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PHYSICOCHEMICAL PROPERTIES OF OIL EXTRACTED FROM THE HOT AND COLD EXTRACTED RED PITAYA (*HYLOCEREUS POLYRHIZUS*) SEEDS

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A B S T R A C T

The red pitaya, *Hylocereus Polyrhizus,* well-known for its tasty pulp attached with sticky and mucilage coated seeds. The seed oils properties are highly influenced by the seed extraction techniques. Therefore, effects of two different seed extraction techniques on some quality characteristics of pitaya seed oil of the red cultivar, *Hylocereus Polyrhizus* were investigated. The techniques are referred as the hot and cold method applied in extracting clear mucilage free red pitaya seeds. The oil obtained from the seeds were analysed for its free fatty acids, peroxide, *p*-anisidine and the totox values. The fatty acids and triacylglycerols separated using analytical chromatographic technique. The free fatty acids value and the oxidation products measure of both methods showed no significant differences. The GC analysis for the hot extracted seed oil showed the presence of palmitic (15.712%), oleic (24.889%) and linoleic acid (48.699%) while the cold extracted seed oil exhibited slightly higher content of palmitic (15.778%), oleic (23.566%) and linoleic acid (49.807%). The HPLC analysis triacylglycerols of the pitaya seed oil showed Linoleic: Linoleic: Linoleic (LLL) and Oleic: Linoleic: Linoleic (OLL) dominated the TAG composition of the oil with the average percentage of 14.065% and 13.990% for the cold extracted seed oil and 15.620% and 15.795% for the hot one. The result shows that different seed extraction techniques influence the oil yield and the seed oils properties.

Keywords: Pitaya seed oil, *Hylocereus polyrhizus,* Essential fatty acids, Linoleic acid, Triacylglycerols.

INTRODUCTION

Pitaya or better known as dragon fruit has become one of the most desirable fruit in the market in recent times due to its sweet taste and texture (Lim *et al*., 2010). The red pitaya, *Hylocereus polyrhizus* (*Hylocereus polyrhizus*) especially has become the most attracted pitaya variety for its red-violet flesh and sweetness that signifies the high carbohydrate content in it (Wichienchot *et al*., 2010). The red pitaya also reported to contain high fiber, protein and minerals (Ruzainah *et al*., 2009). Another study done by Rebecca *et al*. (2010) has revealed the probiotic properties and high anti-oxidant level in red pitaya fruit.

The red pitaya flesh impregnated with minute distinctive seeds which are coated with mucilage. This morphological condition leads to different seed extraction ideas that could serve on gaining clear bright seeds to analyse its potential contents. The principal reason behind this study is to attempt on 2 different seed extraction techniques (hot & cold method) in order to obtain clear pitaya seeds. The seed oil quality can be affected by the seed extraction method and condition especially for pitaya seeds due to its morphological condition. The challenge is extremely in the recovery of seeds from its gel-like coating (mucilage) that envelope every seed. The hot method applies heat hydrolysis of the mucilage with endogenous water. In contrast, the mucilage was hydrolyzed with acid at ambient temperature (25°C) in the cold method (Nemati *et al.,* 2010). Oil extracted from these group of seeds were analysed for its free fatty acids and oxidation products. Besides that, triacylglycerols and fatty acid composition in the oil were also determined.

About 30% of oil yield of the red pitaya seed which is rich in essential fatty acids was reported by Ariffin *et al.* (2009). A complete diet includes a well-balanced intake of essential fatty acids (EFA) and the acids have been

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reported to exhibit multiple nutraceutical roles in human well-being. Meharban Singh (2005) in his review stated that deficiency in fatty acid intake may lead to impaired development in brain cells, especially in infants.

Triacylglycerols (TAGs) are the main constituent of vegetable oil and play an important role as transporters of fatty acids which eventually serve as an energy source. Complete hydrolysis of TAG gives glycerol and fatty acids. In plant seeds oil, the breakdown of TAGs will eventually increase the free fatty acids (FFA) level in the oil thus affecting the oil quality. FFA value is one of the important parameter to evaluate the oil quality as well (Nyam *et al*., 2009).

All the quality analyses relate to the effect of seed extraction techniques that are being implemented in this study. This study is aimed to compare the physicochemical properties of the oil obtained from the respective hot and cold extraction processes.

