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Poster Presentation Proceedings

SP4: Nutrition quality and health benefit of indigenous food
Chemical properties and fatty acid profile of lipids extracted from freshwater fish species

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ABSTRACT

Freshwater fish species from the Mekong sub-region have been considered as the source of lipids for human consumption. Their chemical properties and fatty acid profiles were investigated in order to better understand the nutritional information and potential source of essential oils. Crude fat from head and flab parts of fish was found to be 44.62, 48.47, 58.83, 67.44, and 64.64% for gunther’s walking catfish (*Clarias macrocephalus*), por fish (*Pangasius bocourti*), striped catfish (*Pangasianodon hypophthalmus*), mong fish (*Pangasius conchophilus*), and Mekong giant catfish (*Pangasianodon gigas*), respectively. The oil from fish with high fat content (striped catfish, mong fish and Mekong giant catfish) was isolated using the boiling method. The impurity of isolated oil samples was in the range of 0.0171-0.0185%, while 0.30-0.16% was found to be their moisture contents. Acid value of oil isolated from striped catfish, mong fish, and Mekong giant catfish was 1.14, 0.87, and 1.13 mg KOH/g sample, respectively. In addition, this value was increased for 1.19, 1.41, and 1.06 folds after incubation at 60 °C/13 days. Striped catfish oil showed highest iodine value, suggesting high unsaturated fatty acid content. It was then subjected to analyze for fatty acid composition using gas chromatography. Saturated fatty acid content in striped catfish was 34.8 % and palmitic acid was the most abundant among 8 saturated fatty acids. Oleic acid was the most abundant among 12 species of unsaturated fatty acids, which accounted for 60.28 %. Omega-3 (\(\omega-3\)) and omega-6 (\(\omega-6\)) fatty acid contents were found for 1.96 and 17.45 %, respectively. Linolenic and linoleic acids were the most abundant for the \(\omega-3\) and \(\omega-6\) fatty acids, respectively. Oil from fish species obtained in Mekong sub-region could be the source of essential fatty acids, particular unsaturated fatty acids.

Keywords: freshwater fish species, fatty acid composition, Mekong sub-region, fish oil

Introduction

Freshwater fish species have been considered as the food resource for human consumption for people who’s living along the Mekong region of Thailand particularly, Nong Khai province. Fishes from Mekong river have also been used as the raw material for making the expensive dishes at the popular restaurants, particular fishes in the family of Pangasiidae e.g. por fish (*Pangasius bocourti*), striped catfish (*Pangasianodon hypophthalmus*), mong fish (*Pangasius conchophilus*), and Mekong giant catfish (*Pangasianodon gigas*). These fishes are considered as the oily fish and Mekong giant catfish seems to be the most expensive and popular one.

Fish resource has not been considered as only the source of proteins but also as the source of lipids for human, especially unsaturated fatty acids. Polyunsaturated fatty acids are important for human to prevent the coronary artery disease (Conner, 2000). The general recommendation daily intake of docosahexaenoic acid / eicosapentaenoic acid...
(DHA/EPA) is 0.5 g for infants and 1 g/day for adults and patients with heart disease (Kris-Etherton et al., 2002).

Fish lipids are good source of polyunsaturated fatty acids and contain high levels of long chain polyunsaturated fatty acids, particularly linoleic acid (18:2n6) and arachidonic acid (20:4n6) (Ackman, 1967). Ozogul et al. (2007) reported that fresh water fish species from Lake Seyhan consisted of 10.7-22.7 % monosaturated and 23.2-43.7 % of polyunsaturated fatty acids. In addition, the polyunsaturated fatty acids from marine fish were found to be comparable to that of freshwater fish species (Ozogul et al. 2007). However, fatty acid composition of fish could be varied with different factors e.g. diet, location, gender, season, and environmental condition. Investigation the fatty acid composition of the specific fish should be performed in order to get the exact information regarding the nutritive value of oil from interested fish.

Therefore, the objective of this study was to investigate the chemical properties and fatty acid profiles of oil extracted from economical fish species found in Mekong region at Nong Khai province.

