) methods. In a recent study, the effects of certain solvents including hexane, petroleum ether, chloroform, chloroform:methanol and ethanol were examined in the extraction of PSO (Aruna, Manohar, & Singh, 2018). The authors reported that hexane was the best solvent giving the highest yield whereas ethanol was inefficient for PSO extraction.

3.2. Lipid oxidation and oxidative stability

The PSO obtained by ethanol was not included in the current stage of the present study since the PSO yield was found to be significantly lower than the other three solvents used in the previous stage.

Table 1. Oxidation parameters for techniques-solvents combinations of PSO

Extraction method	Solvent	PV (meq O2/kg)	*p*-AV (meq p-Anisidine/kg)	TOTOX value
Soxhlet	Diethyl ether	5.71±0.44^{b}	20.57±1.42^{b}	31.99±1.63^{c}
	n-Hexane	4.49±1.29^{b}	8.54±1.04^{a}	17.52±2.83^{b}
	Petroleum ether	11.16±0.59^{c}	8.41±1.38^{a}	30.72±1.21^{c}
Stirring	Diethyl ether	4.83±1.17^{b}	18.69±1.92^{b}	28.36±3.75^{c}
	n-Hexane	2.78±0.11^{a}	6.56±1.13^{a}	12.13±1.12^{a}
	Petroleum ether	5.09±0.31^{b}	5.45±2.03^{a}	15.63±2.01^{ab}

†$^{a-c}$ Means within each column with different letters differ significantly ($p<0.05$).

Table 2. Induction periods of PSO and commercial oils at different temperatures

Sample	Induction period (h)							
	60 °C	70 °C	80 °C	90 °C	100 °C	110 °C	120 °C	130 °C
PSO	54.02	18.54	7.82	3.02	1.15	0.58	0.44	0.31
Sunflower oil	-	-	-	-	-	6.73	4.05	-
Canola oil	-	-	-	-	-	10.10	5.90	-

Table 1 shows the oxidation parameters for methods and solvents. The effects of methods and solvents on primary and secondary products in lipid oxidation of PSO were assessed through the PV, *p*-AV, and TOTOX values. PVs and *p*-AVs ranged from 2.78 to 11.16 meq O_2/kg and 5.45 to 20.57 meq *p*-Anisidine/kg depending on the extraction methods and solvents, respectively. The lowest TOTOX value was obtained by stirring with *n*-hexane. Although this could be attributed to the room temperature being convenient for preventing oxidation of polyunsaturated fatty acids, this positive effect was not observed for the other solvents in the stirring method. The chemical composition of the extracted oil can be affected by solvents and methods used in the extraction (Uoonlue & Muangrat, 2018). The oxidative stability of the oil is directly related to the chemical composition of the oil such as the presence or absence of antioxidants in the oil of interest.

Oxidative stabilities of PSO determined by Rancimat at different temperatures are shown in Table 2. The IP values of PSO at 60, 70, 80, 90, 100, 110, 120 and 130 ºC were 54.02, 18.54, 7.82, 3.02, 1.15, 0.58, 0.44 and 0.31 h, respectively. It was also determined the IPs of commercial sunflower oil and canola oil at 110 and 120 ºC for comparison purposes. With high punicic acid content, PSO can be assumed as an unstable oil against thermal oxidation compared to commercial vegetable oils. The IP value of PSO at 110 ºC was shorter than those reported by Habibnia, Ghavami, Ansaripour, and Vosough (2012) (0.73-1.02 h), Basiri (2013) (3.03 h) and Melo et al. (2016) (0.72 h). However, the IP of PSO at 80 ºC was highly longer than those reported by Costa, Silva, and Torres (2019) (0.10-0.22 h) for commercial cold-pressed PSOs. These differences between our results and literature could be due to extraction methods, solvents, and pomegranate varieties as well as operational parameters of Rancimat (Farhoosh, 2007).

