

## Effect of extraction solvents on the oxidative stability of chia seed (*Salvia hispanica* L.) oil stored at different storage temperatures

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### Abstract

This study consists of two parts. The first part is to identify the fatty acid composition of chia seed oils obtained by Soxhlet method using acetone and hexane as extraction solvents with different extraction times including acetone 4 hrs (A4), acetone 8 hrs (A8) and hexane 8 hrs (H8) as a control. Next, the oxidative stability and antioxidant activity of chia seed oils stored at different temperatures (25°C and 40°C) for 18 days were evaluated. From the study, chia seed oil (A8) had the highest content of  $\alpha$ -linolenic acid (67.79%) with significant difference ( $p < 0.05$ ) followed by other oil samples that were extracted using acetone and hexane for 4 hrs (67.54%) and 8 hrs (66.38%), respectively. The oxidative stability of chia seed oil was determined by peroxide value, *p*-anisidine value and TOTOX value. The results revealed that chia seed oils stored at room temperature (25°C) had higher oxidative stability compared to oil samples stored at 40°C. Elevated temperature strongly affected lipid oxidation. The control sample had higher oxidative stability than acetone-extracted chia seed oils. Meanwhile, antioxidant activity using DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity test was also carried out. Antioxidant activity of chia seed oil extracted by acetone had higher radical scavenging activity inhibition ( $p < 0.05$ ) than the control sample at both temperatures (25°C and 40°C). The results confirmed that chia seed oil obtained by acetone had higher polyunsaturated fatty acids and lower oxidative stability than hexane. In conclusion, chia seed oil extracted by hexane showed better oxidative stability at different storage temperatures.

## 1. Introduction

Demand for vegetable oil has increased due to increasing domestic and industrial purposes. Nutritionally, vegetable oil provides calories, vitamins, and essential fatty acids for the human diet (Akinoso and Oni, 2012). Chia seeds (*Salvia hispanica* L.) have a high oil content (30-40%), which is rich in polyunsaturated fatty acids (PUFA), mostly omega-3 ( $\alpha$ -linolenic acid, 54-67%) and omega-6 (linoleic acid, 12-21%) (Coorey *et al.*, 2014). Because of the high PUFA content, the consumption of chia seeds brings some health benefits (Marineli *et al.*, 2015; Sierra *et al.*, 2015; Fonte-Faria *et al.*, 2019). Chia seed oil also contains high levels of tocopherols and phytosterols, as well as a minor concentration of polyphenols, carotenoids, and squalene (Ixtaina *et al.*, 2011; Dąbrowski *et al.*, 2017). However, chia seed oils are susceptible to oxidation due to the

highest amount of unsaturated fatty acids (more than 80%) among all vegetable oils (Timilsena *et al.*, 2017). Thus, lipid oxidation degrades nutritional qualities, sensory properties and shortens storage life (Vaidya and Eun, 2013). Metal ions, higher temperature and exposure to light and oxygen are several factors that could affect lipid oxidation during storage (Bendini *et al.*, 2010). Particularly, the temperature has the most significant effect on oil autoxidation because elevated temperature speeds up oxidation level by decomposing the hydroperoxides (primary oxidation products), to alkoxy radicals and finally to secondary oxidation products (aldehydes and ketones) (Kim and Min, 2008).

Previous studies determined the effect of storage temperature on the oxidative stability of walnut and grape seed oil which is contained predominantly in unsaturated fatty acids (Vaidya and Eun, 2013). The

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authors found out that storage temperature at 25°C on both oil oxidations occurred steadily. However, oxidation parameters of both oils increased as the temperature elevated (40°C and 60°C) due to the formation of secondary products from the decomposition of hydroperoxides. This indicates that storage temperature affects the oxidative stability of edible oils. Oxidation parameters, such as the peroxide value (PV) and *p*-anisidine value (AV) are commonly used as the quality indicators of edible oil (Pristouri *et al.*, 2010). Antioxidant activity is another important quality parameter of edible oil. The method of measuring the scavenging free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was normally used to determine the antioxidant activity of oil (Kalantzakis *et al.*, 2006). Oil extraction from plant seed can be conducted by using different solvent polarities (polar and non-polar solvents). Various studies have been published on the different solvent polarities influenced the oxidative stability of other edible oils from seeds such as *Tecoma stans* and plum (Jedidi *et al.*, 2020; Savic *et al.*, 2020). To the best of our knowledge, the impacts of different temperatures (25°C and 40°C) on oxidative stability and antioxidant activity of chia seed oil obtained by extraction solvents have not been studied yet.

Several widely practised methods for lipid extraction, including distillation procedure, mechanical pressing and solvent extraction are carried out to obtain the oil components from the raw materials. Solvent extraction is the most widely used procedure. The extraction of desirable compounds obtained through the several stages: (1) the solvent enters into the solid matrix; (2) the solute from the solid matrix soluble in the solvents; (3) the solute is diffuse out of the solid matrix; (4) the extracted solutes are collected. Any factor enhancing the diffusivity and solubility of the solutes in the above steps will facilitate the oil extraction. The polarity of the solvent, the particle size of the raw materials, the solvent-to-solid ratio, the temperature and the duration will influence the extraction capability (Yi *et al.*, 2012; Li *et al.*, 2014). The solvent selection is crucial for oil extraction. Polarity, availability, cost and safety are important aspects for the selection of solvents (Zhang *et al.*, 2018).

