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Review Article

Effects of Different Extraction Methods on Yield, Polyunsaturated Fatty Acids, Antioxidants, and Stability Improvement of Chia Seed Oil: A Review

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ABSTRACT

There has been an increasing trend in the use of chia seed oil (CSO) rich in polyunsaturated fatty acids (PUFA), mainly α-linolenic and linoleic acids, accompanied by antioxidants like tocopherols, phytosterols, carotenoids and polyphenols, which may benefit to human health. Various conventional (solvent and mechanical pressing) and alternative extractions (supercritical fluid extraction using carbon dioxide and ultrasound-assisted method) have been utilised to extract oil from chia seed based on selected conditions and types of solvent, which affect the oil yield and nutritional composition. Alternative extractions like ultrasound-assisted with solvent (time: 40 min, temperature: 50°C and solvent-to-seed ratio: 12 ml of ethyl acetate/g of chia seed) and supercritical fluid extraction (SFE) using carbon dioxide enriched with 10% acetone (time: 300 min, temperature: 70°C and pressure: 280 bar) can be applied to improve the amount of oil (up to 27.1% and 33.9% for ultrasound-assisted with solvent and SFE, respectively) and PUFA extracted from the chia seed. SFE is recommended as a highly desirable alternative to traditional methods (Soxhlet and pressing) for extracting tocopherol and phytosterol from CSO. Incorporating acetone with SFE could enhance the number of polyphenols and carotenoids in CSO compared to pure supercritical carbon dioxide. However, CSO is highly susceptible to oxidation due to its high concentration of PUFA (more than 80%). Recent research on the

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nutritional qualities of CSO has explored the impact of improvement techniques to prevent loss of nutritional quality. Therefore, natural antioxidants and blends of vegetable oils have been applied to improve CSO's oxidative stability and shelf life.

Keywords: Chia seed oil, conventional extraction, oxidative stability, polyunsaturated fatty acids, supercritical fluid extraction, tocopherols, ultrasonic-assisted extraction

INTRODUCTION

Chia seed (*Salvia hispanica* L.) is originally from Mexico and Guatemala, where it has been consumed for thousands of years (Ixtaina, Mattea, et al., 2011). Countries such as Australia, Argentina, Columbia, America, and Europe have successfully cultivated chia plants and become the largest chia seed producers over the past few years (Grancieri et al., 2019). The high demand for chia seeds is the reason for its continually high share in the global market. Future Market Insights (2023) has estimated that the overall market value for whole chia seed will reach US$390.3 billion over the prediction period (2023–2033). The demand for chia seeds is predicted to report a 7% Compound Annual Growth Rate (CAGR) (2023–2033), higher than the previous performance (2018–2022) with a CAGR of 2.4% (Future Market Insights, 2023).

Food products enriched with polyunsaturated fatty acids (PUFA) from plant sources have gained customer attention for their significant role in positively affecting human health (Kus-Yamashita et al., 2016). The primary dietary source of PUFA is fish oil, but the current need has led to the overexploitation of certain fish species, impacting global fish stocks (Lenihan-Geels & Bishop, 2016). Furthermore, plant oils are cheaper, have higher oxidation resistance and yield higher than fish oil (Tacon & Metian, 2008). Therefore, vegetable oil is investigated as a sustainable alternative source to fulfil the demand for PUFA worldwide. A higher concentration of PUFA in chia seed oil (CSO) is identified and compared with other known plant food sources. Generally, CSO contains about 63.64% α-linolenic acid (ALA), 19.84% linoleic acid (LA), 7.07% palmitic acid (PA), 5.5% oleic acid (OA) and 2.81% stearic acid (SA) (Shen et al., 2018). CSO is characterised by their high PUFA content and as a good source of antioxidants (Dąbrowski et al., 2018a; Dąbrowski et al., 2016). The extraction of high-quality CSO is a recent development globally, and the demand for CSO is growing.

Different extraction methods (conventional and alternative) influenced the composition of PUFA, antioxidants and oil yield (Dąbrowski et al., 2016). Vegetable oil extractions use traditional methods, including solvent extraction or Soxhlet, distillation, and mechanical pressing (hot and cold). However, these methods involve long extraction times that could remove antioxidant compounds in the extracted oil (Scapin et al., 2017). As a result, many researchers nowadays seek the latest extraction techniques to solve these matters. Novel vegetable oil extraction methods from different seeds, like supercritical fluid extraction

(SFE) and ultrasound-assisted extractions, have been studied lately (Ferrentino et al., 2020; Senrayan & Venkatachalam, 2020).

Lipid oxidation is one of the chemical reactions contributing to the deterioration of vegetable oil quality (Fruehwirth et al., 2020). Different approaches and processes were carried out to improve CSO's oxidative stability from lipid oxidation. To our knowledge, there is a limited review of the bioactive compounds (PUFA and antioxidants) of CSO extracted by conventional and alternative extraction methods. It is also the first review paper to report the different improvement techniques for the oxidative stability of CSO from lipid oxidation. Therefore, this review has summarised an overview of current research in CSO extraction and its effect on yield, PUFA and antioxidants. Moreover, different techniques for improving CSO stability, such as natural antioxidants and vegetable oil blending against lipid oxidation, are discussed.