3.3. Shelf-life prediction

Shelf-life prediction of PSO was conducted using a linear relationship between the temperature in the range of 60-110 ºC and IP by the following equation:

$$IP = 11592.82 * e^{(-0.09*T)} \quad (7)$$

The correlation coefficient (R^2) of Eq. (5) was 0.9972. The shelf lives of PSO at 0, 4, and 20 ºC were estimated as 11593, 8088 and 1916 h, respectively (Fig. 2).

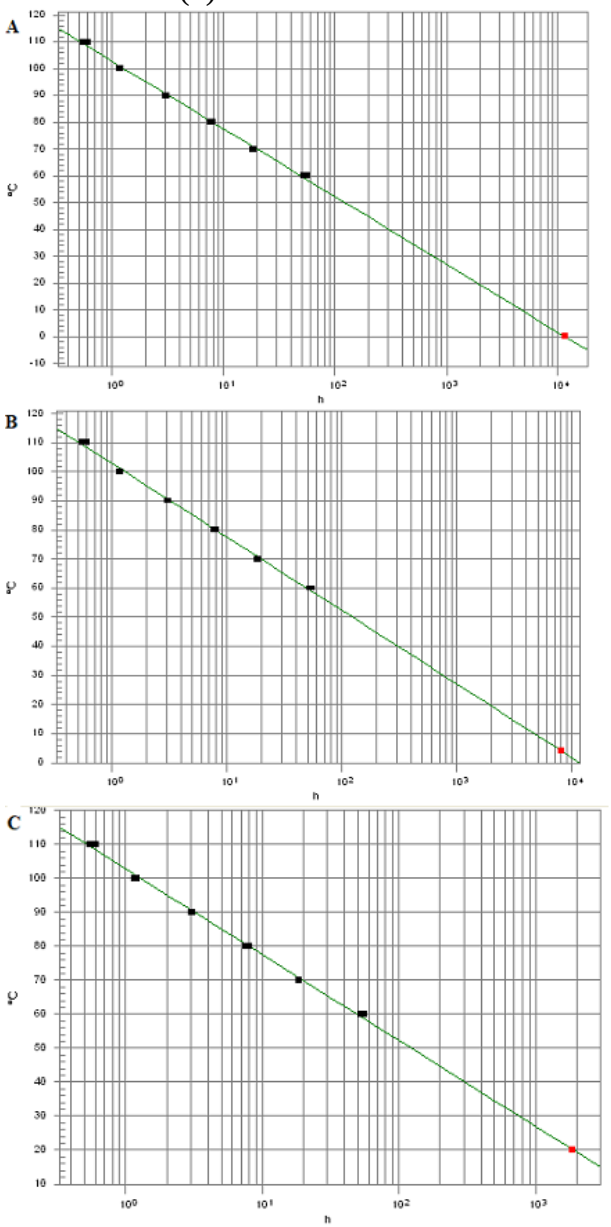

Figure 2. Shelf life prediction of PSO by Rancimat at 0 (A), 4 (B) and 20 (C) °C

There were no comparable shelf life results for PSO. However, shelf life at 20 ºC by the Rancimat method was reported between 4291 and 5687 h for soybean oil (Farhoosh, 2007) and 3789 h for sunflower oil (Upadhyay & Mishra, 2014). It was also reported that shelf lives of sunflower, sesame oils, and their blends at 25 ºC by Rancimat were between 2638 (109.94 days) and 5220 h (217.50 days) without antioxidant addition (Ghosh et al., 2019). This low shelf life at 20 ºC showed that PSO was very prone to oxidative deterioration compared to the above-mentioned studies. Shelf-life prediction by Rancimat can result in either overprediction or

underprediction depending on Rancimat parameters as shown in the study of Farhoosh (2007). Nevertheless, the results obtained by Rancimat are preferable to long-term storage studies because of its shorter analysis time.