Different extraction solvents, such as hexane and acetone, were used to extract oil from chia seeds. Both of these solvents have distinct effects on the health, environment and quality of the extracted oil. The choice of solvents used for extraction plays an essential role in the production of good quality oils regarding the recovery, the composition of fatty acids and the oxidative stability (Okeleye and Betiku, 2019). Since hexane has an aliphatic structure, it is the most suitable

solvent for vegetable oil extraction. However, hexane is extremely flammable. Moreover, it has acute and chronic toxicity for human health through inhalation, ingestion, and contact with eye or skin. Clean Air Act 1990 regulates the law due to the potential health risks from hexane by searching an alternative solvent which is safer and less toxic that could produce high-quality oil (Tir *et al.*, 2012). Therefore, polar solvents such as acetone and ethyl acetate have been recommended as alternative extraction solvents to hexane. Acetone has an excellent reputation for obtaining lipids without proteins degradation. It easily extracts more polar antioxidant components such as polyphenols and flavonoids. Acetone is a renewable solvent and not considered as a hazardous air pollutant under the Clean Air Act 1990 (Tir *et al.*, 2012; Okeleye and Betiku, 2019). This indicates that acetone has the potential to be an alternative solvent to hexane for the oil extractions with less hazardous, more polar antioxidant compounds present and less toxic to human health and environment.

The objective of this study was to extract oil from the chia seeds using various solvents (hexane and acetone) by Soxhlet method at different extraction times and evaluate their fatty acids profile. Then, the effect of different temperatures (25°C and 40°C) on oxidative stability and antioxidant activity of chia seed oil extracted was investigated.

## 2. Materials and methods

### 2.1 Materials

Commercial Australian black chia seeds (*Salvia hispanica* L.) were supplied by Chia Co Australia and stored at 5±2°C. The moisture content of the black chia seeds was 5.3%. The seeds milled for 15 s into fine powder by using a coffee grinder (HanJiaOurs, China) before oil extraction. Acetone (R & M Chemicals) and hexane (QReC) were used as a solvent for oil extraction. All chemicals used in the oxidative stability and antioxidant determinations were analytical grades. Meanwhile, fatty acid standards were obtained from Sigma-Aldrich Chemical Co.

### 2.2 Chia seed oil extraction

Chia seed oil was extracted by Soxhlet method according to AOAC method (1990). Ground chia seed powder was placed into an extraction thimble. Then, the cotton wool was added on the top of extraction thimble containing chia seed powders. The sample was extracted using the selected solvent for this study (200 mL of hexane or acetone) in the Soxhlet apparatus. The extraction was carried out for different extraction times (4 hrs and 8 hrs) using acetone as a polar extraction solvent at 70°C (boiling point for acetone is 56°C).

Meanwhile, chia seed oil extraction also conducted using hexane for 8 hrs as a control sample. Previous studies have shown that different extraction times (8 hrs to 18 hrs) was carried out to extract oil from chia seed by using hexane as standard solvent (Álvarez-Chávez *et al.*, 2008; Ixtaina *et al.*, 2010; Ixtaina *et al.*, 2011; Dąbrowski *et al.*, 2016; Scapin *et al.*, 2017). The hexane extraction in the Soxhlet method for 8 hrs was carried out to minimize the electricity and power consumption of the machine. The hexane extraction should be heated at 80°C based on their boiling point between 63°C to 69°C (Liu and Mamidipally, 2005). After extraction, the solvent was removed using a rotary vacuum evaporator (Büchi, Flawil, Switzerland) for 30 mins at 40°C under a pressure of 199 bar to speed up the solvent removal. The oil was transferred to a dark bottle, flushed with nitrogen gas and stored at 4°C until further analysis. The chia seed oil extraction was conducted with three replicates for each sample tested. The fatty acid composition was conducted on the chia seed oils. Next, the chia seed oil samples were tested for oxidative stability (peroxide value, *p*-anisidine value and Totox value) and antioxidant activity (DPPH radical scavenging activity) at different temperatures (25°C and 40°C) for 18 days.

### 2.3 Fatty acid composition

Composition of the chia seed oil was measured in terms of fatty acid methyl esters (FAME), based on the method of Md Ali and Dimick (1994). First of all, 0.1 g of chia seed oil was mixed with 1.0 mL hexane and 1.0 mL 1 M sodium methoxide (anhydrous methanol) in screw-capped glass tubes. The oil solution was mixed vigorously for 10 secs by using a vortex mixer and then kept for 10 mins until two layers are formed. The bottom layer consists of glycerol and non-methylated fatty acid components. Meanwhile, the supernatant solution formed contains FAME must be taken out carefully by using pipette without mixing from the solutions at the bottom phase. This hexane solution containing FAMES was injected to gas chromatography with a flame ionization detector (GC-2010 Shimadzu). The fatty acid measurements were carried out on a polar-silica capillary column HP-5 (30 m x 0.32 mm; film thickness, 0.25 µm) and flame ionization detector (FID) equipped with integrator (C-R6A Chromatopac). The oven temperature was set at 40°C for 5 mins then increased up to 220°C at 20°C/min. Next, the temperature of the injector and the detector were adjusted at 250°C and 270°C. Fatty acid compositions were measured according to the chromatogram peak of the FAMES with different holding time. The results were stated as the relative percentage of each fatty acid (FA) presents in the oil sample.