MATERIALS AND METHODS

Materials: Mature red pitaya fruits, *H. polyrhizus*[1] were purchased from the local farm located at Sepang, Selangor, Malaysia. All the chemicals used were of analytical grade purchased from Merck (Darmstadt, Germany). The fatty acid methyl ester (FAME) standard mixture was purchased from the Supelco Co. (United States of America).

Methodology:

Seed extraction techniques

Hot method: The fruits purchased were each wrapped with aluminum foil and autoclaved for an hour. The hydrolyzed fruits were cooled and aluminum foil removed before the skins were carefully peeled. The hydrolyzed flesh recovered and mixed vigorously with water to allow complete separation of seeds from the flesh. The fine seeds that settled down at the bottom were collected and air dried (Ariffin *et al*., 2009).

Cold method: The fruits were peeled and the flesh sliced into smaller size. The sliced fruit flesh transferred into the depulper to separate the seeds from the flesh. The seeds each enveloped with the mucilage were collected. The seeds were treated with hydrochloric acid; the mucilage free seeds were water-washed extensively. The hydrolyzed mucilage sieved and the seeds recovered and air dried (Vishwanath *et al*., 2006; Nemati *et al.,* 2010).

Oil extraction: 10 grams of dried seeds were finely ground and the oil extracted by solvent extraction using hexane (boiling point; 69 °C). The solvent then evaporated using the rotary evaporator to obtain the oil (Besbes *et al.,* 2005).

Physicochemical properties: The physicochemical properties of the oil recovered were analysed using the routine AOCS method. The free fatty acids (FFA) were expressed as percentage of oleic acid based on the titration volume. The primary oxidation product, the peroxides value (PV) was expressed in milliequivalents of oxygen per kg of oil (mEq of O_2/kg) while the secondary oxidation level was determined by measuring the p-anisidine value (p-AV). The acid value was calculated from the results obtained from the FFA. Totox value that indicates the total oxidation value (2PV + *p*-AV) using the standard formula and the values obtained from the oxidation measurement tests.

Fatty acid analysis using Gas chromatography: Pitaya oil (100mg) was esterified to fatty acid methyl ester using sodium methoxide and the different esterified fatty acid constituents were separated and determined using gas chromatography (Hewlett-Packard 6890 series) fitted with flame ionization detector using the column BPX-70 (60 m x 0.25 mm, internal diameter: 0.2 µm) from Varian, Inc. with the oven condition of 115 °C that raising to 180 °C at the rate of 8 °C/ minute and held for 10 minutes. It then allowed to reach up to 240 °C at the rate of 8 °C/ minute and held for 10 minutes (Ariffin *et al.*, 2009). The fatty acids were identified by comparing the retention time of the sample peaks with the FAME[3] standard mixture peaks and the result expressed in percentage of distribution (Ixtaina *et al.*, 2011).

Triglycerides: To prepare a 5% (w/v) of the test sample solution, accurately 50µL of oil pipetted into a centrifuge tube (2mL) and dissolved with 950 µL acetone and the tube sealed and vortex for a few seconds. The dissolved fat solution was filtered through the 0.45 µm syringe filter directly into the HPLC vials. The clear sample solution (10 µL) injected into the pre-optimized analytical conditions equipped with a refractive index detector, flow rate of 1.5 ml/min using Hichrom C18 column (15cm, 2.1mm i.d., 5µm) as the stationary phase and mobile phase acetone: acetonitrile (50:50; v/v) mixture. Peak identification achieved by comparing the retention time of the sample peak with the alternative reference peak of soybean oil.

Statistical analysis: The analyses were carried out in triplicates. The data analyzed using Minitab version 14.0

where analysis of variance (ANOVA) was applied to the data comprising FFA, AV, PV, p-AV and Totox measurement. Tukey's test at 5% significance applied to define on the data significant differences.

RESULT AND DISCUSSION

Physicochemical Properties: The physicochemical properties of the oil extracted from the hot and cold extracted seeds were analyzed and tabulated as below;

Table 1: Physicochemical properties of hot & cold extracted RP seed oil.

Physicochemical characteristic	Hot	Cold
Free Fatty Acid, FFA (%)	0.119 ± 0.02[a]	0.108 ± 0.01[a]
Acid value (%)	0.205 ± 0.04[a]	0.189 ± 0.02[a]
Peroxide value (mEq O2 / kg)	0.105 ± 0.01[a]	0.124 ± 0.01[b]
p-Anisidine value	0.349 ± 0.04[a]	0.399 ± 0.04[a]
Totox (2PV + *p*-AV)	0.441 ± 0.04[a]	0.398 ± 0.04[a]

Results are expressed as mean ± SD for 3 replications, Alphabet within the same column were significantly difference $p < 0.05$.