Materials and Methods

Materials

   Freshwater fish, gunther’s walking catfish (Clarias macrocephalus), por fish (Pangasius bocourti), striped catfish (Pangasianodon hypophthalmus), mong fish (Pangasius conchophilus), and Mekong giant catfish (Pangasianodon gigas) were purchased from local market at Nong Khai province. Those fishes were transferred to the laboratory at School of Science and Technology, Nong Khai Campus, Khon Kaen University within 2 days after catch. Fish samples were manually eviscerated and the head and flab parts were minced before keeping at -20 °C until used.

Methods

1. Crude fat determination
   Mince sample was analyzed for their crude fat content using soxtec method according to the AOAC (1995). Petroleum ether was used as the solvent. The crude fat content was calculated from extracted fat after drying.

2. Extraction of fish oil
   Mince sample was mixed with distilled water at the ratio of sample: water of 1:2. The mixture was boiled and stirred continuously for 30 min. The obtained sample was filtered through the three-layers of cheese cloth. The filtrate was then transferred into separatory funnel. Phase separation was allowed to be occurred when sample was left at room temperature for 2 h. The aqueous phase (lower layer) was then drained out and the oil phase (upper layer) was used as oil sample.

3. Determination of moisture content of fish oil
   Oil sample (5 g) was weighed into the beaker covered with watch glass and heated at 70-100 °C until the water vapor was not detected. The sample was then heated rapidly until reach the smoking point. The weight of sample after cooling was used to calculate the moisture content.

4. Determination of impurity of fish oil
   Oil sample was solubilized in kerosene before filtering through the vacuum suction. The retentate was washed twice with warm kerosene before final washing with
petroleum ether. The obtained matter was dried and the weight was used to calculate for percentage of impurity.

5. Determination of acid value of fish oil

The acid value of fish oil sample was determined according to procedure described in Official and Standardized Methods of Analysis (Watson, 1994).

6. Determination of iodine value of fish oil

The iodine value was performed by titration method according to the AOCS method (AOCS, 1992).

7. Fatty acid methyl ester preparation

The striped catfish oil was used to prepare the methyl ester by transmethylation using 2 M KOH in methanol and n-heptane. Oil (5 mg) was dissolved in 2 mL of heptane followed by 4 mL of 2 M methanolic KOH. The mixture was centrifuged at 4,000 rpm for 10 min and the heptane layer was taken for GC analysis.

8. Gas chromatographic analysis

Fatty acid composition was analyzed by GC Clarus 500 with authosampler (Perkin Elmer, USA) equipped with a flame ionization detector and fused silica capillary column (30 m × 0.32 mm, ID 0.25 μm). The oven temperature was controlled at 140 °C for 5 min, raised up to 200 °C at the rate of 4 °C/min, and further heated up to 220 °C at a rate of 1 °C/min. The injector and the detector temperature were set at 220 and 280 °C, respectively. The sample size was 1 μL and the carrier gas was controlled at 16 psi. The split used was 1:100. Fatty acids were indentified by comparing the retention times of FAME with the standard (37 components) FAME mixture (Supelco). The replication of GC analysis was performed.

Results and Discussion

The crude fat content of head and flab parts from freshwater fish with high potential economy in the wet land at the Mekong river of Nong Khai province were found in the range of 44.62-67.44 % (Table 1). This range was much higher (almost 20 folds) than those reported for freshwater fish found in the Lake Seyhan, Turkey (Ozogul et al. 2007). This may be because the different parts of fish samples. The oily organs, head and flab parts, were used as sample for this study, while the whole muscle was used in the previous study. In addition, crude fat content in this study was expressed as the dried basis since all samples were dried completely before determination of crude fat content. However, crude fat content from fish species might be varied due to species and geographical origin (Rasoarahona et al. 2005). In deed, fish species with highest fat content was mong fish, while the lowest fat content was found to be gunther’s walking catfish. Fat content values of mong fish and Mekong giant catfish were not statistically different (p<0.05), while that of Mekong giant catfish and striped catfish were also comparable. Therefore, oil from 3 fish species showing high fat content (striped catfish, mong fish, and Mekong giant catfish) were selected for fish oil extraction using boiling method. The chemical properties of extracted oil were further investigated.
Table 1 Crude fat content from head and flab parts of fish species found in Mekong region at Nong Kha province