### 2.4 Preparation and storage of oil samples

Chia seed oils (20.0±0.1 g) were transferred into 30 mL dark bottles with 10% of headspace and flushed with nitrogen gas to remove oxygen (Mancebo-Campos *et al.*, 2014). The bottles were tightly capped and covered with aluminum foil to protect dirt and air was stored in the dark conditions at 25°C and 40°C. The oil samples were stored in the storage cabinet at 25°C to avoid lights or other interferences from the outside. Meanwhile, chia seed oil was stored under accelerated temperature at 40°C in the natural convection oven (Memmert UN110, Germany). Samples were collected at different time intervals (day of 0, 3, 6, 9, 12, 15, and 18) to detect the lipid oxidation by measuring peroxide value and *p*-anisidine value. Besides these parameters, the total oxidation value (TOTOX) was calculated. The antioxidant activity of chia seed oils during storage was also analyzed. Analyses for three different replicates of the chia seed oil samples were carried out. The sample names for chia seed oils tested for oxidative stability and antioxidant activity during storage are followed as A4-25 (chia seed oil extracted by acetone for 4 hrs), A8-25 (chia seed oil extracted by acetone for 8 hrs) and H8-25 (chia seed oil extracted by hexane for 8 hrs).

### 2.5 Determination of peroxide value (PV)

The PV was measured according to the AOAC procedure 965.33 (2000). Chia seed oils (0.5 g) were dissolved with 10 mL of trichloromethane. Then, 15 mL of acetic acid and 1 mL of an aqueous solution of saturated potassium iodide were added. The sample was slightly stirred for 1 min and kept for 5 mins in the dark. Once the incubation was completed, 75 mL of distilled water was added, and the sample was vigorously shaken. Finally, liberated iodine was titrated with sodium thiosulfate (0.01 N) in an automatic titrator. The PV, expressed as mEq/kg of oil, was calculated according to the formula:

$$PV = \frac{V \times N \times 1000}{W}$$

Where V is the volume (mL) of sodium thiosulfate consumed in the titration, N is the normality of the sodium thiosulfate solution and W is the weight of the sample (g).

### 2.6 Determination of *p*-anisidine value (AV)

AV of the chia seed oils was performed according to the IUPAC method (1987). Chia seed oil (0.5 g) was dissolved in isooctane or hexane in a 25 mL volumetric flask. The sample was then reacted with a *p*-anisidine solution in acetic acid (0.25% w/v) for 10 min to produce a coloured complex. The absorbance of the samples with and without *p*-anisidine solution was measured using a

UV spectrophotometer (Shimadzu UV-1800, Japan) at 350 nm, and the parameter AV was calculated as follows:

$$AV = \frac{25 \times (1.2 \times (E_b - E_a))}{W}$$

where,  $E_b$  is the absorbance value of the oil-solution,  $E_a$  is the absorbance value of the oil-anisidine-solution and  $W$  is the weight of the sample (g).

### 2.7 Determination of total oxidation value (TOTOX)

The overall oxidation state of oil given by the TOTOX was calculated according to the formula:

$$TOTOX = AV + 2PV$$

### 2.8 Determination of antioxidant activity

The antioxidant activity of chia seed oil obtained by different solvents were measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, according to the method of Ruttarattanamongkol and Petrasch (2016) with some modifications. About 0.2 mL of chia seed oil was vigorously mixed with 10 mL of methanol for 1 min. The mixture was centrifuged at 1000×g for 3 mins. Then, 0.6 mL of the supernatant was mixed and thoroughly shaken with 2 mL of freshly prepared 0.1 mmol/L DPPH solution in methanol (39.43 mg DPPH/1000mL of

methanol). The mixture was allowed to stand for 1 hr in the dark. The absorbance of the mixture was then measured at 517 nm by a UV spectrophotometer (Shimadzu UV-1800, Japan). The antioxidant activity of chia seed oil was expressed as percentage inhibition of DPPH radical and was calculated as follows:

$$\% \text{ Antioxidant activity} = \left( \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \right) \times 100$$

### 2.9 Statistical analysis

All experimental data from three replicated chia seed oil samples were expressed as mean±standard deviation. The statistical analyses were conducted using MINITAB software version 9.1. Data were subjected to analysis of variance (ANOVA) at 95% confidence level ( $p < 0.05$ ) to find any significant difference between samples.

## 3. Results and discussion

### 3.1 Fatty acid composition of chia seed oils

The fatty acid profile of chia seed oil obtained by different extraction solvents is presented in Table 1. Oils were mostly rich in  $\alpha$ -linolenic acid (66.38-67.79%), while other fatty acids were in the following order of quantity: linoleic (18.11-18.24%) > palmitic (5.5-5.88%) > oleic (5.11-5.23%) > stearic acid (2.56-3.02%). PUFA,

Table 1. Fatty acid profiles of chia seed oils extracted by different extraction solvents