3.4. Fatty acid composition

The effects of extraction type (Soxhlet or stirring) and transesterification method (potassium hydroxide or sodium methoxide) on the determination of the fatty acid composition of PSO were determined by ANOVA. The general fatty acid composition of PSOs did not change with extraction types and transesterification methods. Fourteen fatty acids were identified with GC-FID in all conditions. The results showed that the extraction type had no significant effects ($p=0.218$), however, the transesterification method had a significant effect ($p=0.001$) on the fatty acid composition of PSO, especially punicic acid. As can be seen in Table 3, when PSO was methylated with catalysts, the content of punicic acid for sodium methoxide and potassium hydroxide was 84.75 and 81.79%, respectively. Besides, the total punicic acid isomer content of PSO changed depending on the catalyst type used. Previous research evaluated the use of acid and base catalysts on the fatty acid profile of PSO (Sassano et al., 2009). The authors suggested the use of a base catalyst (sodium methoxide) in the determination of punicic acid in PSO. Melo et al. (2016) also showed a superior effect of a base catalyst in the determination of punicic acid in PSO compared to acid catalyst. According to our results, sodium methoxide was more effective than potassium hydroxide in the determination of the PSO fatty acid profile.

3.5. Microencapsulation

The effects of emulsion (MD: GA ratio, oil: wall material ratio) and spray drying parameter (Inlet air temperature) on ME and MY of PSO microcapsules are presented in Table 4.

Firstly, PSO microcapsules were produced using MD: GA ratios of 1:1 (T_1) and 2:1 (T_2). The results indicated that MD: GA ratio of 2:1 led to a decrease in ME and MY of PSO microcapsules. This can be associated with the lower emulsifying capacity of MD. It was reported that MD usage in the wall matrix decreased lipid oxidation by reducing oxygen permeability of the wall matrix for microencapsulated avocado oil because of hydrophilicity of MD and hydrophobicity of oxygen (Bae & Lee, 2008).

Secondly, oil: wall material ratio of feed emulsion was changed from 1:2 (T_2) to 1:3 (T_3). The increase in the proportion of the wall matrix, from 1:2 to 1:3, had no significant effects on ME and MY. Besides, this application decreases oil content in the total mass of microcapsules. Microcapsules having high oil content and ME are preferable for the enrichment of food products because a higher amount of oil (active material) can be incorporated into food products by using lower microcapsule powder. On the other hand, the use of less wall material is a cost-effective application.

Lastly, the effect of inlet air temperature on ME and MY of PSO microcapsules was tested. The inlet air temperatures of 140 and 180 ºC showed no significant effect on MY and ME of PSO microcapsules compared to 160 ºC. Tonon et al. (2011) reported that temperatures higher than 170 ºC promoted lipid oxidation during spray drying. Hence, PSO should not be spray-dried at high temperatures because of its polyunsaturated fatty acid content, especially punicic acid. Lavanya, Kathiravan, Moses, & Anandharamakrishnan (2019) showed that increasing outlet temperature led to an increase in PVs of microencapsulated chia and fish oils. In this study, outlet air temperature increased as a function of inlet temperature. High inlet air temperature can accelerate oxidation during spray drying, however, the microcapsules are exposed to outlet air temperature in the collection bottle during the total drying time. Thus, the selection of a lower outlet temperature is important to prevent further oxidation. The inlet air temperature of 140 ºC had no negative effects on MY and ME of PSO microcapsules, and the lowest outlet temperature was observed at this temperature. Hence, it was selected as optimal temperature for microencapsulation of PSO.

Table 3. Effect of extraction methods and catalysts on the fatty acid profile of PSO