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Scientific name</th>
<th>Crude fat content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gunther’s walking catfish</td>
<td><em>Clarias macrocephalus</em></td>
<td>44.62 ± 0.60 ^c</td>
</tr>
<tr>
<td>Por</td>
<td><em>Pangasius bocourti</em></td>
<td>48.48 ± 6.20 ^c</td>
</tr>
<tr>
<td>Striped catfish</td>
<td><em>Pangasianodon hypophthalmus</em></td>
<td>58.83 ± 4.12 ^b</td>
</tr>
<tr>
<td>Mong fish</td>
<td><em>Pangasius conchophilus</em></td>
<td>67.44 ± 2.60 ^a</td>
</tr>
<tr>
<td>Mekong giant catfish</td>
<td><em>Pangasianodon gigas</em></td>
<td>64.64 ± 1.47 ^ab</td>
</tr>
</tbody>
</table>

* The different letters indicate statistic difference (p < 0.05) of crude fat contents.

The insoluble material in isolated oil was slightly observed (Table 2). The maximum value was found in striped catfish oil with the value of 0.0185%. This value was considered as a trace amount in crude oil and could be removed according to the refining process. Since oil was isolated from boiling method, moisture contents in the isolated oil may possibly remain in the oil. The water might be adsorbed to the polar lipids e.g. phospholipids, glycoproteins, and lipoproteins. It can be seen that moisture content in oil obtained from mong fish was the highest and followed by Mekong giant catfish and striped catfish. The results suggested that oil from striped catfish was most stable since it contains less water to accelerate the chemical deterioration.

Table 2 Moisture and insoluble impurity contents of oil extracted from head and flab parts of fish species found in Mekong region at Nong Kha province

<table>
<thead>
<tr>
<th>Fish Species</th>
<th>Insoluble impurity (%)</th>
<th>Moisture content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Striped catfish</td>
<td>0.019 ± 0.0147 ^a</td>
<td>0.357 ± 0.132 ^c</td>
</tr>
<tr>
<td>Mong fish</td>
<td>0.022 ± 0.0062 ^a</td>
<td>1.623 ± 0.230 ^a</td>
</tr>
<tr>
<td>Mekong giant catfish</td>
<td>0.017 ± 0.0171 ^b</td>
<td>0.840 ± 0.269 ^b</td>
</tr>
</tbody>
</table>

* The different letters indicate statistic difference (p < 0.05) of moisture and impurity contents.

Table 3 showed the acid value of oil extracted from fish species. It can be seen that oil from Mekong giant catfish showed highest free fatty acid content, while the lowest value was found in oil from mong fish. All oil samples showed an increase free fatty acid content after incubation at 60 °C for 13 days and the rate of free fatty acid liberation was found to be highest in oil extracted from mong fish. It has been reported that free fatty acid of fish oil was also increased when storage at either 4 or -18 °C (Boran et al., 2006). Generally, free fatty acid was more susceptible to oxidation rather than that of mono-, di-, or triglyceride. It could be postulated that oil from mong fish would be most susceptible to oxidation during storage. The results suggested that oil from striped catfish and Mekong giant catfish showed the comparable stability at high temperature.

Iodine value is another important property in order to determine the quality of oil. It represents the degree of unsaturation of fatty acid within mono-, di-, or triglyceride. The iodine value of oil extracted from striped catfish was found to be the highest value, suggesting the highest degree of unsaturation. Oil from mong fish and Mekong giant catfish showed the comparable degree of unsaturation (Table 3).

According to these data, oil extracted from striped catfish showed the potential for being a good quality of fish oil in term of moisture content, insoluble impurity, free fatty acid, and iodine value.
acid as well as degree of saturation of fatty acid. Therefore, it was selected for further fatty acid composition analysis using gas chromatographic technique.

