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ANTIOXIDANT PROPERTIES OF TOTAL LUTEIN CONTENT IN DIFFERENT PARTS OF PUMPKIN *(Cucurbita maxima)*

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Abstract: Pumpkin (Cucurbita maxima) is a vegetable crop which is commonly consumed as vegetables or incorporated into food products. Pumpkin flesh was reported abundant with carotenoid compounds includes α-carotene, β-carotene, β-cryptoxanthin, lutein and zeaxanthin. As this antioxidant related to the colour pigment, these nutrients highly potential to be in other parts of pumpkin such as peel and seed. Therefore, the aim of this was to determine the total lutein content in different parts of pumpkin and their antioxidant properties. The pumpkin would be collected and evaluated at the commercial maturity stage (60% to fully orange-yellow of fruit peel). The presence of the lutein properties using DPPH, FRAP and ABTS assays in different parts of pumpkin was tested by using microplate spectrophotometer and analysed statistically with SPSS version 20. Among of all pumpkin fruit parts, flesh presented the highest concentration of potential lutein extracts, followed by peel and seed, respectively. On the contrary, potential lutein extracts from the pumpkin peel had the highest antioxidant activity in terms of DPPH, FRAP and ABTS assays when compared to the lutein extracts in flesh and seeds. This study indicates that the potential lutein from Cucurbita maxima especially peel may be the alternative to be used as both natural antioxidants in food products due to increasing demand for natural food preservatives. Therefore, this study may act as a source for others to further study to optimize the usage of pumpkin by-products

Keywords: Pumpkin, peel, flesh, seed, lutein, antioxidant properties

Introduction

Pumpkin (*Cucurbita maxima*) is native to warm-temperate and temperate regions. This species produce round, flattened or oval pumpkin-like fruit with more yellow than orange skin (Rahman *et al*., 2006). According to the statistic reported by Food and Agriculture Organization of the United Nations [FAO] (2017), the production quantity of pumpkin in Malaysia increased from 11185 tons in 2009 to 164494 tons in 2016 which indicate the increasing usage of pumpkin products. Pumpkin flesh was normally consumed as bread, soup and pies (Norfezah *et al*., 2011). The consumption of pumpkin surely produce large quantities of pumpkin by-product such as peel and seeds. This causes the problem in waste management due to disposal of pumpkin waste.

Pumpkin is rich in carotenoid compound. Previous study reported the nutritive value of pumpkin flesh with the intense yellow-orange colour of pumpkin (*Cucurbita* spp.) flesh indicates abundance of carotenoids included α-carotene, β-carotene, β-cryptoxanthin, lutein and zeaxanthin (Durante *et al*., 2014). Furthermore, the pumpkin by-product such as peels and seeds produced by fruit and vegetable processing is found to be rich in phenolic compounds, flavonoids and carotenoids which depending on the species and varieties of plants (Gowe, 2015). In addition, Kim *et al*. (2012) reported that β-carotene concentration in three species of pumpkin such as *Cucurbita pepo*, *Cucurbita moschata* and *Cucurbita maxima* are found to be high in peels compared to flesh and seeds. Therefore, utilization of by-product as a source of nutritive components will lead the food industry towards an economic benefit.

Lutein is the only carotenoids (other than zeaxanthin) that mainly found in the macula lutea of the eye where the light is focused by the lens (Stringham *et al*., 2005). Lutein is a dietary carotenoid which also abundance in the

grains, fruits and vegetables of various colours such as kiwi fruit, orange juice, spinach, maize, orange pepper and different kinds of squash (Sommerburg *et al*., 1998). Besides, flowers such as marigold (*Tagetes erecta* L.) is a plant source that contained the richest and purest of lutein (Tsao *et al*., 2004). Although lutein is the only compound other than zeaxanthin that can improve the human eye health, the affordable source of this antioxidant is limited in Malaysia. The main source of the commercial lutein was only originated from Marigold flower (*Targetes erecta*) which is not widely planted in tropical country. Therefore, this study is highly required to prove the existence of valuable lutein in discarded waste.