Lipid oxidation is one of the chemical reactions contributing to the deterioration of vegetable oil quality (Fruehwirth et al., 2020). Different approaches and processes were carried out to improve CSO's oxidative stability from lipid oxidation. To our knowledge, there is a limited review of the PUFA and antioxidants in CSO extracted by conventional and alternative extraction methods. It is also the first review paper to report the different improvement techniques for the oxidative stability of CSO from lipid oxidation. Therefore, this review summarises the research on CSO extraction and its effect on yield, PUFA, and antioxidants. Moreover, different techniques for improving CSO stability, such as natural antioxidants and vegetable oil blending against lipid oxidation, are discussed.

EXTRACTION METHODS OF CHIA SEED OIL

The extraction process is one of the vital stages in oil production from seeds. The conventional oil extraction processes are mechanical and solvent extraction. Research groups have evaluated the potential of using alternative extraction technologies to improve CSO yield and nutritional value. Table 1 summarises the advantages and drawbacks of various CSO extraction methods.

Pressing

Pressing is a commonly used technique for the industrial production of CSO. Screw pressing is less expensive than solvent extraction because it does not require costly chemicals to squeeze the oil from chia seed (Martínez et al., 2012). In addition, mechanical pressing can be carried out at a low temperature, preserving the PUFA, tocopherols and carotenoids of CSO. However, pressing is slow and laborious and provides incomplete oil extraction production, thus producing a low yield of CSO (Dąbrowski et al., 2016; Fernandes et al., 2019; Ixtaina, Martínez, et al., 2011). Another disadvantage of pressing is that it accelerates the process of CSO oxidation (Ali et al.,

Table 1
Advantages and drawbacks of methods to extract chia seed oil

Extraction method	Advantage	Drawback	References
Pressing	• Less expensive than solvent extraction (does not require solvent) • Simple technology • Cold pressing conducted at low temperatures to preserve the quality of the CSO	• Slow and time-consuming • Lower oil yield (20.11%–26.3%) than solvent extraction (19.28%–35.8%) • Produces high peroxide value of oil, especially at a higher temperature	Fernandes et al. (2019); Dąbrowski et al. (2016); Ali et al. (2012); Martínez et al. (2012); Ixtaina, Martínez, et al. (2011)
Solvent extraction (Soxhlet and Folch)	• Extract more lipids than other extraction methods •A straightforward methodology and little training needed • Elevated temperature extracted a higher amount of oil from chia seed	• High flammability and toxicity • Long time for extraction (up to 18 hr) • Large volume of solvent wasted, which causes environmental problems	Dąbrowski et al. (2016)
Ultrasound-assisted extraction	• Better penetration of solvent into cellular materials • Improves extraction yield and PUFA content with shorter extraction time compared to solvent extraction and SFE (without ultrasound-assisted)	• Prolonged sonication may lead to degradation of bioactive compounds and changes in extracted oil from chia seed	Rosas-Mendoza et al. (2017)
Supercritical fluid extraction (Fluid: carbon dioxide)	• Environmentally friendly solvent • Supercritical carbon dioxide (SC-CO_2) is readily available • SC-CO_2 has a high solvation power and diffusivity for better penetration to extract lipid components (oils and bioactive compounds) • Polar co-solvent (acetone) can be applied together with SC-CO_2 to extract higher oil yield, polyphenols and carotenoids	• Unsuitable for the extraction of polar antioxidants (polyphenols) • Require more expensive equipment than other conventional methods • Lower oil yield (9.5%–33.9%) than Soxhlet extraction	Bubalo et al. (2018); Dąbrowski et al. (2018b); Bubalo et al. (2015)

2012) due to the high friction force applied during extraction, leading to hydroperoxide formation and triggering oxidative reactions.

A high peroxide value (PV) of 10.98 mEq O_2/kg oil was observed in CSO obtained by pressing, exhibiting a high vulnerability to lipid oxidation (Fernandes et al., 2019). The result obtained for PV of pressed CSO agreed with those reported by previous authors (Martínez et al., 2012), who found that higher pressing temperatures (50°C–70°C) presented a higher susceptibility towards oxidation compared to lower temperatures (20°C and 30°C). As a result, the PV is higher than those allowed by the Commission Implementing Regulation (EU) 2014, which declares that PV for CSO obtained by cold pressing must not be more than 10 mEq O_2/kg oil.

The yield of pressed CSO (20.01%–26.3%) was lower than other extraction methods like Soxhlet, SFE and ultrasound-assisted extraction (Dąbrowski et al., 2016; Fernandes et al., 2019; Ixtaina, Martínez, et al., 2011). Based on the oil yield extracted by different mechanical pressing, hot screw pressing conducted at a high temperature (110°C) for 1 hr could maximise the yield of CSO (26.27%) compared to cold pressing without using high temperature (24.1%) (Dąbrowski et al., 2016). However, the different values of CSO yield obtained are not significant ($p \geq 0.05$). It is similar to Martínez et al. (2012), who stated that different pressing temperatures (30°C–70°C) had no significant effect on CSO yield. Pressing temperature negatively affected the peroxide value of the CSO, but the different temperatures did not significantly influence the oil yield.