Extraction method	**Soxhlet**		**Stirring**	
Catalyst type	**Sodium methoxide**	**Potassium hydroxide**	**Sodium methoxide**	**Potassium hydroxide**
Fatty acids	**Area (%)**	**Area (%)**	**Area (%)**	**Area (%)**
C14:0	0.02±0.01	0.41±0.32	0.02±0.01	0.54±0.07
C15:0	0.03±0.01	0.10±0.03	0.02±0.01	0.10±0.01
C16:0	2.42±0.02	3.74±1.03	2.42±0.03	4.02±0.26
C16:1	0.05±0.01	0.13±0.05	0.07±0.02	0.13±0.03
C17:0	0.07±0.01	0.12±0.07	0.06±0.02	0.11±0.00
C18:0	1.75±0.03	2.07±0.21	1.74±0.02	2.18±0.11
C18:1	4.79±0.08	5.51±0.47	4.73±0.02	6.23±0.95
C18:2	4.44±0.06	4.41±0.02	4.28±0.02	4.59±0.60
C20:0	0.44±0.01	0.41±0.05	0.45±0.02	0.43±0.00
C18:3 n-3	0.08±0.02	0.16±0.09	0.08±0.01	0.10±0.00
C20:1	0.67±0.04	0.67±0.01	0.64±0.04	0.64±0.02
C18:3 n-5	84.15±0.11^{a}	81.79±2.10^{b}	84.67±0.08^{a}	80.56±2.24^{b}
C18:3 n-5 isomer 1	0.80±0.05	0.29±0.13	0.59±0.04	0.29±0.19
C18:3 n-5 isomer 2	0.29±0.01	0.21±0.03	0.24±0.02	0.10±0.03

†$^{a, b}$ Means within each column with different letters differ significantly ($p<0.05$).

Table 4. Experimental conditions and responses for microencapsulation of PSO

Treatments	**MD (g)**	**GA (g)**	**Oil (g)**	**MD:GA (g/g)**	**Oil: wall material (g/g)**	**T_i (°C)**	**T_o (°C)**	**MY (%)**	**ME (%)**
T_1	20	20	20	1:1	1:2	160	90±2.0	29.2±8.8^{a}	73.4±2.5^{b}
T_2	26.7	13.3	20	2:1	1:2	160	90±1.0	27.4±0.9^{a}	69.4±1.9^{b}
T_3	22.5	22.5	15	1:1	1:3	160	94.5±2.5	31.9±2.9^{a}	89.8±2.2^{a}
T_4	20	20	20	1:1	1:2	180	105±3.0	31.1±0.6^{a}	77.8±1.8^{ab}
T_5	20	20	20	1:1	1:2	140	72±1.0	28.7±8.9^{a}	74.1±7.2^{ab}

† $^{a, b}$ Means within each column with different letters differ significantly ($p<0.05$). MD: Maltodextrin; GA: Gum Arabic; T_i: Inlet temperature; T_o: Outlet temperature; MY: Microencapsulation yield; ME: Microencapsulation efficiency.

3.6. Effect of antioxidants on microencapsulation

PSO microcapsules were further obtained at 140 °C with MD: GA ratio of 1:1 and oil: wall material ratio of 1:2 with the addition of synthetic and natural antioxidants to check the effects of antioxidants on PSO microcapsules. Butylated hydroxyanisole (BHA) and pomegranate peel phenolics were used in the same ratios (0.02%) in feed solutions. The control solution was prepared without any antioxidant. The proportion of BHA (0.02%) was selected according to the maximum permitted amount of this synthetic antioxidant in food products (Fortin, 2016). Although there is no limit for pomegranate peel phenolics, the same amount (0.02%) was used in the preparation of emulsions for a meaningful comparison. The addition of antioxidants into emulsions showed a statistically insignificant effect on lipid oxidation during the spray drying of PSO as shown in Table 5.

Table 5. Effect of natural and synthetic antioxidants on oxidative stability of PSO

PSO microcapsules	PV (meq O_2/kg)	*p*-AV (meq *p*-anisidine/kg)	TOTOX
Control	5.20±1.55[a]	8.45±1.03[a]	18.85±3.74[a]
PSO microcapsules with BHA	5.68±1.75[a]	7.04±1.06[a]	18.39±4.57[a]
PSO microcapsules with pomegranate peel phenolics	7.83±2.42[a]	5.44±1.50[a]	21.11±5.35[a]

†Means within each column with the same letters ([a]) are not significantly different (p>0.05).