Fatty acid composition (%)	A4	A8	H8
Octanoic acid C8	0.01±0.00 <sup>b</sup>	0.01±0.00 <sup>b</sup>	0.03±0.00 <sup>a</sup>
Myristic acid C14	0.03±0.00 <sup>b</sup>	0.03±0.00 <sup>b</sup>	0.04±0.00 <sup>a</sup>
Pentadecanoic acid C15	0.02±0.00 <sup>a</sup>	0.01±0.00 <sup>a</sup>	0.02±0.00 <sup>a</sup>
Palmitic acid C16	5.54±0.00 <sup>b</sup>	5.50±0.01 <sup>c</sup>	5.88±0.01 <sup>a</sup>
Palmitoleic acid C16:1	0.05±0.00 <sup>a</sup>	0.05±0.00 <sup>a</sup>	0.05±0.00 <sup>a</sup>
Margaric acid C17	0.03±0.00 <sup>b</sup>	0.03±0.00 <sup>b</sup>	0.04±0.00 <sup>a</sup>
Stearic acid C18	2.62±0.01 <sup>b</sup>	2.56±0.04 <sup>b</sup>	3.02±0.02 <sup>a</sup>
Oleic acid C18:1N9C	5.12±0.04 <sup>ab</sup>	5.11±0.05 <sup>b</sup>	5.23±0.01 <sup>a</sup>
Linoleic acid C18:2N6C	18.24±0.01 <sup>a</sup>	18.13±0.01 <sup>b</sup>	18.11±0.04 <sup>b</sup>
$\gamma$ -linolenic acid C18:3N6G	0.05±0.00 <sup>a</sup>	0.04±0.01 <sup>a</sup>	0.05±0.01 <sup>a</sup>
$\alpha$ -linolenic acid C18:3N3A	67.54±0.06 <sup>b</sup>	67.79±0.03 <sup>a</sup>	66.38±0.04 <sup>c</sup>
Arachidic acid C20	0.17±0.01 <sup>b</sup>	0.15±0.01 <sup>b</sup>	0.20±0.01 <sup>a</sup>
Eicosenoic acid C20:1N9	0.09±0.00 <sup>a</sup>	0.08±0.00 <sup>a</sup>	0.09±0.01 <sup>a</sup>
Eicosadienoic acid C20:2	0.05±0.01 <sup>a</sup>	0.05±0.00 <sup>a</sup>	0.05±0.01 <sup>a</sup>
Heneicosanoic acid C21	0.03±0.00 <sup>a</sup>	0.04±0.00 <sup>a</sup>	0.03±0.00 <sup>a</sup>
Eicosapentaenoic acid C20:5N3	0.07±0.00 <sup>ab</sup>	0.06±0.00 <sup>b</sup>	0.07±0.01 <sup>a</sup>
Erusic acid C22:1N9	0.13±0.00 <sup>b</sup>	0.14±0.01 <sup>b</sup>	0.49±0.03 <sup>a</sup>
Nervonic acid C24:1N9	0.22±0.00 <sup>a</sup>	0.22±0.00 <sup>a</sup>	0.22±0.01 <sup>a</sup>
SFA (%)	8.44	8.33	9.27
MUFA (%)	5.66	5.65	6.13
PUFA (%)	85.9	86.02	84.6
Total (%)	100	100	100

Values are expressed as mean±standard deviation. Values with different superscript letters indicate significant difference ( $p < 0.05$ ). A4: Chia seed oil extracted by acetone for 4 hrs, A8: Chia seed oil extracted by acetone for 8 hrs, H8: Chia seed oil extracted by hexane for 8 hrs, SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid.



monounsaturated fatty acid (MUFA), and saturated fatty acid (SFA) represented for 84.6-86.02%, 5.65-6.13%, and 8.33-9.27% of the total share, respectively. These results agree with the previous studies by Dąbrowski *et al.* (2018) and Timilsena *et al.* (2017). According to Table 1, chia seed oil obtained by acetone for 8 hrs significantly extracted higher amount of  $\alpha$ -linolenic acid ( $p < 0.05$ ) than other chia seed oils (A4 and H8). Next, the second-largest content of fatty acid is linoleic acid. A4 contained significantly higher linoleic acid content ( $p < 0.05$ ) than other chia seed oils. Chia seed oils extracted by acetone at different extraction times obtained slightly higher content of PUFA (85.9–86.02%) than hexane-extracted chia seed oil (84.6%). The content of PUFA in the present study was higher than those determined by Dąbrowski *et al.* (2017) who reported that the lower PUFA level of chia seed oil (81%) obtained by hexane and acetone at 18 hrs of extraction. The present study showed that shorter extraction time of chia seed oil (within 4 hrs to 8 hrs) to obtain a higher amount of polyunsaturated fatty acids compared to previous work. Ixtaina *et al.* (2010) cited that significant effect of extraction time on the share of linoleic and  $\alpha$ -linolenic acids was found. There was an increase in  $\alpha$ -linolenic acid at furthering extraction times. In contrast, higher levels of linoleic acid were seen at intermediate times. Such revelation by the previous study had a good agreement with the content of PUFA in chia seed oil extracted by acetone at different time. Even though the chia seed oil is nutritious food due to the high content of PUFA, it may result in poor oxidative stability (Ixtaina *et al.*, 2012). The fatty acid analysis is the main parameter for determining the oil susceptibility to oxidation (Tańska *et al.*, 2016). The more unsaturated and less saturated of the fat, the oxidation reaction proceeds faster

(Liu and White, 1992; Costa de Camargo *et al.*, 2016). Maszewska *et al.* (2018) reported that  $\alpha$ -linolenic has poor resistance to external oxidation factors. Huang *et al.* (2012) stated that high concentration of PUFA in soybean oils are more vulnerable to oxidation, which leads to loss of quality in terms of volatile products formation from lipid oxidation. Therefore, efforts must be made by comparing different storage temperatures to determine the oxidative stability of chia seed oils extracted by various extraction solvent.