Materials and Methods

Samples Preparation

Pumpkin fruits (*Cucurbita maxima*) with maturity stage which were ranged from 4 to 6 were purchased from local market in Terengganu. The pumpkin fruits were washed and cut manually using a knife. Peels, flesh and seeds of the pumpkin were separated from each other and cut to reduce the size (Montesano *et al*., 2018). Approximately 2kg of the small pieces of raw pumpkin flesh was dried at 50°C for 3 days in the cabinet dryer (Protech-FSD380) and ground using a universal grinder (SmartGrind Deluxe, Black and Decker, Mirama, FL, USA). The pumpkin peels and the pumpkin seeds were cleaned to remove impurities and dried at 50°C for 3 days in the cabinet dryer. Samples were ground into a fine powder (Hamed, 2017). All samples were collected and stored in HDPE plastic bag at -20°C for further analysis to preserve the sample quality with three replications for each treatment.

Standard Solution Preparation

The pure xanthophyll solution (≥70% of purity) was used as the standard. According to Šivel *et al*. (2014), 10µL of xanthophyll (1.0mg/mL) was dissolved in 100µL of 95% ethanol to form 0.10mg/mL standard xanthophyll. Eleven different solutions of known concentrations of standard xanthophyll between 0.01mg/mL to 0.10mg/mL were prepared. The calibration curve of absorbance against various concentrations of standard lutein solution was plotted based on the reading by spectrophotometry at 448nm.

Solvent Extraction of Lutein

Ten grams of the pumpkin flesh powder sample were homogenized in 40mL of acetone (Hajare *et al*., 2013). The sample was stored at room temperature for 2 hours (dark) and filtered using filter paper (Smith 101). The residue of the sample was re-extracted twice by soaking in the acetone solution (Rong & Yang, 2005). The organic solvent of the combined filtrate was removed in a rotary evaporator (BUCHI Rotavapor R-200) at 40˚C (Aruna *et al*., 2009). The concentrated layer as the extracted lutein sample was then collected and stored in an amber glass bottle at 4˚C. The collected extract (g) was calculated as the percentage (%) of yield by dividing the weight of extract to the total amount of sample (g). The procedure was repeated for the pumpkin peel and pumpkin seed powder samples respectively.

Spectrophotometry Analysis

Total lutein content of the different parts of pumpkin samples were analysed using Microplate Spectrophotometers (Thermo Scientific Microplate reader Vatioscan Flash, Denmark). The wavelength of 448nm was used to measure the absorbance of the lutein aliquots from different parts of pumpkin samples (Biehler *et al*., 2010). The concentration of the lutein content in the pumpkin samples was determined by using the plotted standard curve.

Antioxidant Properties

DPPH Radical-Scavenging Activity Test

A total of 100µL of 1mg/mL of sample lutein aliquots was mixed with 100µL of 0.1mol/L DPPH in ethanol in 96 well microplates. The 96 well plates were incubated for 30 min in the dark at room temperature (Shi *et al*., 2013). After incubation, absorbance of samples was measured

at 517nm immediately with Microplate Spectrophotometer (Thermo Scientific Microplate reader Vatioscan Flash, Denmark). The percentage of the DPPH radical scavenging was expressed as the percentage of inhibition by using the equation below (Siriamornpun *et al*., 2012).

$$\%\ of\ DPPH\ radicals\ scavenged = \frac{A_{blank} - A_{sample}}{A_{blank}} \text{x}100$$

where A_{blank} = Absorbance of blank at t=0 min

A_{sample} = Absorbance of sample at t= 20min

Equation 1: Percentage of DPPH radicals scavenged

Ferric ion Reducing Antioxidant Power (FRAP) Assay

FRAP assay was done based on the method by Hayes *et al*. (2011). A total of 100µL of lutein aliquots from different parts of pumpkin was added to 100µL of FRAP reagent solution in 96 well plates and incubated at 37˚C for 15 min in dark. The absorbance of each lutein aliquots was read at 595nm by using a Microplate Spectrophotometer (Thermo Scientific Microplate reader Vatioscan Flash, Denmark). Ferric (II) sulphate standard was used for the calibration curve to determine the antioxidant status of the samples. Besides, antioxidant potential of similar concentration of synthetic antioxidants butylated hydroxytoluene (BHT) were also determined by using the same method.