The result was consistent with previous findings of Binsi et al. (2017). The authors reported that the addition of sage polyphenols to feed solution did not show a protective effect against oxidation during spray drying. However, the authors reported the protective effects of incorporated sage polyphenols during the post-storage of the microcapsules. A previous study was also reported that the addition of the mixture of 0.05% rosemary, 1% broccoli sprout, and 1% citrus extracts to feed solutions effectively reduced lipid oxidation during post-storage of microencapsulated seed oil (Ahn, Kim, & Kim, 2012). In another study, Yeşilsu and Özyurt (2019) evaluated the antioxidant effect of rosemary, thyme, and laurel extracts on lipid oxidation in fish oil microcapsules, and 1500 ppm rosemary extract addition was the most effective one. Although the oils rich in polyunsaturated fatty acids are known to be prone to thermal oxidation, they are exposed to inlet air temperature for only a few seconds during spray drying. Thus, it can be concluded that the use of antioxidants during spray drying had no or minute effects on the oxidation of microcapsules. The positive effects of antioxidants might be apparent during long time storage of microcapsules. Another possible explanation is the exposure of oil particles to oxidation before the formation of microcapsules (Binsi et al., 2017), specifically in the emulsification process.

4. Conclusions

Extraction methods and solvents had significant effects on the oxidation of PSO. Rancimat analysis showed that PSO was highly susceptible to oxidation compared to commercial oils. PSO was characterized by a high level of punicic acid, a polyunsaturated fatty acid. The derivatization procedure affected identifiable punicic acid content and the highest content of punicic acid was found in esterification with sodium methoxide. Although inlet air temperature, oil: wall ratio, and MD: GA ratio affected ME, they showed no significant effect on MY. The addition of synthetic and natural antioxidants to the feed solution did not prevent oxidation during spray drying. Further storage studies are necessary to attain better knowledge regarding the effect on the shelf life of PSO microcapsules of oxidation.

5. References

Abbasi, H., Rezaei, K., Emamdjomeh, Z., & Mousavi, S. M. E. (2008). Effect of

various extraction conditions on the phenolic contents of pomegranate seed oil. *European Journal of Lipid Science and Technology*, *110*(5), 435–440. https://doi.org/10.1002/ejlt.200700199

Abbasi, H., Rezaei, K., & Rashidi, L. (2007). Extraction of Essential Oils from the Seeds of Pomegranate Using Organic Solvents and Supercritical CO2. *Journal of the American Oil Chemists' Society*, *85*(1), 83–89. https://doi.org/10.1007/s11746-007-1158-x

Ahn, J.-H., Kim, Y.-P., & Kim, H.-S. (2012). Effect of natural antioxidants on the lipid oxidation of microencapsulated seed oil. *Food Control*, *23*(2), 528–534. https://doi.org/10.1016/j.foodcont.2011.08.026

Anwar, S. H., & Kunz, B. (2011). The influence of drying methods on the stabilization of fish oil microcapsules: Comparison of spray granulation, spray drying, and freeze drying. *Journal of Food Engineering*, *105*(2), 367–378. https://doi.org/10.1016/j.jfoodeng.2011.02.047

AOCS. 1997. Official methods and recommended practices of the American oil chemists' society Method Ce 2-66. Champaign: AOCS Press.

AOCS. 1998. Official methods and recommended practices of the American oil chemists' society Method Cd 18-90. Champaign: AOCS Press.

Arain, S., Sherazi, S. T. H., Bhanger, M. I., Talpur, F. N., & Mahesar, S. A. (2009). Oxidative stability assessment of Bauhinia purpurea seed oil in comparison to two conventional vegetable oils by differential scanning calorimetry and Rancimat methods. *Thermochimica Acta*, *484*(1–2), 1–3. https://doi.org/10.1016/j.tca.2008.11.004

Aruna, P., Manohar, B., & Singh, R. P. (2018). Processing of pomegranate seed waste and mass transfer studies of extraction of pomegranate seed oil. *Journal of Food Processing and Preservation*, *42*(5), e13609. https://doi.org/10.1111/jfpp.13609

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. https://doi.org/10.1080/02652040802075682

