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Abstract. This research aims to determine chemical compositions and characteristics of pumpkin seed oils (PSO) in comparison with the characteristics of extra virgin olive oil (EVOO) and palm olein. The results showed pumpkin seed oil has high unsaturated fatty acid (81.70%) with major fatty acid was linoleic acid (47.80%), extra virgin olive oil has oleic acid 77.70% and palm olein rich palmitate acid 37.20%. Chemical characteristics of PSO, EVOO and palm oil show such as iodine value were 111.74 g I_2 / 100g, 76.85 g I_2 / 100g and 59.34 g I_2 / 100g. Total phenolic content were 58.27 mg gallic acid equivalent / g oil, 176.09 mg gallic acid equivalent / g oil and 117.10 mg gallic acid equivalent / g oil. Total tocopherol were 240.18 µg/g oil, 459.07 µg/g oil and 559.78 µg/g oil. Antioxidant activity were 35.50%, 51.83% and 73.30%. Pumpkin seed oil has good oxidation stability to be utilized in the food industry, high unsaturated fatty acids and tocopherol is ability to using in pharmaceutical, nutraceutical, cosmetics and other industrial application.

1. Introduction

Pumpkin is a plant that can found in Indonesia. Pumpkin was originated from Central and South America. Pumpkin seeds always considered an agricultural industrial waste, but pumpkin seeds are also an organic source that has a unique bioactive compounds, so that it can be used as nutraceutical product, functional foods and medicinal raw materials. Many people in Canada, Mexico, USA, Europe and China always consumed pumpkin seeds in roasted, salted snack and it has uniquely flavoured with nutty taste [1]. Pumpkin seeds contained crude protein (20.21%), crude fat (24.27%), ash (0.68%), crude fibre (31.48%), moisture (4.32%), steroids, alkaloids, flavonoids and tannins [2, 3]. Pumpkin seed oil has excellent benefit for human health to cure several diseases such as prostate disease prevention, retardation of hypertension progression, reduction of bladder and urethral pressure, urinary disorders prevention and the alleviation of diabetes by promoting hypoglycaemic activity [4]. Pumpkin seed oils has total phenolics content (3.96-5.82 mg gallic acid equivalent/100 g), total phytosterols (782.1-805.2 mg/100 g), squalene (591.3-632.5 mg/100 g oil), tocols (94.29-97.79 mg/100 g oil), carotenoids (6.95-7.60 mg/100 g oil) and total antioxidant activity (26.67-38.89 mg GAE/100 g) values [5]. Although PSO has good characteristics can use for industrial application and contribution to healthy human, but PSO is not widely used commercially. It would be better if

usability of animal fats was replaced with vegetable oils. In contrast, EVOO and palm olein were popular and widely used commercially because EVOO has higher oleic acid and micronutrient with strong antioxidant activity and also delicious in taste, in addition, palm olein was used for 90 % food industry and 10 % for oleochemical products [6]. Palm olein has an unique fatty acid composition and triglyceride profile with a balanced comparison of saturated fatty acids and unsaturated fatty acids, so that it has a semi-solid fraction that is widely used and applied in the food industry, palm olein contains good micronutrients such as carotenoid (500-700 ppm), tocopherol (500-600 ppm) and tocotrienol (1000-1200 ppm), which were important to oxidation stability and nature antioxidant [7,8]. Because pumpkin seed oils may potentially become the source nature antioxidant, it would be in interest to analyse pumpkin seed oils characteristics in comparison with extra virgin olive oil and palm olein.

2. Materials and methods

2.1. Materials

The materials used in this research were commercial pumpkin seed with Guang Ban Zhong Variety from China. Extra virgin olive oil (Selva brand) and palm olein (Family brand). The chemicals materials used are gallic acid (Sigma), ethanol p.a, aquadest, Folin Ciocialteu reagent (Merck), Na_2CO_3 7%, N-hexane, toluene, bipyridine, $FeCl_3$, ethanol 95%, DPPH, NaOH, BF_3-Methanol, heptane, saturated NaCl solution, sodium sulfate anhydrous. The equipment used in this study were analytical balance, micrometer pipette (Bio Rad), filter papers, spectrophotometry UV-VIS (Bio Rad serial number 273 BR 07090), oven, rotary evaporator, water bath, blender, GC (HP-5890, Waldborn, Germany) and analyse glasses.

2.2. Pumpkin seeds extraction

Dried pumpkin seeds samples were ground using blender. Oil was extracted from the seed flours in maceration method using n-hexane as solvent and then solvent was evaporated under reduced pressure using a rotary evaporator at 60^{o}C. The residue was weighed and dissolved in hexane. The PSO was stored in refrigerator at 4^{o}C until use.