ABTS Radical Scavenging Assay

A total of 100µL of lutein aliquots from samples was mixed with 100 µL of $ABTS^{\cdot+}$ solution in 96 well plates and incubated for 6 min at 25˚C in darkness respectively. The absorbance was read at a wavelength of 734nm using Microplate Spectrophotometer (Thermo Scientific Microplate reader Vatioscan Flash, Denmark). The similar analysis was also done on a similar concentration of synthetic antioxidant (BHT). The antioxidant capacity is expressed as the percentage of inhibition by using the equation below (Okoh *et al*., 2014):

$$\%\ of\ DPPH\ radicals\ scavenged = \frac{A_{blank} - A_{sample}}{A_{blank}} \text{x}100$$

where A_{blank} = Absorbance of blank at t=0 min

A_{sample} = Absorbance of the sample at t= 6min

Equation 2: Antioxidant capacity in ABTS assay

Statistical Analysis

All the data were presented as mean± standard deviation. Data analysis were done by using IBM Statistical Package for Social Science (SPSS) software version 20. Besides, one-way ANOVA with comparison between equal variance was studied to determine the antioxidant and antimicrobial properties of total lutein in different parts of pumpkin (*Cucurbita maxima*). Besides, Duncan post hoc test was used with the degree of significant ($p<0.05$).

Results and Discussion

Potential Lutein Extract from Different Parts of Pumpkin (Cucurbita maxima)

Among the different parts of pumpkin (*Cucurbita maxima*), pumpkin seed gave the highest percentage yield of potential lutein content by using acetone solvent extraction with 24.69±2.52% (Figure 1). The percentage yield of potential lutein extract from pumpkin seed showed there was a significant difference with pumpkin peel and pumpkin flesh whereas there was no significant difference between the percentage yield of potential lutein extract from pumpkin peel (1.09±0.42%) and pumpkin flesh (1.24±0.18%). The chemical structure of acetone that contained groups of non-polar methyl group and polar carbonyl group make it acts as a good solvent for extraction provided significantly better result for both yield of extract and antioxidant activity (Do *et al*., 2014). Consequently, according to Kim *et al*. (2012), pumpkin (*Cucurbita maxima*) seed which had significantly more fat (524.34 ± 1.32 g/kg raw weight) than the peel (8.69 ± 0.99 g/kg raw weight) and flesh (4.20 ± 0.23 g/kg raw

weight) of pumpkin (*Cucurbita maxima*) could be extracted by acetone solvent with higher percentage of yield.

The highest percentage yield of potential lutein extract from the pumpkin seed did not represent the highest concentration of lutein extract in this sample. The concentration of lutein extract was found significantly highest in pumpkin flesh compared to other parts. The lowest lutein content in the seed extract compared to other parts is maybe due to the germination that occurred in the seed that normally distracts the carotenogenesis in the seed (Sun *et al.* 2018). Instead of lutein, the higher extracted yield of the seed may contain other lipophilic compounds such as tocopherol and tocochromanol (Fernandez-Marin *et al.* 2017).

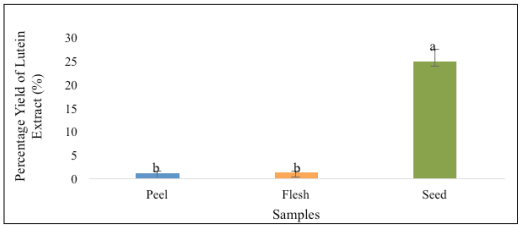

Figure 1: Yield of lutein extract (%) in different parts of the pumpkin (Cucurbita maxima).