### 3.2 Peroxide value of chia seed oils

The oxidation degree on chia seed oil was determined by measuring peroxide value (PV) at 25°C and 40°C for 18 days. The influence of extraction solvents during storage on PV in the chia seed oils is shown in Figures (1 and 2). Results showed that PV of chia seed oils increased significantly ( $p < 0.05$ ) with storage time at different temperatures. PV is one of the most widely used methods to measure the concentration of hydroperoxides formed in the early stages of lipid oxidation (Zhang *et al.*, 2010). The value of hydroperoxides in chia seed oils increased from beginning to the end of the experiment at both temperatures. A8-25 sample had the highest PV ( $29.20 \pm 0.92$  mEq/kg), being significantly higher ( $p < 0.05$ ) than A4-25 sample ( $26.27 \pm 2.01$  mEq/kg) and H825 ( $7.33 \pm 0.31$  mEq/kg) at the end of storage (25°C). On the 12<sup>th</sup> days of storage, chia seed oils extracted by acetone obtained higher acceptable limit of PV (10.0 mEq/kg) established by the Codex Alimentarius Commission (1999). However, hexane-extracted chia seed oil maintained below the level of PV (10.0 mEq/kg) until the end of storage at 25°C. Meanwhile, a greater increase in PV for chia seed oils stored at 40°C. The PV of A4-40

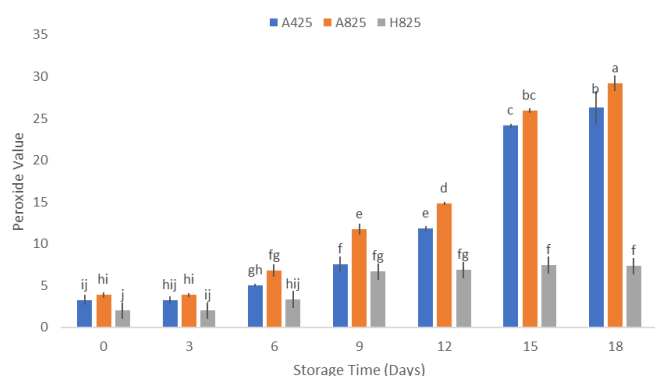


Figure 1. Peroxide values of chia seed oil obtained by different extraction solvents and times stored at 25°C for 18 days. Values are expressed as mean of three independent batches ( $n = 3$ ) and error bars indicate standard deviation. Bars with different letters indicate that there were significant differences between the means ( $p < 0.05$ ). A4-25: Chia seed oil extracted by acetone for 4 hrs, A8-25: Chia seed oil extracted by acetone for 8 hrs, H8-25: Chia seed oil extracted by hexane for 8 hrs.

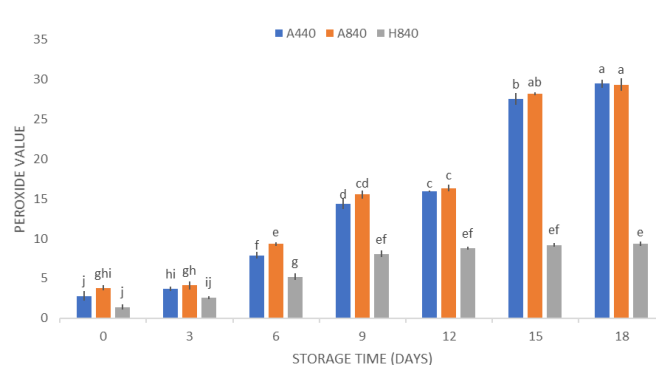


Figure 2. Peroxide values of chia seed oil obtained by different extraction solvents and times stored at 40°C for 18 days. Values are expressed as mean of three independent batches ( $n = 3$ ) and error bars indicate standard deviation. Bars with different letters indicate that there were significant differences between the means ( $p < 0.05$ ). A4-25: Chia seed oil extracted by acetone for 4 hrs, A8-25: Chia seed oil extracted by acetone for 8 hrs, H8-25: Chia seed oil extracted by hexane for 8 hrs.

(29.33±0.81 mEq/kg) and A8-40 (29.01±1.2 mEq/kg) were significantly higher ( $p < 0.05$ ) than H8-40 (9.33±0.31 mEq/kg) until 18 days of storage, indicating chia seed oils extracted by acetone had lower oxidative stability towards higher temperature at 40°C compared to the chia seed oil extracted by hexane. Storage at elevated temperature resulted in a higher degree of primary oxidation products formation. It was in agreement with the findings of Shao *et al.* (2015), who stated that the lipid autoxidation rate expanded rapidly with an increase in temperature when stored in darkness. Chia seed oil samples extracted by acetone stored after ninth-day of storage at both temperatures were passed over the permitted level of PV. However, the PV of chia seed oil extracted by hexane presented lower than the acceptable level until the end of storage at both temperatures (25°C and 40°C). These results indicated that the chia seed oils extracted using hexane has higher oxidative stability as compared to acetone-extracted oils. Yet at the same time, both MUFA and SFA of chia seed oil extracted by hexane (H8) were also higher than those oils extracted by acetone (A4 and A8) which also contributed to the higher oxidative stability of H8. In addition, relatively high PV of acetone-extracted chia seed oil samples at the initial day that undoubtedly would influence the progress of lipid oxidation. It can be seen that the utilization of different solvents significantly affected the oxidative stability of the oil. Therefore, it can be concluded that the sensitivity of chia seed oils extracted by acetone to autoxidation was higher as compared to oil obtained by hexane. The possible reason could be the presence of high PUFA content in chia seed oil obtained by acetone, as the autoxidation rate of  $\alpha$ -linolenic acid is twice higher than linoleic acid (Kim and Min, 2008).