Table 3 Free fatty acid and iodine values of oil isolated from head and flab parts of commercial fish species with high potential oil content

<table>
<thead>
<tr>
<th>Fish Species</th>
<th>Free fatty acid (mg KOH/g sample)</th>
<th>Iodine value (mg KOH/g sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Room Temperature 60 °C/13 days</td>
<td></td>
</tr>
<tr>
<td>Striped catfish</td>
<td>1.14 ± 0.052 a</td>
<td>1.37 ± 0.064 a</td>
</tr>
<tr>
<td>Mong fish</td>
<td>0.87 ± 0.012 b</td>
<td>1.21 ± 0.012 b</td>
</tr>
<tr>
<td>Mekong giant catfish</td>
<td>1.32 ± 0.020 b</td>
<td>1.40 ± 0.022 b</td>
</tr>
</tbody>
</table>

* The different letters indicate statistical difference (p < 0.05) of free fatty acid and iodine values.

Gas chromatographic technique has been applied to identify fatty acids, which is based on the retention time of methyl ester derivatives of each fatty acid. Fatty acid composition within striped catfish oil was analyzed and several fatty acids were observed (Table 4). It can be seen that the unsaturated fatty acids within striped catfish oil was found to be 60.28%, which was higher than saturated fatty acid (34.84%). The similar trend was observed in oil from sea water fish species e.g. blue fish (*Pomatomus saltator*), sea bream (*Sparus auratus*), sea bass (*Dicentrarchus labrax*), and Marbled spinefoot (*Siganus rivulatus*).

Table 4 Fatty acid composition of oil extracted from head and flab parts of striped catfish (*Pangasianodon hypophthalmus*) as assessed by gas chromatography

<table>
<thead>
<tr>
<th>Saturated fatty acid</th>
<th>Amount (%)</th>
<th>Unsaturated fatty acids</th>
<th>Amount (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauric acid (C12:0)</td>
<td>1.05</td>
<td>Palmitoleic acid (C16:1n7)</td>
<td>2.14</td>
</tr>
<tr>
<td>Myristic acid (C14:0)</td>
<td>2.36</td>
<td>-cis-9-Oleic acid (C20:1n11)</td>
<td>37.84</td>
</tr>
<tr>
<td>Pentadecanoic acid (C15:0)</td>
<td>0.18</td>
<td>-cis-11-Eicosenoic acid (C20:1n11)</td>
<td>0.79</td>
</tr>
<tr>
<td><strong>Palmitic acid (C16:0)</strong></td>
<td><strong>23.59</strong></td>
<td>Erucic acid (C22:1n9)</td>
<td>0.10</td>
</tr>
<tr>
<td>Heptadecanoic acid (C17:0)</td>
<td>0.35</td>
<td>-cis-9,12-Linoleic acid (C18:2n6)</td>
<td>15.91</td>
</tr>
<tr>
<td>Stearic acid (C18:0)</td>
<td>6.70</td>
<td>-γ-Linolenic acid (C18:3n6)</td>
<td>0.21</td>
</tr>
<tr>
<td>Arachidic acid (C20:0)</td>
<td>0.16</td>
<td>-α-Linolenic acid (C18:3n3)</td>
<td>1.13</td>
</tr>
<tr>
<td>Tricosanoic acid (C23:0)</td>
<td>0.45</td>
<td>-cis-11,14 - Eicosadienoic acid (C20:2)</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-cis-8,11,14-Eicosatrienoic acid (C20:3n6)</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-cis-11,14,17-Eicosatrienoic acid (C20:3n3)</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-cis-5,8,11,14,17-Eicosapentaenoic acid (C20:5n3)</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-cis-4,7,10,13,16,19-Docosahexaenoic acid (C22:6n3)</td>
<td>0.43</td>
</tr>
</tbody>
</table>