Basiri, S. (2013). Evaluation of antioxidant and antiradical properties of Pomegranate (Punica granatum L.) seed and defatted seed extracts. *Journal of Food Science and Technology*, *52*(2), 1117–1123. https://doi.org/10.1007/s13197-013-1102-z

Binsi, P. K., Nayak, N., Sarkar, P. C., Jeyakumari, A., Muhamed Ashraf, P., Ninan, G., & Ravishankar, C. N. (2017). Structural and oxidative stabilization of spray dried fish oil microencapsulates with gum arabic and sage polyphenols: Characterization and release kinetics. *Food Chemistry*, *219*, 158–168. https://doi.org/10.1016/j.foodchem.2016.09.126

Bustamante, A., Hinojosa, A., Robert, P., & Escalona, V. (2017). Extraction and microencapsulation of bioactive compounds from pomegranate (Punica granatum var. Wonderful) residues. *International Journal of Food Science & Technology*, *52*(6), 1452–1462. https://doi.org/10.1111/ijfs.13422

Çam, M., Erdoğan, F., Aslan, D., & Dinç, M. (2013). Enrichment of Functional Properties of Ice Cream with Pomegranate By-products. *Journal of Food Science*, *78*(10), C1543–C1550. https://doi.org/10.1111/1750-3841.12258

Çam, M., İçyer, N. C., & Erdoğan, F. (2014). Pomegranate peel phenolics: Microencapsulation, storage stability and potential ingredient for functional food development. *LWT - Food Science and Technology*, *55*(1), 117–123. https://doi.org/10.1016/j.lwt.2013.09.011

Carneiro, H. C. F., Tonon, R. V., Grosso, C. R. F., & 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. https://doi.org/10.1016/j.jfoodeng.2012.03.033

Costa, A. M. M., Silva, L. O., & Torres, A. G. (2019). Chemical composition of commercial cold-pressed pomegranate (Punica granatum) seed oil from Turkey and Israel, and the use of bioactive compounds for samples' origin preliminary discrimination. *Journal of Food Composition and Analysis*, *75*, 8–16. https://doi.org/10.1016/j.jfca.2018.09.004

Curtis, J. M., Berrigan, N., & Dauphinee, P. (2008). The Determination of n-3 Fatty Acid Levels in Food Products Containing Microencapsulated Fish Oil Using the One-Step Extraction Method. Part 1: Measurement in the Raw Ingredient and in Dry Powdered Foods. *Journal of the American Oil Chemists' Society*, *85*(4), 297–305. https://doi.org/10.1007/s11746-008-1194-1

Đurđević, S., Milovanović, S., Šavikin, K., Ristić, M., Menković, N., Pljevljakušić, D., … Bogdanović, A. (2017). Improvement of supercritical CO 2 and n -hexane extraction of wild growing pomegranate seed oil by microwave pretreatment. *Industrial Crops and Products*, *104*, 21–27. https://doi.org/10.1016/j.indcrop.2017.04.024

Eikani, M. H., Golmohammad, F., & Homami, S. S. (2012). Extraction of pomegranate (Punica granatum L.) seed oil using superheated hexane. *Food and Bioproducts Processing*, *90*(1), 32–36. https://doi.org/10.1016/j.fbp.2011.01.002

Fadavi, A., Barzegar, M., & Hossein Azizi, M. (2006). Determination of fatty acids and total lipid content in oilseed of 25 pomegranates varieties grown in Iran. *Journal of Food Composition and Analysis*, *19*(6–7), 676–680. https://doi.org/10.1016/j.jfca.2004.09.002

Farhoosh, R. (2007). The Effect of Operational Parameters of the Rancimat Method on the Determination of the Oxidative Stability Measures and Shelf-Life Prediction of Soybean Oil. *Journal of the American Oil Chemists' Society*, *84*(3), 205–209. https://doi.org/10.1007/s11746-006-1030-4

Fortin, N. (2016). Food Regulation: Law, Science, Policy, and Practice, Second Edition. John Wiley & Sons, Inc. https://doi.org/10.1002/9781119341178