Error bars represent standard deviation values. n= 9 Different letters indicate significant differences between products at $p < 0.05$.

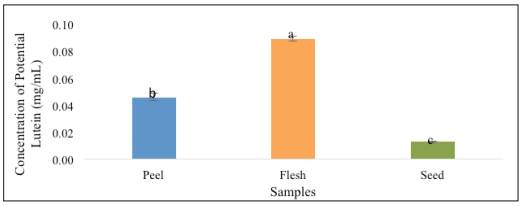

Figure 2: Concentration of lutein content against different parts of pumpkin (*Cucurbita maxima*)

Error bars represent standard deviation values. n= 9 Different letters indicate significant differences between products at $p < 0.05$

Antioxidant Properties

DPPH Radical-Scavenging Activity Test

Figure 3 showed the antioxidant activity in scavenging DPPH free radical for potential lutein extracts from different parts of the pumpkin and synthetic antioxidant. In the DPPH assay, the ability of potential lutein extracts from different parts of the pumpkin and the synthetic antioxidant was measured via the reduction of purple colour intensity to yellow colour. There were significant difference ($p<0.05$) among all samples. The potential lutein from pumpkin peel and seed exhibited the highest (98.84±0.34%) and lowest value (40.96±5.13%), respectively ($p<0.05$) of antioxidant activity. Higher potential lutein in pumpkin peel and flesh compared to synthetic antioxidant, BHT showed the potential of natural antioxidant to mitigate the antioxidant damage that could be subjected to the type of free radical species (Abdel-Aal & Rabalski, 2013).

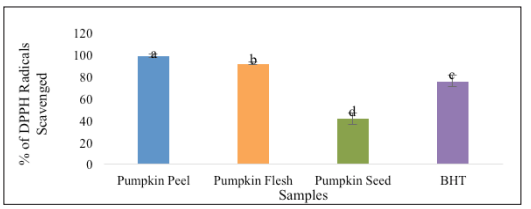

Figure 3: Antioxidant activity in scavenging DPPH free radical for potential lutein extracts from different parts of pumpkin (*Cucurbita maxima*) and synthetic antioxidant.

Error bars represent standard deviation values. n= 9 Different letters indicate significant differences between products at $p < 0.05$.

Ferric ion Reducing Antioxidant Power (FRAP) Assay

Ferric reducing ability (FRAP) was another indicator that used for measuring the antioxidant capacity of the matter where iron (II) sulphate\\e was used as the standard.

The FRAP value which was shown in Figure 4 had a similar trend as in DPPH free radical scavenging activity except for the synthetic antioxidant, BHT. The FRAP value of

potential lutein in pumpkin peel was the highest compared to other parts ($p<0.05$) and similar ($p>0.05$) to BHT.

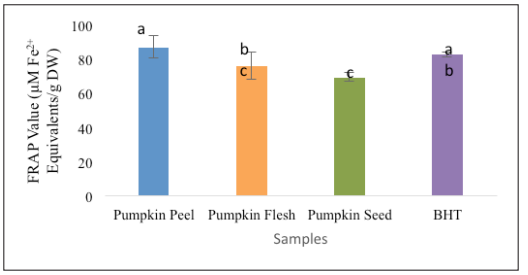

Figure 4: Ferric Reducing Ability Equivalent in potential lutein extracts from different parts of pumpkin and synthetic antioxidant (mM Iron (II) Sulphate/g DW)

Error bars represent standard deviation values. n= 9 Different letters indicate significant differences between samples at $p < 0.05$. DW represent dry weight.

On the other hand, the antioxidant activity of potential lutein extracts from different parts of pumpkin was studied through the ABTS radical scavenging assay. The synthetic antioxidant, BHT with the highest antioxidant activity (71.20±1.57%) showed a significant difference compared with the different parts of the pumpkin. Figure 5 showed the ABTS radical scavenging assay in the descending order of pumpkin peel (25.86±8.04%) > pumpkin flesh (18.79±2.22%) > pumpkin seed (12.88±1.40%). Lutein could bind to compound ABTS as lutein was non-polar, soluble in organic solvents and slightly soluble in lipid, moreover, ABTS method could be used to measure the radical activity of hydrophilic and lipophilic in the pure compound (Kusmiati & Agustini, 2017). The efficiency of lutein to quench ABTS•+ radical cation was influenced by the increasing polarities of the functional groups in the terminal rings (Miller *et al*., 1996).