Based on the results displayed in table 1, there are no significant differences between the free fatty acids, acid and p-anisidine value of both the hot and cold method. The oil contains 0.119% and 0.108% of free fatty acids for hot and cold method respectively (Refer Table 1). The FFA value is considerably lower for any crude purified seed oil. This indicates the absence of enzymatic reaction (lipase) which would contribute to elevated FFA level in plant seed oil. Sometimes the FFA value of unrefined seed oil can go up to 3% depending upon their extraction method. The peroxide value is the index of rancidity, which is substantially lower for the red pitaya seed oil. This may be due to auto-oxidation that take place during the sample handling prior to analysis. The secondary stage of oxidation that occurs when the hydroperoxides decompose to form aldehydes was observed in this seed oil. However the anisidine value in this case is considerably lower in both methods based on the quality specifications for plant oils.

Fatty acid composition: Fatty acids in the pitaya oil separated using gas chromatography and reported as percentage of distribution. The peaks were identified based on the retention time on the standard chromatogram composed of 19 components peak. The figures below show the fatty acid distribution in both hot and extracted seed oil.

The chromatograms of the fatty acids of the *H. polyrhizus*[1] hot and cold extracted *H. polyrhizus* seeds oil are shown in fig. 1 and fig.2. Both exhibit the similar range of fatty acid distribution. The linoleic acid content is the major fatty acid composition in the seed oil analyzed with the average percentage of 48% for the hot method and 49% of the cold one. These results are similar as reported by Ariffin *et al.* (2009) and Lim *et al.* (2012). There were no significant differences in the other fatty acid constituents of the hot and cold oil extracts with oleic at 24: 25% (hot: cold), palmitic 15:16% (hot: cold) and linolenic 1.3:1.2% (hot: cold).

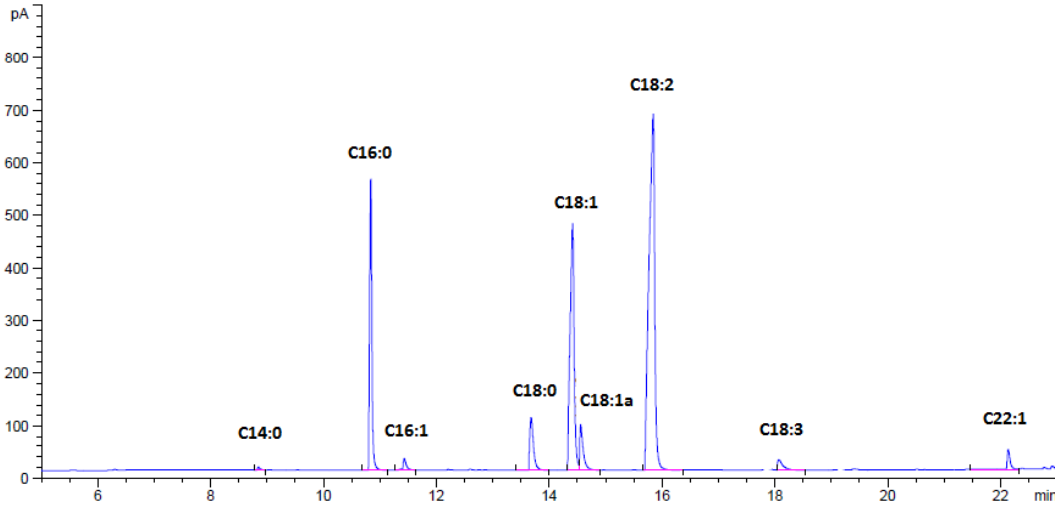

Figure 1: Chromatogram of fatty acids of hot extracted seed oil.