Ghosh, M., Upadhyay, R., Mahato, D. K., & Mishra, H. N. (2019). Kinetics of lipid oxidation in omega fatty acids rich blends of sunflower and sesame oils using Rancimat. *Food Chemistry*, *272*, 471–477. https://doi.org/10.1016/j.foodchem.2018.08.072

Goula, A. M. (2013). Ultrasound-assisted extraction of pomegranate seed oil – Kinetic modeling. *Journal of Food Engineering*, *117*(4), 492–498. https://doi.org/10.1016/j.jfoodeng.2012.10.009

Goula, A. M., & Adamopoulos, K. G. (2012). A method for pomegranate seed application in food industries: Seed oil encapsulation. *Food and Bioproducts Processing*, *90*(4), 639–652. https://doi.org/10.1016/j.fbp.2012.06.001

Goula, A. M., & Lazarides, H. N. (2015). Integrated processes can turn industrial food waste into valuable food by-products and/or ingredients: The cases of olive mill and pomegranate wastes. *Journal of Food Engineering*, *167*, 45–50. https://doi.org/10.1016/j.jfoodeng.2015.01.003

Grossmann, M. E., Mizuno, N. K., Schuster, T., & Cleary, M. P. (2010). Abstract A72:

Punicic acid, a fatty acid from pomegranate seed oil, inhibits breast cancer cell proliferation. *Chemoprevention and Biological Therapies*. American Association for Cancer Research. https://doi.org/10.1158/1940-6207.prev-09-a72

Habibnia, M., Ghavami, M., Ansaripour, M., & Vosough, S. (2012). Chemical evaluation of oils extracted from five different varieties of Iranian pomegranate seeds. Retrieved from https://www.sid.ir/en/Journal/ViewPaper.aspx?ID=245873

Khoddami, A., Man, Y. B. C., & Roberts, T. H. (2014). Physico-chemical properties and fatty acid profile of seed oils from pomegranate (Punica granatumL.) extracted by cold pressing. *European Journal of Lipid Science and Technology*, *116*(5), 553–562. https://doi.org/10.1002/ejlt.201300416

Lansky, E. P., & Newman, R. A. (2007). Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *Journal of Ethnopharmacology*, *109*(2), 177–206. https://doi.org/10.1016/j.jep.2006.09.006

Lavanya, M. N., Kathiravan, T., Moses, J. A., & Anandharamakrishnan, C. (2019). Influence of spray-drying conditions on microencapsulation of fish oil and chia oil. *Drying Technology*, *38*(3), 279–292. https://doi.org/10.1080/07373937.2018.1553181

Liu, G., Xu, X., Hao, Q., & Gao, Y. (2009). Supercritical CO2 extraction optimization of pomegranate (Punica granatum L.) seed oil using response surface methodology. *LWT - Food Science and Technology*, *42*(9), 1491–1495. https://doi.org/10.1016/j.lwt.2009.04.011

Loughrill, E., Thompson, S., Owusu-Ware, S., Snowden, M. J., Douroumis, D., & Zand, N. (2019). Controlled release of microencapsulated docosahexaenoic acid (DHA) by spray–drying processing. *Food Chemistry*, *286*, 368–375. https://doi.org/10.1016/j.foodchem.2019.01.121

McNamee, B. F., O'Riorda, E. D., & O'Sullivan, M. (2001). Effect of Partial Replacement of Gum Arabic with Carbohydrates on Its Microencapsulation Properties. *Journal of Agricultural and Food Chemistry*, *49*(7), 3385–3388. https://doi.org/10.1021/jf001003y

Melo, I. L. P. De, Carvalho, E. B. T. De, Sılva, A. M. De O. E., Yoshıme, L. T., Sattler, J. A. G., Pavan, R. T., & Mancını-Fılho, J. (2016). Characterization of constituents, quality and stability of pomegranate seed oil (Punica granatum L.). *Food Science and Technology*, *36*(1), 132–139. https://doi.org/10.1590/1678-457x.0069