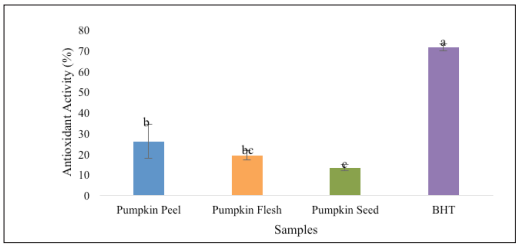

Figure 5: Antioxidant activity in ABTS Radical Scavenging Assay for potential lutein extract from different parts of pumpkin (*Cucurbita maxima*) and synthetic antioxidant.

Error bars represent standard deviation values. n= 9 Different letters indicate significant differences between samples at $p < 0.05$.

According to Pisoschi and Negulescu (2012), there were difference reaction among the analysis of antioxidant activities. DPPH assay was the antioxidant reaction with an organic radical, ABTS assay applied the principle which reacts with an organic cation radical whereas the principle of the method FRAP assay was an antioxidant reaction with a Fe(III) complex. Thus, these three protocols had been proposed to give comparable results for the antioxidant activity measured in the potential lutein extracts from different parts of the pumpkin. The concentration of potential lutein extracts from different parts of pumpkin was correlated positively with DPPH assay with a p-value of 0.021 and Pearson correlation of 0.744. This result indicated a mild relationship between concentration of potential lutein extracts in different parts of pumpkin and their free radical scavenging abilities. Therefore, it was assumed that the higher the concentration of potential lutein extracts, the higher the antioxidant activity (DPPH).

As a summary, the potential lutein extracts from the pumpkin peel showed higher antioxidant activity ($p<0.05$) than the lutein extracts from the pumpkin flesh and pumpkin. Typically for plant materials, the antioxidant activity would follow a similar trend where the scavenging activity of the extract was concentration-dependent (González-Montelongo *et al*., 2010). However, this was not

the case in this study for potential lutein extracts in pumpkin peel. Even though the concentration of potential lutein in pumpkin peel was lower than that of the pumpkin flesh, the antioxidant activities of potential lutein extracts from non-edible pumpkin peel were greater than the edible pumpkin flesh. These findings were in line with previous study whereby Avila *et al*. (2018), reported that there had bioactive compounds such as phenolics and flavonoids contributed to the higher antioxidant activity in the peel of pumpkin *Cucurbita maxima*. In addition, the acetone extracted higher polyphenolic content in the peel of cucurbit fruit than in the pulp of cucurbit fruit, hence the peel exhibited higher antioxidant activity compared to the pulp (Singh *et al*., 2016). Moreover, Hassan & Bakar (2013) studied the peel of banana *C. betacea* displayed higher antioxidant capacity as compared to the flesh of the fruit which was suspected to be contributed by the ascorbic acid and phenol compounds in the peel. Yang *et al*. (2016) also reported a similar trend with the present study where lotus rhizome peel had a higher DPPH radical scavenging activity than flesh. The antioxidant properties underlying the activities of carotenoids towards free radicals and their scavenging effects (Miller *et al*., 1996).

Conclusion

The highest concentration of lutein was found in pumpkin flesh, followed by pumpkin peel and seed and antioxidant activity was found higher in peel. In addition, the concentration of lutein extracts from the pumpkin by-product such as peel and seed was comparative with the concentration of lutein extracted from the Marigold flower, which suggested the pumpkin by-product could be the alternative and affordable source of lutein to improve the eye health. This study can be extended into the incorporation of pumpkin by-product into other food products and with the storage study.

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