### 3.3 *p*-Anisidine value of chia seed oils

There were increases in *p*-anisidine (AV) value for all samples at a temperature of 25°C and 40°C with inconsistent patterns throughout the storage period (Figure 3 and 4). AV is the measurement of the secondary lipid oxidation products (carbonyl, aldehydes and ketones) produced after the hydroperoxide decomposition. This is the phase that leads to oil rancidity (O'Keefe and Pike, 2010). As we can notice, the AV is strongly affected by oxidation time (Kim *et al.*, 2013) and significant differences ( $p < 0.05$ ) were found during storage at 25°C for chia seed oils. A8-25 (6.32±0.21) had the highest AV, which is significantly higher ( $p < 0.05$ ) than A4-25 (6.00±0.39) and H8-25 (4.82±0.30) at the 18 days of storage. Meanwhile, AV for all of chia seed oils further increased at 40°C. The AV of A8-40 (9.08±0.12) and A4-40 (8.22±0.52) were much more significant ( $p < 0.05$ ) than H8-40 (5.20±0.47) until 18 days of storage. However, the rise of AV in H8-40 was not statistically significant ( $p > 0.05$ ) throughout the storage period. It indicated that the chia seed oil extracted by hexane is the best solvent in retarding the production of secondary oxidation products during storage at both temperatures compared to acetone-extracted chia seed oils. Based on the results, AV of chia seed oils stored at 40°C showed a high AV, indicating high levels of secondary oxidation products after hydroperoxides decomposition due to elevated temperature during storage (Vaidya and Eun, 2013). Wang *et al.* (2010) stated that AV for seed oils (walnut and grape) remained stable during storage at 25°C. It is indicated that oxidation rates occur slowly at low temperatures. However, AV of oil could increase even at low temperatures due to the exposure to light or moisture (Gotoh and Wada, 2006). In addition, Gotoh and Wada (2006) reported that PV and AV increase concurrently

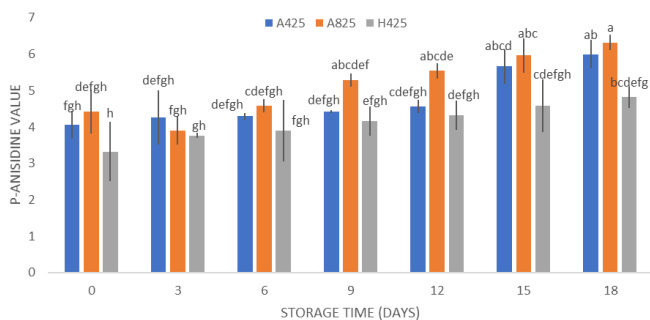


Figure 3. *p*-anisidine values of chia seed oil obtained by different extraction solvents and times stored at 25°C for 18 days. Values are expressed as mean of three independent batches ( $n = 3$ ) and error bars indicate standard deviation. Bars with different letters indicate that there were significant differences between the means ( $p < 0.05$ ). A4-25: Chia seed oil extracted by acetone for 4 hrs, A8-25: Chia seed oil extracted by acetone for 8 hrs, H8-25: Chia seed oil extracted by hexane for 8 hrs.

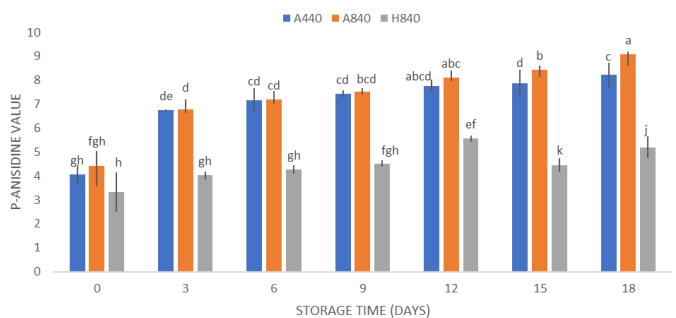


Figure 4. *p*-anisidine values of chia seed oil obtained by different extraction solvents and times stored at 40°C for 18 days. Values are expressed as mean of three independent batches ( $n = 3$ ) and error bars indicate standard deviation. Bars with different letters indicate that there were significant differences between the means ( $p < 0.05$ ). A4-25: Chia seed oil extracted by acetone for 4 hrs, A8-25: Chia seed oil extracted by acetone for 8 hrs, H8-25: Chia seed oil extracted by hexane for 8 hrs.

during the storage period, which proved that there is a positive correlation between both parameters.

### 3.4 Total oxidation of chia seed oils

The total oxidation of the oil sample can be determined from the calculated PV and AV values. These values are reported as TOTOX value. TOTOX value measures primary and secondary oxidation products, reflecting the early and final phases of the oil oxidation. Therefore, it provides a better assessment of the total oxidation of the oil (O'Keefe and Pike, 2010). Lower TOTOX value shows better stability of oil sample against lipid oxidation (Shahidi and Wanasundara, 2002). The TOTOX values of all chia seed oils increased in a consistent pattern, as shown in Figures 5 and 6. Based on Figure 5, the TOTOX value of A8-25 ( $64.72 \pm 1.69$ ) at the end of storage period recorded significantly higher ( $p < 0.05$ ) than A4-25 ( $58.53 \pm 4.23$ ) and H8-25 ( $19.49 \pm 0.33$ ). Meanwhile, TOTOX value of A4-40 ( $67.15 \pm 0.72$ ) and A8-40 ( $67.75 \pm 1.73$ ) at  $40^\circ\text{C}$  on the day of 18 indicated no significant difference ( $p > 0.05$ ) each other, but both chia seed oils were significantly higher ( $p < 0.05$ ) than the TOTOX value of H8-40 ( $23.87 \pm 0.79$ ). The results revealed that chia seed oil extracted by hexane had the lowest of TOTOX value at both storage temperatures. It can be concluded that the TOTOX value for chia seed oils stored at  $40^\circ\text{C}$  had lower oxidative stability. This can be attributed to oil degradation at the increased storage temperature, thus enhancing TOTOX value and reducing oil stability (Maestri et al., 2006).