Solvent Extraction

The Soxhlet and Folch procedures are the standard methods for total lipid extraction from food materials. Solvent extraction methods are the most effective, with 98% of total oil recovery (Dąbrowski et al., 2016). The different solvents used in extracting CSO include hexane, petroleum ether, acetone and a mixture of chloroform and methanol. Hexane is the most common solvent used for CSO extraction. It is highly effective for vegetable oil extraction due to its high oil recovery, low boiling point temperature (63°C–69°C), and excellent non-polar nature (Liu & Mamidipally, 2005). However, hexane has led to several severe impacts, such as hazardous pollution to the environment, toxicological effects on human health, and residual solvents in the final product (Kumar et al., 2017).

According to their polarity, four solvents were also used to extract CSO in isolating bioactive compounds and oil recovery. These solvents include petroleum ether, chloroform, methanol, and acetone. The oils extracted are strongly affected by the solubility and extractability of lipids in the solvents (Ramluckan et al., 2014). However, less oil yield was obtained from polar solvents due to the difficulty in molecular interaction between substances in the sample matrix and solvent. The yield of CSO from solvent extraction methods was proven to be higher than other alternative extractions (19.28%–36.1%)

(Dąbrowski et al., 2016; Dąbrowski et al., 2018a; Dąbrowski et al., 2018b; Fernandes et al., 2019; Ishak, Ghani, Yuen, 2020; Oteri et al., 2023; Rosas-Mendoza et al., 2017; Shen et al., 2018; Timilsena et al., 2017).

Few studies reported the low yield of CSO obtained using cold extraction without applying the Soxhlet apparatus at room temperature. Low oil recovery of CSO was observed using hexane and petroleum ether (19.28% and 26.6%, respectively) under cold extraction conditions (Fernandes et al., 2019; Timilsena et al., 2017). High oil yield from chia seed (more than 30%) was achieved with hexane under hot extraction conditions using the Soxhlet apparatus. In general, lipids' solubility can be improved with increased temperature by breaking the strong molecular oil interactions and enhancing the extraction rate of oils from the molecule (Ritcher et al., 1996). These findings suggested that the oil from chia seed under hot extraction conditions is more efficient than cold extraction.

Ultrasound-assisted Solvent Extraction

Using ultrasound technology in various food industries has numerous potentials for efficiency, yield and selectivity (Chemat et al., 2017). Ultrasound-assisted extraction (UAE) is the latest technique with many advantages to improve CSO yield. UAE is a rapid method to provide higher extraction oil yield than conventional extraction procedures without using ultrasound-assisted. Generally, ultrasonic power above 20 kHz leads to cavitation, which involves microbubble shock formed by excess vibration and generating pressure until the bubble explodes on the contact surface between the sample matrix and a liquid solvent (Rosas-Mendoza et al., 2017). Thus, such reactions break the sample's cell wall, and the extraction of lipid components into the solvent is enhanced (Chemat et al., 2017).

Applying ultrasound on chia seed samples in ethyl acetate and petroleum ether resulted in significantly higher oil yield than extraction without ultrasound (de Mello et al., 2017; Rosas-Mendoza et al., 2017). The ultrasonic extraction using optimal conditions at 40 min, temperature of 50°C and solvent-to-seed ratio (12 ml of ethyl acetate/g of seed) produced a CSO yield of 27.19% higher than without ultrasound (22.12%) (de Melo et al., 2017). Meanwhile, the extraction oil yield of chia seed is increased by 3.2% after being treated with ultrasonic power at 40 kHz for 90 min compared to the stirring method (Rosas-Mendoza et al., 2017). Therefore, the organic solvents used for CSO extraction assisted by ultrasound treatment are appropriate for replacing hexane. The warming effect during ultrasound treatment also affects the oil recovery from chia seed.

In comparison with the study (de Melo et al., 2017; Rosas-Mendoza et al., 2017), the yield of CSO was slightly higher at hot extraction 50°C (27.19%) compared to cold extraction without heat treatment (25.0%). It shows that using an ultrasound bath, the CSO extraction has improved with increased temperature. An increase in extraction temperature enhanced the speed rate of microbubble breaking, and this process would promote the penetration of solvent into the cell wall of the sample and increase the extraction rate of lipid compounds into the solvent (Li et al., 2016). Applying ultrasound as a sample pre-treatment before SFE is the best option to improve CSO yield for a shorter extraction time. Fernandes et al. (2019) reported that the use of ultrasound treatment on chia seed for 15 min before SFE increased the total oil yield (24.5%) compared with SFE without using ultrasound (22.2%). The authors also examined the different durations of the ultrasound process on chia seed oil extraction at 30 and 60 min, which showed similar oil content of chia seed (24.5%). Extending the extraction time using ultrasound may lead to a decrease or no changes in the CSO yield due to the maximum release of lipid components at the beginning of the extraction. After a certain period, the CSO extraction process was completed. Therefore, using ultrasonic treatment with appropriate extraction time and temperature would improve the CSO recovery using extraction methods.