2.3. Determination of fatty acid composition

Determination of fatty acid composition with two stages was sample preparation and analysis fatty acids. Stage of sample preparation is methylation process of fatty acid into fatty acids methyl ester (FAME). Fatty acids methyl ester and fatty acid composition were determined using procedure as describe American Oil Chemists Society method [9,10]. Sample was analysed with gas chromatography (GC; Hewlett-Packard-5890 series, Waldborn, Germany) with flame ionisation detector and a select FAME fused silica capillary column (25-100 m, 0.20-0.35 mm 1.d) with helium (column head pressure 286 kPa, flow rate 1.0 mL/min) as the carrier gas. Inject 1 µL of a solution containing 20-25 mg/ml methyl ester, equivalent to 15-20 µg of fatty acids. Suitable GC operating conditions are injection port temperature 250^{o}C, detector temperature 250^{o}C and oven temperature 180^{o}C. Fatty acid composition was calculated as:

$$\frac{A_i}{\Sigma A_i} \times 100\% \quad (1)$$

Where ΣA_i is sum of the areas under all the peaks.

2.4. Determination of iodine value

Determinant of iodine value was calculated from result fatty acid composition using procedure as describe American Oil Chemists Society (AOCS) method [11].

2.5. Determination of total tocopherol

Total tocopherol was determined by absorbance of light in dissolved subtances in the sample using spectrophotometry UV-VIS [12]. Oil samples are weighed accurately as much as one hundred ± 10 mg into a 10 mL volumetric flask. Oil samples were added to 5 mL toluene with micrometer pipettes and the oil taken into solution. Then as much three and one-half mL of 2.2 bipyridine (0.07% w/v/ in 95% aqueous ethanol) and 0.5 mL of $FeCl_3$. $6H_2O$ (0.2% w/v in 95% aqueous ethanol) are added in that order. Taken 10 mL of 95% aqueous ethanol with micrometer pipettes to make a solution. Then, the solution was standing for one min the absorption at 520 nm and it will determined using as a reference a blank solution, prepared as above but omitting. The Solutions must be covered from strong light during colour development because strong light will disturb absorption process. The method was calibrated by preparing standards containing 0-240 µg of pure α-tocopherol in 10 mL of toluene and analysing as above. The concentration of tocopherol in the sample was calculated as

$$\text{Total tocopherol} = \left(1 - \frac{\text{A-B}}{\text{M.W}}\right) \tag{2}$$

Where *A* is sample absorption in 10 mm cell, *B* is blank absorption in 10 mm cell, *M* is gradient of a absorbance vs weight graph for α-tocopherol calibration was ca 8.0 x 10^3, *W* is sample weight in g.

2.6. Determination of total phenolic content

Total phenolic contents (TPC) of the oils were analysed by the Folin-Ciocalteu method [13]. In brief, samples were taken as much as 10 mg, the Folin-Ciocalteu reagent (0.1 mL) and the Na_2CO_3 reagent (2.0 mL) were added into a test tube and made to a volume 0.1 mL with 95% ethanol. Then, sample was mixture in vortex until 3 min. The mixture was stored at dark room for 30 min. the absorbance at 750 nm of the mixture was using spectrophotometry UV-VIS and the total phenolic content was calculated as gallic acid equivalent (mg GAE/g) from the calibration curve. The total phenolic content in the sample was calculated as:

$$\text{TPC} = \left(\frac{\text{C.V. DF}}{\text{g}}\right) \times 100\ \% \tag{3}$$

Where V is volume of sample in mL, *C* is concentration in mg GAE/ml, DF is dilution factors, g is weight of sample in mg.

2.7. Determination of antioxidant activity

Antioxidant activity was determined using free radical reduce method using DPPH [14]. Briefly, DPPH solution (1 mL, 0.1 mmol/L) was mixed with oil samples (0.5 mL) dissolved in suitable solvent (ethanol p.a). The solution was mixture to make homogeny solution. The mixture was kept at dark room with temperature 25°C for 30 min and then absorption at 519 nm was measured on a spectrophotometry UV-VIS using ethanol p.a as the blank. The percentage inhibition of samples was calculated as:

$$\text{Inhibition (\%)} = \left(1 - \frac{\text{A}}{\text{A}_0}\right) \times 100 \tag{4}$$

Where A_0 is the absorbance at 519 nm of DPPH without sample and *A* is the absorbance at 519 nm of containing DPPH and sample.

3. Results and Discussions

3.1. The yield of PSO

Yield of PSO was found in the range of 38-41%. Variety of plant, areas climate, ripening stage and the extraction method can affect oil yield of seeds. The freshly PSO was dark green to red ochre in colour with typical nutty, slightly green, fatty and special odour.

3.2. Chemical characteristic of oils

Fatty acid composition in PSO by comparison oils with EVOO and palm olein is present in table 1.