**Summary** 34.84  **Summary** 60.28

The major species of saturated fatty acid from striped catfish oil were palmitic acid (C16:0) and stearic acid, which were found to be 23.59 and 6.7%, respectively. Palmitic acid was found in oil extracted from North American catfish (*Clarias gariepinus*), common carp (*Cyprinus carpio*), Wels catfish (*Siluris glanis*), and Tench (*Tinca tinca*) for 18.2, 15.9, 18.1, and 17.6%, respectively (Ozogul et al, 2007). The palmitic acid in striped catfish seemed to be higher than those data reported previously. This was because the different environmental condition between Mekong river, Thailand and Lake Seyhan, Turkey.
The unsaturated fatty acid in striped catfish was detected for 60.28%. The major unsaturated fatty acids were cis-9-oleic acid (C20:1n11) and cis-9, 12-Linoleic acid (C18:2n6), which were observed for 37.84 and 15.91%, respectively (Table 4). Ozogul et al. (2007) also reported that cis-9, 12-linoleic acid (C18:2n6) was the major polyunsaturated fatty acid from American catfish. The ratio of polyunsaturated fatty acids to the saturated fatty acids was 0.56, which was higher than that of Nile tilapia with skin (de Castro et al, 2007). However, this value seemed to be lower than that of freshwater and marine fish species found in Turkey (Ozogul et al. 2007). The different fatty acid composition was due to the fish behavior e.g. herbivorous, omnivorous or carnivorous (Sargent et al., 1995). Saito et al. (1999) indicated that environmental condition, especially temperature influences lipid content and fatty acid composition of fish oil.

It can be seen that EPA and DHA were also detected in oil extracted from striped catfish (Table 4). The comparable essential fatty acid contents between freshwater and seawater fish species was also reported (Ozogul et al. 2007). This indicated that oil from striped catfish could be the alternative source of essential/polyunsaturated fatty acids for human consumption.

Conclusions

The head and flab parts of Mekong giant catfish, striped catfish, and mong fish contained high crude fat content, suggesting the high potential for being the source of fish oil. The chemical properties of oil extracted from striped catfish showed the highest content of unsaturated fatty acids. The omega 3 and 6 fatty acids (DHA, EPA) were also detected. Therefore, consumption of striped catfish oil would likely benefit for human health.

References


AOAC (1992). Official methods and recommended practices of the American oil chemist’s society. 4th ed., Champaign, American Oil Chemist’s Society, IL: USA.


Characterization of cold pressed organic Jasmine rice bran oil
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Surindra Rajapat University, Muang, Surin 32000, Thailand
*Corresponding e-mail address: p_kaewkool@yahoo.com

ABSTRACT

This study aims to focus on characterization of cold pressed organic jasmine rice bran oil. The cold pressed organic jasmine rice bran oil was obtained from Rice Fund Surin Organic Agriculture Cooperative Ltd. (RFSOAC). Different chemical-physical parameters were characterized. The refractive index, acid value, peroxide value and iodine value were recorded as 1.7294, 1.36 KOH/mg/g oil, 3.12 mg/kg oil and 93.02 mg/g, respectively. The fatty acid profile showed palmitic acid (21.79%), stearic acid (1.86%), oleic acid (44.85%), linoleic acid (31.32%) and linolenic acid (0.19%) as major fatty acids. In this research, γ-oryzanol is an important component in rice bran oil, it occurs in cold pressed organic jasmine rice bran oil at a high level up to 1.298%, where it serves as a natural antioxidant.

Keywords: chemical-physical properties, cold pressed organic jasmine rice bran oil

Introduction

Jasmine rice (Oryza sativa L. cv. Khao Hom Dawk Mali) is the most important agricultural product in Thailand, since it is the basic food for most of the population in this region. Rice milling yields 70% of rice (endosperm) as the major product, and by-products consist of 20% rice husk, 8% rice bran, and 2% rice germ. Rice bran can be used as feed or as a source of rice bran oil [1]. An earlier study reported that rice bran oil is an edible oil of unsaturated fatty acid (mono-unsaturated fatty acid 45% and poly-unsaturated fatty acid 37%) [2, 3].