Supercritical Fluid Extraction

Most SFE has been performed with supercritical carbon dioxide (SC-CO_2) as a supercritical solvent due to its critical conditions (temperature: 31.1°C and pressure: 73.8 bar), non-explosive, inexpensive, user-friendly and easy to remove from the product. Thus, it can be applied to food (Bubalo et al., 2015). SC-CO_2 has low viscosity and high diffusivity due to the high pressure, which promotes better transport properties by rapidly penetrating the sample molecules, resulting in faster extraction rates than liquids. SC-CO_2 is suitable for extracting less polar compounds like fatty acids (Herrero et al., 2006). The oil content of chia seed extracted by SFE highly varied (9.5%–33.9%) according to the different extraction conditions (Dąbrowski et al., 2018b; Fernandes et al., 2019; Ishak et al., 2021; Ixtaina et al., 2010; Ixtaina, Mattea, et al., 2011).

The extraction pressure is the main parameter that influences the extraction efficiency of CSO, as reported by Ishak et al. (2021), Fernandes et al. (2019), Dąbrowski et al. (2018b), Ixtaina, Vega, et al. (2011) and Ixtaina et al. (2010). Increased extraction pressure typically enhances the desired compound's solubility (Bubalo et al., 2018). An increase in pressure resulted in higher extraction oil yields from chia seed, which is elevated from approximately 3.9% at 150 bar to almost 17.45% at 250 bar (Fernandes et al., 2019). High pressure generally improved the oil yield due to increased SC-CO_2 density (Soh et al., 2018). Higher pressure would then facilitate the oil movement into the surface of the sample matrix (Ghoreishi et al., 2016). Extraction temperature and time also affected the oil recovery from chia seed. At the constant pressure (250 bar), a higher amount of CSO (17.45%) is obtained at a lower temperature (40°C) compared to oil extracted at 60°C (11.96%) (Fernandes et al., 2019). The authors stated that an increase in temperature reduced the SC-CO_2 density, which led to a decrease in the oil yield.

Meanwhile, the yield of CSO improved by 5% after extending the extraction time from 60 to 75 min at constant pressure (250 bar) and temperature (40°C) (Fernandes et al., 2019). The range of extraction time to recover the oil from chia seed based on previous studies is 75–300 min (Dąbrowski et al., 2018b; Fernandes et al., 2019; Ixtaina et al., 2010; Ixtaina, Mattea, et al., 2011; Scapin et al., 2017). The extraction consists of two periods, which include a fast extraction period (initial linear and transition phase) and a slow extraction period (second linear phase) (Ixtaina, Mattea, et al., 2011; Ixtaina et al., 2010). Most CSO extraction mainly occurred in the fast extraction period based on the accumulated extraction curves for oil removal by SC-CO_2 (Ixtaina, Mattea, et al., 2011). The result showed that the total extraction time for CSO extracted by higher pressure (450 bar) at 40°C and 60°C were 135 and 138 min, respectively. In contrast, oil extracted at a lower pressure (250 bar) indicated longer total extraction time at 40°C and 60°C (285 and 423 min, respectively). CSO extracted by the SFE process varied from 9.5% (60 min with SC-CO_2) to 33.9% (300 min with SC-CO_2 enriched by 10% acetone) with the pressure of 280 bar at 70°C (Dąbrowski et al., 2018b).

An increased extraction time from 60–300 min for pure SC-CO_2 significantly improved CSO yield (9.5%–32.7%). Moreover, acetone yielded more CSO and minimised the extraction time. The extraction enriched with 10% acetone obtained a higher yield of CSO (22.2%) than pure SC-CO_2 (9.5%) at 60 min of extraction time. These findings agree with Fernandes et al. (2019), who reported that the addition of 30% ethanol as a polar co-solvent in the SFE process produced more oil yield (25.10%) than the pure SC-CO_2 (22.24%) for 75 min of extraction. SC-CO_2 polarity has difficulty extracting polar substances (phenolic compounds). Ethanol and acetone are mainly used as co-solvents to improve the polarity of SC-CO_2, enhancing its solvating power to extract phenolic compounds (Bubalo et al., 2015). Besides that, different particle sizes of ground chia seed influenced the oil extraction using SC-CO_2 (Ishak et al., 2021). The authors reported that intermediate particle sizes of ground chia seed (100–400 μm) based on the grinding time of 10 s has higher surface area (0.0350 m^2/g) to contact with SC-CO_2 compared to larger particle sizes of ground sample (0.066 m^2/g), resulting in oil extracted quickly from the surface of the sample matrix. Therefore, the overall fat content of chia seed (9.5%–35.8%) is significantly affected by conditions and parameters employed during oil extraction, such as temperature, solvent type, extraction time, pressure, co-solvent and particle size of the ground sample.

EFFECTS OF EXTRACTION METHODS ON POLYUNSATURATED FATTY ACIDS AND ANTIOXIDANTS OF CHIA SEED OIL

Lipids contain PUFA and antioxidants (phytosterols and tocopherols), which are soluble in non-polar organic solvents but poorly soluble in polar solvents. Polyphenols are the minor antioxidant compounds in CSO. The composition of CSO (fatty acids and antioxidants) is based on the different extraction methods presented in Table 2.