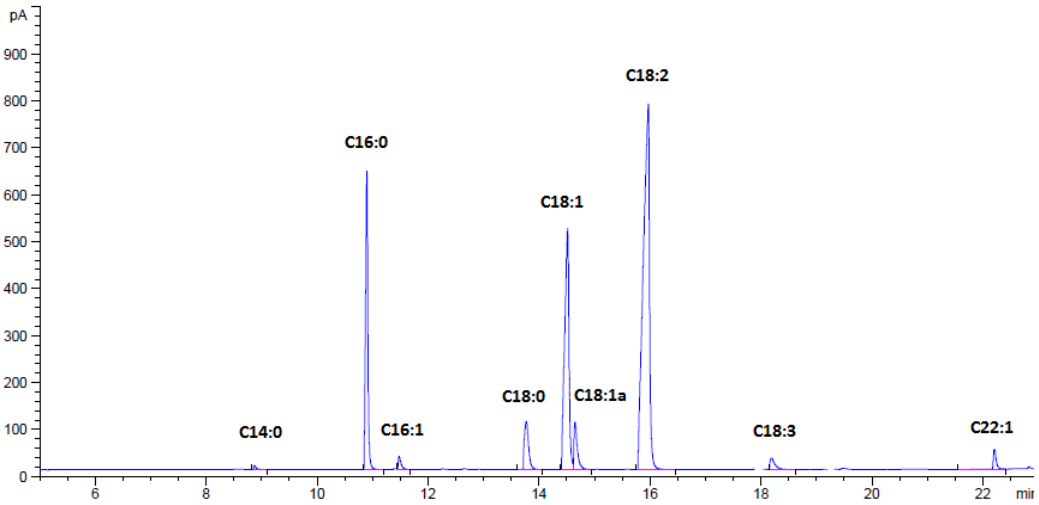

Figure 2: Chromatogram of fatty acids of cold extracted seed oil.

These values are comparable with other seed oil such as the grape seed oil which was reported to have about 60% of linoleic acids and the hempseed about 52% - 62% of the essential omega- 6. The red pitaya seed oil contains higher palmitic acid compared to melon seed oil that contains only about 8.1% (De Mello *et al.,* 2001). It can be emphasised here that high level of unsaturated fatty acids in our diet contributes in lowering of high blood cholesterol level (Baydar and Akkurt, 2001).

Other fatty acids identified in the *H. polyrhizus*[1] seed oil are the remaining long-chain fatty acid, erucic acid which presents between the ranges of 0.8% to 1.2%. Study done by Lim *et al*. (2010) showed the identification of erucic acid, calculated to be slightly lower compared to their report. The statistical analysis of the data showed that there are no significant differences of the palmitic, stearic and linoleic acid of both the hot and cold extracted seed oil. In contrast, the myristic, palmitoleic, oleic, cis-vaccenic and linolenic acids showed significant differences between the 2 techniques with the p value < 0.05. The numerical value of each fatty acid is presented in Table 2.

Table 2: Fatty acid composition of hot & cold extracted seed oil.

Fatty acids	Hot method	Cold method
Myristic (C14:0)	0.155 ± 0.01[a]	0.1717 ± 0.00[a]
Palmitic (C16:0)	15.712 ± 0.10[a]	15.778 ± 0.69[a]
Palmitoleic (C16:1)	0.691 ± 0.05[a]	0.841 ± 0.00[b]
Stearic (C18:0)	4.832 ± 0.16[a]	4.579 ± 0.01[a]
Oleic (C18:1)	24.889 ± 0.13[a]	23.566 ± 0.23[b]
Cis- Vaccenic (C18:1a)	3.251 ± 0.04[a]	3.630 ± 0.01[b]
Linoleic (C18:2)	**48.969 ± 0.46[a]**	**49.087 ± 0.41[b]**
Linolenic (C18:3)	0.899 ± 0.03[a]	1.048 ± 0.03[b]
Erucic (C22:1)	0.908 ± 0.04[a]	1.196 ± 0.04[b]

Results are expressed as mean ± SD for 3 replications, Alphabet within the same column were significantly difference p < 0.05.

Triacylglycerols: TAG was resolved by HPLC and was reported in relative percentage. Identification of the glycerides made based on the comparison to the reference chromatogram according to its elution carbon number (ECN) as well. Based on the relative percentage (%) tabulated below, the Oleic: Linoleic: Linoleic (OLL) and Linoleic: Linoleic: Linoleic (LLL) content are relatively higer than other TAGs combination. The OLL content in the purified crude pitaya seed oil is examined to be 15.620% & 15.795% while LLL, 14.065% and 13.990% for both the hot and cold extraction method respectively (Refer Table 3). Linoleic: Linolenic: Linolenic (LLnLn) was determined to be the least in oil analysed with the percentage of 0.805 and 0.695 for both the techniques respectively (Refer Table 3). This is comparable to other seed oil, for instant corn oil and sunflower oil was reported to have LLL of , 16.16% and 12.41% respectively (Andrikopoulos et al., 2001).