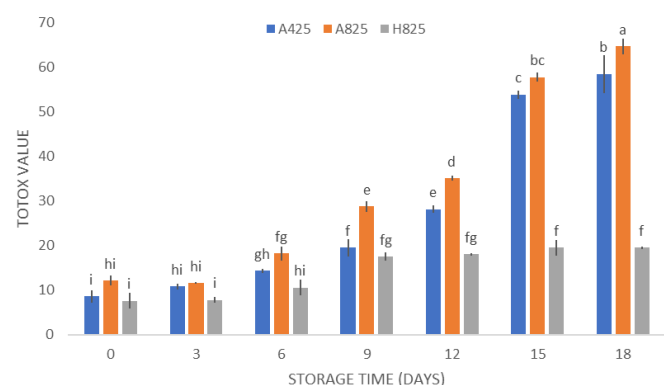


Figure 5. TOTOX Values of chia seed oil obtained by different extraction solvents and times stored at  $25^\circ\text{C}$  for 18 days. Values are expressed as mean of three independent batches ( $n = 3$ ) and error bars indicate standard deviation. Bars with different letters indicate that there were significant differences between the means ( $p < 0.05$ ). A4-25: Chia seed oil extracted by acetone for 4 hrs, A8-25: Chia seed oil extracted by acetone for 8 hrs, H8-25: Chia seed oil extracted by hexane for 8 hrs.

### 3.5 Antioxidant activity of chia seed oils (radical scavenging activity toward DPPH)

Figures 7 and 8 showed a gradual decrease in the antioxidant activity of chia seed oils for both temperatures throughout the storage time as determined by DPPH radical scavenging activity. From the data presented in Figures 7 and 8, it can be observed that the highest DPPH radical scavenging activity obtained by A4-25 followed by A8-25 and H8-25 stored at room temperature throughout the shelf life. The results showed that the antioxidant activity of A4-25 was markedly decreased from 54.22% to 17.37% ( $p < 0.05$ ), while the antioxidant activity of chia seed oils also declined significantly ( $p < 0.05$ ) with the value from 40.70% to 14.15% (A8-25) and 32.53% to 6.3% (H8-25). The results showed that higher antioxidant activity of chia seed oils extracted by acetone was observed when stored at room temperature than the control sample. Meanwhile, the antioxidant activity of chia seed oils stored at a high temperature considerably lower than oil samples kept at room temperature. During storage at  $40^\circ\text{C}$ , the antioxidant activity of A4-40 was dropped from 54.22 to 12.18% ( $p < 0.05$ ). At the same time, the antioxidant activity of other chia seed oils also declined sharply with the value of 40.70% to 1.96% (A8-40) and 32.53% to 3.36% (H8-40). The antioxidant activity of chia seed oils dropped markedly ( $p < 0.05$ ) when exposed to a higher temperature ( $40^\circ\text{C}$ ). This could be attributed to oils with a high content of PUFA deteriorated after exposing to accelerated temperature and the concentration of antioxidant compounds present (Kalantzakis et al., 2006). According to Allothman et al. (2009), the effectiveness of antioxidant depends on the high polarity of the solvent. This may suggest that the antioxidant

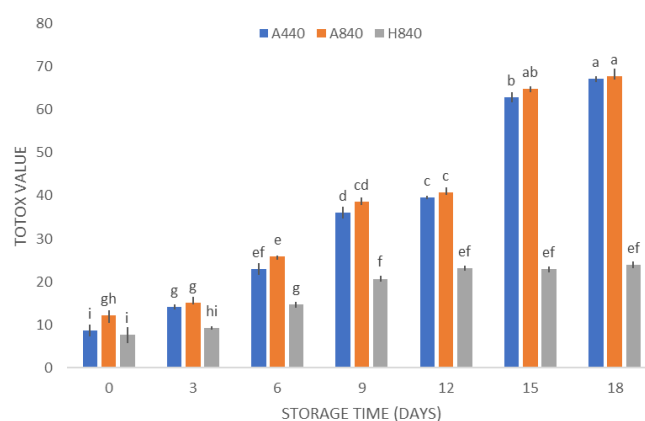


Figure 6. TOTOX values of chia seed oil obtained by different extraction solvents and times stored at  $40^\circ\text{C}$  for 18 days. Values are expressed as mean of three independent batches ( $n = 3$ ) and error bars indicate standard deviation. Bars with different letters indicate that there were significant differences between the means ( $p < 0.05$ ). A4-25: Chia seed oil extracted by acetone for 4 hrs, A8-25: Chia seed oil extracted by acetone for 8 hrs, H8-25: Chia seed oil extracted by hexane for 8 hrs.