Table 2
Composition of chia seed oil (fatty acids and antioxidants) based on the different extraction methods

Composition	Unit	Range (minimum-maximum)	Extraction methods	References
Fatty acids				
Polyunsaturated fatty acids	%	73.69–89.84	Soxhlet (hexane and acetone), petroleum ether extraction using an orbital shaker, Folch procedure (chloroform and methanol), mechanical pressing (hot and cold), SFE (CO_2 and acetone), ultrasound-assisted extraction	Oteri et al. (2023); Ishak et al. (2021); Ishak, Ghani and Nasri (2020); Dąbrowski et al. (2018a); Shen et al. (2018); Hrnčič et al. (2018); Scapin et al. (2017); Ixtaina, Mattea, et al. (2011)
Saturated fatty acids	%	7.76–16.09		
Monounsaturated fatty acids	%	2.43-10.53		
Antioxidants				
Tocopherols	mg/kg	100–1244	Soxhlet, mechanical pressing (hot and cold) and SFE (SC-CO_2, acetone and *n*-propane)	Ishak et al. (2021); Dąbrowski et al. (2018a); Dąbrowski et al. (2018b); Shen et al. (2018); Dąbrowski et al. (2016); Zanqui et al. (2015); Ciftci et al. (2012); Ixtaina, Mattea, et al. (2011); Álvarez-Chávez et al. (2008)
Phytosterols	mg/kg	2272–12600		
Polyphenols	mg/kg	0–172		

Note. SFE=Supercritical fluid extraction, SC-CO_2=supercritical carbon dioxide, UAE=Ultrasound-assisted extraction

Polyunsaturated Fatty Acids

CSO is recommended as one of the healthiest oils in the market due to its high PUFA content (73.69%–89.84%) which consists of ALA (53.67%–69.3%) and LA (16.6%–23.21%) (Dąbrowski et al., 2018a; Hrnčič et al., 2018; Ishak, Ghani, Nasri, 2020; Ixtaina, Mattea, et al., 2011; Oteri et al., 2023; Scapin et al., 2017; Shen et al., 2018). Furthermore, saturated fatty acids (SFA), including PA (5.5%–11.5%) and SA (0.29%–7.89%), were calculated for 7.76%–16.09% of total fatty acids. The minor concentration of OA (2.43%–10.53%) is classified as monounsaturated fatty acids (MUFA), also present in CSO (Ciftci et al., 2012; Ixtaina et al., 2010; Segura-Campos et al., 2014). The CSO is rich in PUFA with more than 80% regardless of conventional extraction methods (Dąbrowski et al., 2016; Fernandes et al., 2019; Ixtaina, Martínez, et al., 2011).

Cold (conducted at room temperature) and hot mechanical pressing (110°C) showed no significant differences in the fatty acid composition of CSO. The PUFA content in cold-pressed CSO (82.2%) was higher ($p \geq 0.05$) than in hot-pressed CSO (81.7%) (Dąbrowski et al., 2016). Most CSO extracted by different solvent extraction methods (Soxhlet, Folch and mixing) had a level of PUFA (more than 80%). This can be explained by the high operational temperature and solvent recycling related to the higher solute solubility and the interaction of the sample and the solvent during extraction (Abdolshahi et al., 2015). It was also observed that non-polar solvents such as hexane and petroleum ether are excellent choices for non-polar lipids such as fatty acids. Therefore, the concentration of PUFA was not affected by different methods and solvents with polarity differences used to extract oil from the chia seed.

Applying ultrasound-assisted solvent extraction using ethyl acetate provided higher PUFA content in CSO than other extraction methods. The results showed that UAE extracted 82% PUFA content with less solvent used (1 g of chia seed in 12 ml of ethyl acetate), reduced extraction time (40 min) and higher selectivity (de Mello et al., 2017). Previous studies have reported that UAE provided better extraction of bioactive compounds in seeds by destroying plant cells due to the strong impact of ultrasonic waves (Tian et al., 2013). The application of UAE in the CSO industry is considered the best option to improve extraction efficiency by performing low operation temperatures, which may prevent heat damage to the oil and preserve the nutritional qualities of the oil.

Several authors have reported the research on the fatty acid composition of CSO extracted by SC-CO_2 compared with the conventional method (Ixtaina, Mattea, et al., 2011; Ixtaina, Martínez, et al., 2011). Ixtaina et al. (2010) are the first authors to report on the fatty acids of CSO extracted by SFE at different conditions of pressure (250, 350 and 450 bar), temperature (40°C, 60°C and 80°C) and time (60, 150 and 240 min). The results showed that wide variations were observed in the amount of each fatty acid in CSO, including ALA (44.4%–63.4%), LA (19.6%–35%), PA (6.8%–14%), SA (2.5%–13%) and OA (3.9%–11.1%) based on the different SFE operating conditions and extraction time significantly affected ($p \leq 0.05$) the concentration of ALA and LA. The highest oil yield of chia seed based on the optimal conditions (80°C, 450 bar and 300 min) was compared with hexane extraction for the fatty acid composition. It is indicated that the fatty acid profile for both oils obtained was not significantly different ($p \geq 0.05$). Ixtaina et al. (2011a) also revealed that no significant differences ($p \geq 0.05$) were detected in the fatty acid composition of CSO obtained by both extraction methods, except that the amount of LA was significantly higher in oil extracted by SFE (pressure: 250 and 450 bar and temperature: 40°C and 60°C) compared to solvent extraction. Dąbrowski et al. (2018b) conducted a study to show the impact of acetone as a modifier at different concentrations and times with SC-CO_2 on the fatty acid composition of CSO. Results showed that the CSO were relatively similar in PUFA level (78.5%–82.2%) regardless of acetone addition and extraction time. Therefore, adding acetone as a high-polarity solvent in the SFE systems did not change the concentration of the fatty acid profile in the CSO.