In comparison with most vegetable oils, Rice bran oil has a qualitatively different composition of bioactive minor components, such as γ-oryzanol, tocotrienols, and phytosterols. γ-Oryzanol derivatives in particular are found in only a very limited number of oils [1]. γ-Oryzanol is a mixture containing compound ferulic acid ester of sterols and triterpene alcohols [4]. It has antioxidant properties. Various of studies have found that γ-oryzanol has various beneficial properties such as decreasing plasma cholesterol, decreasing cholesterol absorption and decreasing disorders of menopause. [5-8]

The overall composition, nutrition profile, functional characteristics of rice bran oil reveals its wide application in healthy diet due to low saturated fat and cholesterol lowering activity in humans [9]. The inherent fatty acid profile of rice bran oil makes it a natural candidate for use as frying oil without hydrogenation need, which is typically accompanied by the development of trans-fats and healthy food.

Surin Province is a good source of planting of fragrant rice or Khao Hom Dok Mali in Thailand. This Province has set a pilot of planting of organic jasmine rice, established the Rice Fund Surin Organic Agriculture Cooperative Ltd. (RFSOAC),
promoted the farmer to plant the organic jasmine rice as well as received the reward of Organic Agriculture Certification Thailand (ACT). Moreover, it produces the cold pressed organic jasmine rice bran oil. To promote the cold pressed organic jasmine rice bran oil as organic agriculture product or healthy food in the future, it is necessary to understand the characterization of cold pressed organic jasmine rice bran oil. The present research work was designed to extract and study different chemical-physical parameters of cold pressed organic jasmine rice bran oil.

**Materials and Methods**

**Materials**

The cold pressed organic jasmine rice bran oil was purchased from Rice Fund Surin Organic Agriculture Cooperative Ltd. (Surin, Thailand). Heptadecanoic acid (99% purity) was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). Sulfuric acid was analytical grade from Fluka (Buchs, Switzerland). Solvents were analytical grade from Lab Scan Co. (Bangkok, Thailand). Standard γ-oryzanol (98% purity) was obtained from Tsuno Rice Fine Chemicals Company (Wakayama, Japan). BPX-70 (70% cyanopropyl polysilphenylenesiloxane) was purchased from SGE International Pty, Ltd. (Victoria, Australia).

**Methods**

The present study was conducted at food science and technology laboratory and science and applied science center laboratory. The cold pressed organic jasmine rice bran oil was analyzed for refractive index, peroxide value, and iodine value and free fatty acids by following their respective methods outlined in AOAC [10].

1. **GC Analysis**

   Gas chromatographic (GC) analysis was carried out on a Shimadzu model 14B equipped with a flame ionization detection (FID) and a split-splitless injector. BPX-70 (70% cyanopropyl polysilphenylenesiloxane) capillary column (15m×0.25mm i.d.) was purchased from SGE International Pty, Ltd. (Victoria, Australia). The chromatographic conditions were as follows: Helium carrier gas flow 2mL/min, Nitrogen makeup gas flow 14 mL/min: detector/injector, 230°C: split ratio, about 50:1.

2. **UV-spectrophotometer Analysis**

   UV-spectrophotometer was carried out on a Shimadzu model UV-1601 and spectrum 315 nm.

3. **Transmethylation**

   About 10-20 mg of cold pressed organic jasmine rice bran oil in a 10mL screw-capped tube and added were 1 mL of heptadecanoate acid (inter standard) 0.4mg/mL was dissolved in toluene and 0.5 mL of 2M H2SO4 was dissolved in methanol. The tube was capped tightly and heated in water bath at 70°C for 4h. After cooling, 0.2mL of 4% Na2CO3 was added and the mixture was washed three times each with 1mL of distilled water, dried over anhydrous Na2SO4 and evaporated to dryness under a stream of nitrogen. The esters were re-dissolved in approximately of hexane and were ready to be analyzed by GC.