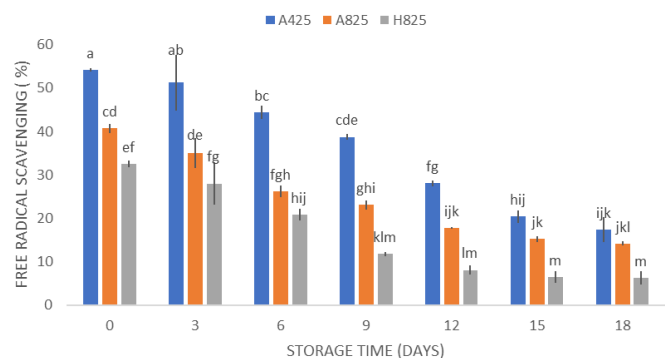


Figure 7. Antioxidant activity assessed by DPPH free radical scavenging capacity of chia seed oil obtained by different extraction solvents and times stored at 25°C for 18 days. Values are expressed as mean of three independent batches ( $n = 3$ ) and error bars indicate standard deviation. Bars with different letters indicate that there were significant differences between the means ( $p < 0.05$ ). A4-25: Chia seed oil extracted by acetone for 4 hrs, A8-25: Chia seed oil extracted by acetone for 8 hrs, H8-25: Chia seed oil extracted by hexane for 8 hrs.

capacity of chia seed oil extracted by acetone is higher and better than extracted oil by hexane during storage period at different temperatures. Several previous studies supported the antioxidant activity results obtained. Barchan *et al.* (2014) reported that oil obtained by polar solvents (methanol and water), which contained the highest amount of total phenolic contents showed strong antioxidant activity as compared to oil extracted by non-polar solvent (hexane). O'Sullivan *et al.* (2011) and Ozsoy *et al.* (2008) also stated that high polarity solvent extracted higher concentration of phenolic compounds which contributed to the increased in antioxidant activity as compared to non-polar solvents including hexane. Generally, antioxidants are compounds that can retard the lipid oxidation process as oxygen receptors and iron-binding agents (Shyamala *et al.*, 2005). However, the results obtained based on the oxidative stability methods for the chia seed oils extracted by acetone showed the least effective against lipid oxidation during storage at the different temperatures (25°C and 40°C), even though these chia seed oils showed higher antioxidant activity than hexane-extracted chia seed oil. This can be explained by oil extracted by polar solvent contained a higher content of free fatty acids and other undesirable compounds such as proteins, carbohydrates, Maillard reaction products, phospholipids and water-soluble components (Al-Hamamre *et al.*, 2012). As a result, the antioxidant activity of chia seed oil extracted by acetone decreased sharply especially at a higher temperature at 40 °C due to the presence of the other polar compounds that are responsible for the acceleration of lipid oxidation during storage. Moreover, free fatty acids are susceptible to oxidation which resulted in reducing the oil stability (Predojevic Zlatka, 2008). Therefore, high oxidative stability chia seed oil extracted by hexane was observed

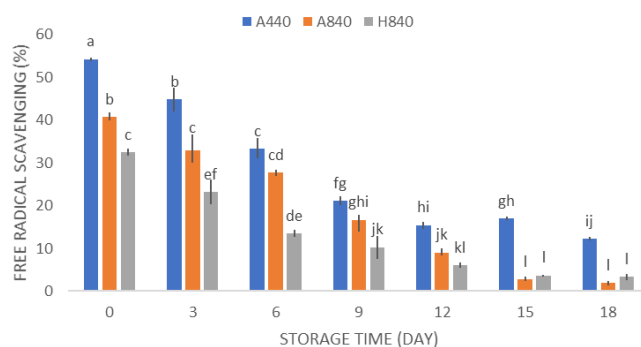


Figure 8. Antioxidant activity assessed by DPPH free radical scavenging capacity of chia seed oil obtained by different extraction solvents and times stored at 40°C for 18 days. Values are expressed as mean of three independent batches ( $n = 3$ ) and error bars indicate standard deviation. Bars with different letters indicate that there were significant differences between the means ( $p < 0.05$ ). A4-40: Chia seed oil extracted by acetone for 4 hrs, A8-40: Chia seed oil extracted by acetone for 8 hrs, H8-40: Chia seed oil extracted by hexane for 8 hrs.

without the presence of unwanted polar compounds, and a lower amount of PUFA obtained during storage at different temperatures despite the fact that lower antioxidant activity was found than acetone-extracted chia seed oils.

#### 4. Conclusion

Chia seed oils obtained by using acetone and hexane as extraction solvents in the Soxhlet method with different extraction times including acetone 4 hrs (A4), acetone 8 hrs (A8) and hexane 8 hrs (H8) as control were carried out in the present study. Chia seed oils extracted by acetone at different extraction times obtained more abundant content of PUFA (85%) compared to hexane-extracted chia seed oil. Meanwhile, the oxidation degree on chia seed oils was determined by measuring PV and AV at 25°C and 40°C for 18 days. The oxidation parameters (PV and AV) of chia seed oils increased significantly ( $p < 0.05$ ) with storage time at different temperatures. The elevated storage temperature of chia seed oil at 40°C resulted in the higher total oxidation than oils stored at room temperature. Results indicated that the chia seed oil extracted using hexane has higher oxidative stability at both storage temperatures as compared to acetone-extracted oils. The antioxidant activity of chia seed oils showed a gradual decrease at both temperatures throughout the storage time. It can be observed that the highest antioxidant activity obtained by chia seed oils extracted by acetone throughout the shelf life. However, the antioxidant activity for all of chia seed oils dropped markedly during the storage at both temperatures due to oils with a high content of PUFA that are susceptible to oxidation. Thus, the utilization of an appropriate solvent in terms of polarity strongly



affected the fatty acid composition, oxidative stability and antioxidant activity of chia seed oils.

### Conflict of interest

The authors declare no conflict of interest.

### Acknowledgements

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