Tocopherols and Phytosterols

Generally, oil extracted from plant seeds contains tocopherols and phytosterols, considered natural antioxidants (Hussain et al., 2021; Gharby et al., 2017). Tocopherols and phytosterols in CSO varied from 100–1244 mg/kg and 2998.8–12600 mg/kg, respectively. Tocopherols in CSO consist mainly of *γ*- with more than 90% of the total, followed by a minor amount of *α*- (about 5%) and *δ*- (around 3%). Meanwhile, the main representative of phytosterols was β-sitosterol (1829–7960 mg/kg), followed by stigmastanol (2180–2770 mg/kg), stigmasterol (173–1830 mg/kg), campesterol (271-924.6 mg/kg), 25-hydroxy-24-methylcholesterol (430.1–683.2 mg/kg), Δ5-avenasterol (355 mg/kg), 24-methylenecycloartanol (127.4–146.8 mg/kg) and other sterol compounds (2180–2770 mg/kg) was present in CSO (Álvarez-Chávez et al., 2008; Ciftci et al., 2012; Dąbrowski et al., 2016; Dąbrowski et al., 2018a; Ishak et al., 2021; Ixtaina, Mattea, et al., 2011; Ixtaina, Martínez, et al., 2011; Shen et al., 2018; Zanqui et al., 2015). The variability is highly dependent on the oil extraction method and the origin of the seeds.

Tocopherols and phytosterols were observed in CSO for alternative and conventional extraction methods. Dąbrowski et al. (2016) reported the composition of tocopherols and phytosterols of CSO obtained by Soxhlet extraction using different solvents (hexane and acetone) and screw pressing (cold and hot conditions). CSO obtained by hexane extraction has significantly higher total phytosterol contents than other extraction methods, indicating that hexane is the best non-polar solvent to extract the highest amount of phytosterols. Meanwhile, both oils extracted at different pressing conditions contained the lowest total phytosterols. *β*-sitosterol is the primary compound with 61% of the total content. Other studies also reported that *β*-sitosterol is the major sterol compound in CSO (Álvarez-Chávez et al., 2008; Ciftci et al., 2012; Shen et al., 2018). Tocopherol of CSO extracted by conventional methods showed *γ*-tocopherol is the main compound obtained regardless of the extraction method used. CSO extracted by cold pressing showed significantly higher total tocopherols than Soxhlet using different solvents and hot pressing at 110°C (Dąbrowski et al., 2016) due to no heat involved from cold pressing and did not degrade tocopherols (Wang et al., 2010).

A higher yield of tocopherols and phytosterols extraction was found in CSO obtained by SFE compared to conventional methods (Dąbrowski et al., 2016). The results showed that the extraction of phytosterols in CSO was higher in SFE at 70°C. In contrast, the higher temperature at 90°C increased the greatest yield of tocopherols at 300 min of extraction time. They reported that high temperatures in the Soxhlet extraction and hot pressing degraded the number of tocopherols and phytosterols in CSO. Furthermore, acetone's addition as co-solvent at different percentages with SC-CO_2 decreased the extraction of tocopherols and phytosterols in the CSO compared to pure SC-CO_2, indicating these compounds are diluted with the presence of acetone (Dąbrowski et al., 2018b).

The extraction pressure and temperature of SFE can be varied to select the highest amount of targeted bioactive compounds (Follegatti-Romero et al., 2009). Ixtaina, Mattea, et al. (2011) reported that CSO extracted at a higher pressure (450 bar) contained significantly higher tocopherol contents compared to lower pressure (250 bar). Peng et al. (2020) stated that applying high pressure (300 bar) would improve the interaction between SC-CO_2 and the sample matrix, contributing to the higher diffusivity of γ-tocopherol in CSO during extraction. It is also related to the internal mass transfer rate and diffusivity enhanced during the extraction process, thus improving the tocopherol levels (Wang et al., 2017). The CSO extraction by compressed liquefied petroleum gas (LPG) and SC-CO_2 to evaluate the quality of oils obtained regarding α-tocopherol, β-sitosterol and antioxidant activity. CSO obtained by SC-CO_2 at lower pressure and temperature (100 bar and 20°C) achieved higher antioxidant activity (87%), α-tocopherol (22.95 mg 100 g^{-1}) and β-sitosterol (77.10 mg 100 g^{-1}) than CSO extracted by LPG. Different parameters for both solvents influenced the qualities of CSO (Scapin et al., 2017). However, LPG is classified as toxic and highly flammable compared to SC-CO_2, which is generally considered safe (Abaide et al., 2017). Therefore, the extraction of CSO using SC-CO_2 is the best choice to obtain more lipophilic antioxidant compounds than LPG.