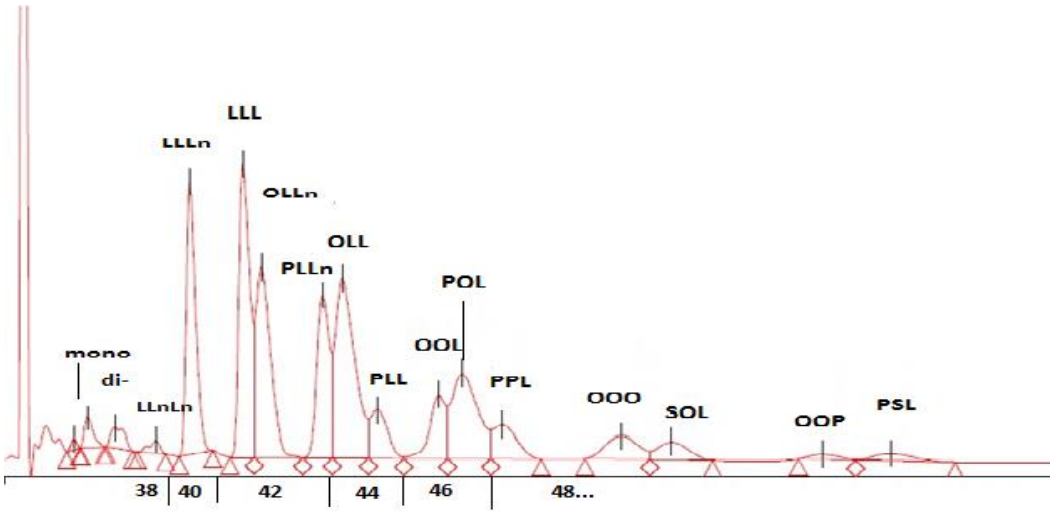

Figure 3: Chromatogram of RPSO.

Table 3: Relative percentage of triacylglycerol present in RPSO.

Triaclyglycerols (Relative Percentage, %)	Hot	Cold
LLnLn	**0.805 ± 0.08[a]**	**0.6950 ± 0.09[a]**
LLLn	10.795 ± 1.04[a]	11.515 ± 0.02[a]
LLL	**14.065 ± 0.39[a]**	**13.990 ± 0.85[a]**
OOLn	12.065 ± 0.0[a]	12.155 ± 0.01[b]
PLLn	9.445 ± 0.19 [a]	9.325 ± 0.01[a]
OLL	**15.620 ± 0.26[a]**	**15.795 ± 0.11 [a]**
PLL	3.475 ± 0.05 [a]	3.570 ± 0.03 [a]
OOL	4.755 ± 0.01[a]	4.490 ± 0.03[b]
POL	10.875 ± 0.22[a]	11.085 ± 0.02[a]
PPL	3.410 ± 0.08 [a]	3.485 ± 0.09 [a]
OOO	3.840 ± 0.07 [a]	3.800 ± 0.04 [a]
SOL	2.720 ± 0.07 [a]	2.785 ± 0.01 [a]
OOP	1.240 ± 0.03 [a]	1.260 ± 0.03 [a]
PSL	1.520 ± 0.01 [a]	1.575 ± 0.05 [a]

P= Palmitic, S=Stearic, O=Oleic, L=Linoleic, Ln=Linolenic.

Results are expressed as mean ± SD for 3 replications. Alphabet within the same column were significantly difference $p < 0.05$.

The statistical analysis of the TAG composition showed significant differences between the hot and cold for Oleic: Oleic: Linolenic (OOLn) and Oleic: Oleic: Linoleic (OOL) respectively while there are no significant differences among other TAG reported (Refer Table 3).

CONCLUSION

The study has shown that different seed extraction techniques may influence the seed oil composition and quality. Application of high temperature during the seed extraction does not affect the oil quality of the seed. The fatty acid composition of hot and cold extracted seed oil showed no significant differences. Moreover, higher seed yield was observed in hot procedure compared to the cold method since seed separation using depulper contributed greater seed loss and was time consuming. The hot method is a one step process for the recovery of clean seeds. Analysis of other properties of the hot and cold extracted seed oil such as the anti-oxidant compounds may draw a better conclusion.

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