Table 1. Percentage of fatty acid composition of PSO, EVOO and palm olein

Fatty acid (FA)	PSO	EVOO	Palm Olein
Caproic (C6:0)	-	-	-
Caprylic (C8:0)	-	-	-
Capric (C10:0)	-	-	-
Lauric (C12:0)	-	-	0.40
Myristic (C14:0)	0.10	0.20	1.20
Palmitic (C16:0)	11.80	11.00	37.20
Palmito oleic (C16:1)	0.10	0.90	-
Stearic (C18:0)	5.80	3.90	3.90
Oleic (C18:1)	33.60	77.70	43.50
Linoleic (C18:2)	47.80	5.10	12.50
Linolenic (C18:3)	0.20	0.60	0.30
Arachidic (C20:0)	0.40	0.40	0.40
Behenic (C22:0)	0.10	0.10	0.10
Total saturated fatty acid	18.20	15.60	43.20
Total unsaturated fatty acid	81.70	84.30	56.30
Monounsaturated fatty acid	33.70	78.60	43.50
Polyunsaturated fatty acid	48.00	5.70	12.80

Table 1 shows that total unsaturated fatty acid of PSO was 81.70% and comprises of 33.60 oleic acid, 47.80% linoleic acid as the dominant fatty acid and 0.20% linoleic acid. In addition, PSO has 18.20% total saturated fatty acid consisting 11.80% palmitic acid and 5.80 stearic acid. The presence of high amounts of linoleic acid give suggested that these oil are highly nutritious, could be used as a good source of essential fatty acid and due to their ability to reduce free radical. Total unsaturated fatty acids of EVOO was 84.30% with oleic acids as the major fatty acids. Both of PSO and EVOO are rich in oleic acid and linoleic acid, therefore they may be used as edible cooking or salad oils or for margarine manufacture. Palm olein contains 56.30% unsaturated fatty acids and 43.20% saturated fatty acids causing palm olein is good on oxidation stability [7]. The high level of unsaturation fatty acids causes the oil to become more easily oxidized. Therefore, the level of saturation will decreases because the double bond has been broken, so the iodine value gets smaller. Chemical characteristic properties are shown in table 2.

Table 2. Chemical characterization of PSO, EVOO and palm olein

Characteristics	PSO	EVOO	Palm olein
Iodine Value (g I_2/100g)	111.74	76.85	59,34
Total phenolic contents (mg GAE/g oil)	58.27±1.11	176.09±8.27	117.10±2.10
Total Tocopherol (µg/g oil)	240.18±3.79	459.07±11.51	559.78±7.93
Antioxidant activity (%)	35.50±1.48	51.83±1.11	73.30±2.60

Note : Data is the means of two replication

Table 2 shows that iodine value of PSO was found to be 111.74 (g I_2/100g) which is higher than the iodine value of EVOO (76.85 g I_2/100g) and palm olein (59.34 g I_2/100g). Poly unsaturated fatty acid can be consistently related to indication of high unsaturated fatty causes high iodine value. Total phenolic contents of PSO, EVOO and palm olein were 58.27 mg GAE/g oil, 176.09 mg GAE/g oil, 117.10 mg GAE/g oil, respectively. Total tocopherol of PSO, EVOO and palm olein were 240.18 µg/g oil, 459.07 µg/g oil and 559.78 µg/g oil, respectively. Tocopherol is odourless, colourless and resistant to heat and acid, but does not resist alkali, UV and oxygen. Tocopherol can disturb oil oxidation process by inhibiting the formation of peroxide chain or decomposition process and inhibiting aldehyde formation [7]. Antioxidant activity of PSO, EVOO and palm olein were 35.50 %, 51.83 % and 73.30 %, respectively. The genetic factors, extraction and storage conditions of seed oils are critical factors for the content of bioactive compounds [15]. High percentage inhibition of a sample causes antioxidant activity to be better at inhibiting free radicals. Flavonoid of crude extract have a glycoside group which cause decrease antioxidant activity [16]. The research result shows that antioxidant activity is positively correlated with TPC and total tocopherol. The higher value of TPC and total tocopherol can make higher ability to reduce free radicals and inhibit oil oxidation. The high oil stability was attributed to the high content of total polyphenol content and to a high antioxidant activity. The high content of these bioactive compounds such as phenolics, tocopherols and carotenoids both highlights their nutritional and medicinal values and provides a high protection against oxidative stress [5].

The low ability of PSO in inhibiting oil oxidation due to the high level of total unsaturated fatty acids (81.70%). Rancidity was caused by high unsaturated fatty acids in the oil. In contrast, palm olein has the same amount of total unsaturated fatty acids and saturated fatty acids, causing palm olein to be more stable in inhibiting oil oxidation damage. In addition, inhibition of oil oxidation can occur due to the presence of tocopherols that act as antioxidants by breaking various free radical chain reactions. Tocopherol has the ability to move hydrogen phenolics to free radicals from polyunsaturated fatty acids.

4. Conclusion

Pumpkin seed oils had linoleic acid as major fatty acid (47.80%). Iodine value 111.74 g I_2/100g, total phenolic contents 58.07 mg GAE/g oil, total tocopherol 240.18 µg/g oil and antioxidant activity 35.50%. Natural antioxidant of PSO has lower than EVOO and palm olein, so that PSO was more susceptible to oxidation damage in the further study, PSO can researched of physical characteristics and oxidation stability of PSO.

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