Polyphenols

Polyphenols exhibit various positive human health effects, such as anti-inflammatory, anti-cancer, and antioxidant activities (Yeo et al., 2015). Polyphenol in CSO ranged from 0 mg/kg (cold pressing) to 172 mg/kg (Soxhlet using acetone). The polyphenolic compounds present in CSO extracted by different extraction methods were caffeic acid, chlorogenic acid, myricetin, quercetin and kaempferol (Ixtaina, Mattea, et al., 2011; Ixtaina, Martínez, et al., 2011). Even though lower levels of phenolic compounds are found in oils than in whole seeds, the same compounds are detected in chia whole seeds (Reyes-Caudillo et al., 2008). Hence, this may be due to polyphenols being polar and hydrophilic, which are not soluble in lipids (Ixtaina, Mattea, et al., 2011a).

SC-CO_2 alone may not be able to extract polar compounds such as polyphenols. Co-solvents are employed during extraction at a small percentage (1%–10%) for better extraction of polyphenols. The modifiers with higher polarity than CO_2 by expanding the range of targeted compounds (Bimakr et al., 2011). Generally, the modifiers used with CO_2 extraction are ethanol, methanol, acetone and mixtures of several solvents (de Melo et al., 2014). Dąbrowski et al. (2018b) reported that incorporating acetone as a modifier in the SFE system progressively increased the polyphenol concentration of CSO. The higher modifier (up to 10%) added into the SC-CO_2 for a short period at 1 hr improved the polyphenol concentrations to the maximum level until 33 mg/kg of CSO. Acetone is commonly used as a polar solvent to extract plant polyphenols (Haminiuk et al., 2014). According to the extraction method, CSO extracted by the Soxhlet method using acetone obtained 24 times higher total phenolic contents than oil extracted by SFE at 90°C (Dąbrowski et al., 2016). It can be concluded that extraction methods using acetone as modifiers to produce CSO contain relatively high polarity of polyphenols known for their antioxidant activity.

STABILITY IMPROVEMENT OF CHIA SEED OIL

Previous research has reported that the induction period (IP) of the bulk CSO is short due to the high concentration of PUFA (>80%), as these are highly unsaturated and can quickly oxidise when subjected to high temperature and overflow air-based conditions (Dąbrowski et al., 2016; Dąbrowski et al., 2018a; Ishak, Ghani, Nasri, 2020; Ixtaina, Mattea, et al., 2011; Ixtaina, Martínez, et al., 2011; Shen et al., 2018; Timilsena et al., 2017). The shorter the IP of oils, the more decomposition products containing fatty acids are released from lipid oxidation (Santos et al., 2013). Generally, lipid oxidation causes many challenges during food processing and storage, including off-flavours in food by the formation of secondary oxidation products (aldehydes and ketones) (Let et al., 2005), reducing nutritional qualities of the oils (essential fatty acids and lipid-soluble vitamins) (Arab-Tehrany et al., 2012) and possessing adverse effect on human health (Jacobsen et al., 1999). Regarding the high content of PUFA in CSO, the formation rate of primary oxidation products increased by higher unsaturated double bonds present in oil (Maszewska et al., 2018). Thus, immediate oxidation occurs in the CSO, which is related to the highest amount of PUFA, with the most prominent fatty acid composed of ALA (53.67%–69.3%). Therefore, the oxidative stability of CSO can be improved by adding antioxidants and in combination with other vegetable oils.

Addition of Antioxidants

Natural or synthetic antioxidants can improve the shelf life of food products by delaying lipid oxidation. Natural antioxidants from plant sources are considered the best option to replace synthetic antioxidants due to their potential health benefits and safety (Lourenço et al., 2019). Lipid oxidation commonly occurs in food products when exposed to oxygen, heat, and light (Carocho et al., 2014). Natural plant extracts proved to be thermally stable during processing and exhibited antioxidant activity (Taghvaei & Jafari, 2015). A few research have evaluated the effectiveness of various natural antioxidants on the oxidative stability of CSO by pressing and analysing using the Rancimat method and storage study (Aliabadi et al., 2023; Bodoira et al., 2017; Ixtaina et al., 2012; Jung et al., 2021).

Aliabadi et al. (2023) reported that the highest concentration of oregano (*Origanum vulgare* L.) extract (1800 ppm) had the highest oxidative stability index of CSO (8.31 hr) compared to oil containing yarrow (*Achillea millefolium*) extracts (600–1800 ppm) according to the Rancimat conditions (110°C and an airflow rate of 20 L/hr). Furthermore, CSO enriched with oregano extract at 1800 ppm had the lowest acid, peroxide, anisidine and total oxidation values under accelerated oxidation conditions for five days in the oven at 90°C. Ixtaina et al. (2012) reported that applying different antioxidants improved CSO's shelf life. The best antioxidant effects recorded in CSO with the addition of ascorbyl palmitate (AP) (2,500 and 5,000 ppm), rosemary extract at the highest concentration studied (5,000 ppm) and the mixture of green extract and rosemary extract (1:1) (Ixtaina et al., 2012). However, adding tocopherols (more than 1500 ppm) decreased the IP of CSO, showing that tocopherols are less effective against lipid oxidation than other antioxidants tested. It can be explained mainly by the polar paradox, which is based on the different antioxidant polarities in which tocopherols might be passive in bulk CSO and sensitive to high temperatures (Ixtaina et al., 2012).