Pardauil, J. J. R., Souza, L. K. C., Molfetta, F. A., Zamian, J. R., Rocha Filho, G. N., & da Costa, C. E. F. (2011). Determination of the oxidative stability by DSC of vegetable oils from the Amazonian area. *Bioresource Technology*, *102*(10), 5873–5877. https://doi.org/10.1016/j.biortech.2011.02.022

Puértolas, E., & Barba, F. J. (2016). Electrotechnologies applied to valorization of by-products from food industry: Main findings, energy and economic cost of their industrialization. *Food and Bioproducts Processing*, *100*, 172–184. https://doi.org/10.1016/j.fbp.2016.06.020

Sahin-Nadeem, H., & Afşin Özen, M. (2014). Physical properties and fatty acid composition of pomegranate seed oil microcapsules prepared by using starch derivatives/whey protein blends. *European Journal of Lipid Science and Technology*, n/a-n/a. https://doi.org/10.1002/ejlt.201300355

Sassano, G., Sanderson, P., Franx, J., Groot, P., van Straalen, J., & Bassaganya-Riera, J. (2009). Analysis of pomegranate seed oil for the presence of jacaric acid. *Journal of the Science of Food and*

Agriculture, *89*(6), 1046–1052. https://doi.org/10.1002/jsfa.3552

Sun-Waterhouse, D., Zhou, J., Miskelly, G. M., Wibisono, R., & Wadhwa, S. S. (2011). Stability of encapsulated olive oil in the presence of caffeic acid. *Food Chemistry*, *126*(3), 1049–1056. https://doi.org/10.1016/j.foodchem.2010.11.124

Symoniuk, E., Ratusz, K., Ostrowska-Ligęza, E., & Krygier, K. (2017). Impact of Selected Chemical Characteristics of Cold-Pressed Oils on their Oxidative Stability Determined Using the Rancimat and Pressure Differential Scanning Calorimetry Method. *Food Analytical Methods*, *11*(4), 1095–1104. https://doi.org/10.1007/s12161-017-1081-1

Tengku-Rozaina, T. M., & Birch, E. J. (2015). Thermal oxidative stability analysis of hoki and tuna oils by Differential Scanning Calorimetry and Thermogravimetry. *European Journal of Lipid Science and Technology*, *118*(7), 1053–1061. https://doi.org/10.1002/ejlt.201500310

Tonon, R. V., Grosso, C. R. F., & 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. https://doi.org/10.1016/j.foodres.2010.10.018

Uoonlue, N., & Muangrat, R. (2018). Effect of different solvents on subcritical solvent extraction of oil from Assam tea seeds (Camellia sinensis var. assamica): Optimization of oil extraction and physicochemical analysis. *Journal of Food Process Engineering*, *42*(2), e12960. https://doi.org/10.1111/jfpe.12960

Upadhyay, R., & Mishra, H. N. (2014). Multivariate Analysis for Kinetic Modeling of Oxidative Stability and Shelf Life Estimation of Sunflower Oil Blended with Sage (Salvia officinalis) Extract Under Rancimat Conditions. *Food and Bioprocess Technology*, *8*(4), 801–810. https://doi.org/10.1007/s11947-014-1446-z

Wrolstad, R. E., Acree, T. E., Decker, E. A., Penner, M. H., Reid, D. S., Schwartz, S. J., … Sporns, P. (2004). Handbook of Food Analytical Chemistry. John Wiley & Sons, Inc. https://doi.org/10.1002/0471709085

Yeşilsu, A. F., & Özyurt, G. (2019). Oxidative stability of microencapsulated fish oil with rosemary, thyme and laurel extracts: A kinetic assessment. *Journal of Food Engineering*, *240*, 171–182. https://doi.org/10.1016/j.jfoodeng.2018.07.021

Zhang, A., & Chen, Z. Y. (1997). Oxidative stability of conjugated linoleic acids relative to other polyunsaturated fatty acids. *Journal of the American Oil Chemists' Society*, *74*(12), 1611–1613.

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