Other studies also supported these findings, where only tocopherol added (200 mg/kg) in the CSO showed the least performance as an antioxidant compared to other antioxidants and their combinations (Bodoira et al., 2017). However, the oxidative stability of CSO improved significantly by the synergistic effect between the mixture of tocopherols (200 mg/kg) and rosemary extract (8000 mg/kg). In contrast, the addition of natural antioxidants in low concentrations (200 mg/kg) demonstrated no significant effect ($p \geq 0.05$) on the oxidative stability of CSO obtained by hexane extraction (Souza et al., 2017). However, tert-butylhydroquinone (TBHQ), a synthetic antioxidant applied in the CSO, was the most efficient against lipid oxidation. The synergistic antioxidant effect between TBHQ and rosemary extract at the concentration of 200 mg/kg increased the oxidative stability of CSO (IP: 11.43 hr) significantly compared to the addition of rosemary extract alone (IP: 1.53 hr). Thus, the increased IP of CSO with enhanced concentrations and combinations of selected antioxidants was observed (Bodoira et al., 2017; Ixtaina et al., 2012; Souza et al., 2017).

Blending of Vegetable Oils

Blending edible oils from plants is one of the most straightforward procedures to enhance stability and nutritional properties at affordable prices (Bordón et al., 2019). The blending of cold-pressed CSO with other vegetable oils (walnut, almond, virgin and roasted sesame oils) based on the different proportions (20:80, 30:70 and 40:60) were compared in terms of their oxidative stability by conducting the Rancimat method (flow rate: 20 L/hr and temperature: 100°C) and accelerated stability test (40°C for 12 days) (Bordón et al., 2019). Pure CSO contained the lowest oxidative stability index ($p \leq 0.05$) compared to other CSO blended with other vegetable oils according to the Rancimat method. Sesame oil blends (both virgin and roasted) with CSO were observed as the most stable against oxidation, while walnut oil mixed with CSO had the lowest oxidative stability among all oil blends (Bordón et al., 2019).

Meanwhile, similar trends were observed for the oxidative stability of CSO blends using accelerated storage conditions. The low stability of oil blends (chia seed and walnut) was observed due to the high content of PUFA (more than 70%). The oil blends with almond and sesame oil (both virgin and roasted) presented higher oxidative stability by yielding a small number of oxidation products based on the peroxide values lower than 3 mEq O_2/kg throughout the entire accelerated study for 12 days (Bordón et al., 2019). The oxidative stability of the mixture of sunflower and cold-pressed chia seed oil (90:10 and 80:20) with the addition of natural antioxidants was evaluated at different storage temperatures (4°C and 20°C) for 360 days. CSO blends with rosemary extract, and AP presented the best oxidative stability throughout the storage study (360 days) compared to other CSO samples without antioxidants (Guiotto et al., 2014). The authors suggested that the addition of antioxidants in the oil blends increased the activation energy and decreased the rate constant, showing an improvement in the oxidative stability of the CSO. Therefore, increased storage temperature and unsaturated fatty acids significantly reduced the oxidative stability of oil blends (sunflower and CSO) during oxidation (Guiotto et al., 2014).

CONCLUSION

CSO has been extracted using various methods such as pressing (hot and cold), solvent extraction (Soxhlet and Folch), SC-CO_2 and ultrasound-assisted extraction. The conventional techniques heated at boiling temperature according to the different types of solvent obtained a higher yield of CSO than other extraction methods. However, mechanical pressing produced the lowest CSO recovery. The improvement in CSO yield can be achieved by conducting an ultrasound extraction system using conventional or alternative methods. Meanwhile, most CSOs contain more than 80% PUFA, obtained by different extraction methods. CSO mainly composes ALA and LA, which may help reduce cardiovascular disease risk. The content of PUFA in CSO was not affected by solvent extraction with different polarity and the addition of acetone in the SFE method. It was found that the application of ultrasound-assisted solvent extraction improved the PUFA level of CSO. Meanwhile, CSO extraction using SC-CO_2 has a high tocopherol and phytosterol content comparable to oils extracted by hexane and LPG. The addition of acetone with SC-CO_2 significantly improved the level of polyphenols in CSO. CSO has emerged as a new source of edible oil with functional properties that can be applied to food products and used as healthy supplements rich in PUFA. The stability of CSO is the primary concern that needs to be focused on since it is high in PUFA, which is very susceptible to oxidation, thus shortening its shelf life when subjected to improper storage temperature. This problem can be solved by adding natural antioxidants in combination with other vegetable oils to maintain the nutritional quality and improve the oxidative stability of CSO. In future, extensive research should be conducted to reveal more applications of recent extraction methods such as aqueous enzymatic, microwave or pulsed electric field-assisted to produce higher oil yield and several lipophilic compounds, including PUFA, tocopherols, phytosterols and polyphenols in chia seed.

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