4. **Calibration curve of γ-oryzanol**
γ-Oryzanol (stock standard) 10mg was dissolved in hexane 100mL as shown in Figure 1.

\[ y = 0.0246x \]
\[ R^2 = 0.9984 \]

**Figure 1** Calibration curve of γ-oryzanol was dissolved in hexane

**Results and Discussion**

The results on chemical-physical characteristics of cold pressed organic jasmine rice bran indicated that refractive index value was 1.7294 (Table 1). The acid, peroxide and iodine values were recorded to be 1.36 KOHmg/g oil, 3.12mg/kg oil and 93.02mg/g, respectively. TIS 44-2516 (1973) Rice bran oil [11] and TIS 47-2533 (1990) Edible oil [12] which reported that quality characteristics of properly nature rice bran oil showed maximum of acid values (4 KOHmg/g oil) peroxide values (10mg/kg oil) and iodine values (115mg/g). The present results are in line with this report.

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Refractive index</td>
<td>1.7294</td>
</tr>
<tr>
<td>2</td>
<td>Acid value</td>
<td>1.36 KOHmg/g oil</td>
</tr>
<tr>
<td>3</td>
<td>Peroxide value</td>
<td>3.12mg/kg</td>
</tr>
<tr>
<td>4</td>
<td>Iodine value</td>
<td>93.02mg/g</td>
</tr>
</tbody>
</table>

**Table 2** Fatty acid composition of cold pressed organic jasmine rice bran oil

<table>
<thead>
<tr>
<th>No.</th>
<th>Fatty acid</th>
<th>Values (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Palmitic acid; C_{16:0}</td>
<td>21.79</td>
</tr>
<tr>
<td>2</td>
<td>Stearic acid; C_{18:0}</td>
<td>1.86</td>
</tr>
<tr>
<td>3</td>
<td>Oleic acid; C_{18:1}</td>
<td>44.85</td>
</tr>
<tr>
<td>4</td>
<td>Linoleic acid; C_{18:2}</td>
<td>31.32</td>
</tr>
<tr>
<td>5</td>
<td>Linolenic acid; C_{18:3}</td>
<td>0.19</td>
</tr>
</tbody>
</table>
Figure 2 Gas chromatogram of fatty acid esters of cold pressed organic jasmine rice bran oil on a BPX-70 capillary column (15m×0.25mm i.d.). Oven temperature: 160 °C. Peaks is labeled names of fatty acid esters.

The fatty acid composition of cold pressed organic jasmine rice bran oil (Table 2 and Figure 2) indicated that oleic and linoleic acids were about 76.17%. Sugano and Tsuji [13] and Tahira et.al.[14] and Van Hoed et.al. [1] reported that oleic and linoleic fatty acids constitute more than 70% of fatty acids of glycerides.

The rice bran oil is similar to peanut oil in fatty acid composition having oleic acid 49.9% and linoleic acid 35.4% [15]. Lee et al. [3] also reported higher percentage of oleic, linoleic and palmitic acids in rice bran oil. The saturation level is slightly higher than conventional soybean oil[16]. The linolenic acid constituted only 0.19 percent of rice bran oil, and this lower linolenic acid content makes rice bran oil more resistant to oxidation than soybean oil [14].

Table 3 Amount γ-oryzanol of cold pressed organic jasmine rice bran oil

<table>
<thead>
<tr>
<th>OD 315 nm</th>
<th>γ-oryzanol (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5277*</td>
<td>1.2980</td>
</tr>
</tbody>
</table>

*Dilution 1,000 times

The γ-oryzanol of cold pressed organic jasmine rice bran oil (Table 3) was about 1.298%. Krishna et.al. [17] and Xu and Godber [18] reported that γ-oryzanol is a mixture of ferulic acid ester of sterol and trierpene alcohols, it occurs in rice bran oil at a level of 1 to 2%, where it serves as natural antioxidant.
Conclusions

In this research work, the chemical-physical parameters of cold pressed organic jasmine rice bran oil were investigated based on Thai Industrial Standards (TIS) 44-2516 (1973) Rice bran oil [11] and TIS 47-2533 (1990) Edible oil [12]. The results of the fatty acid composition in cold pressed organic jasmine rice bran oil indicated that oleic and linoleic acids were 76.17%. In addition, it was found that the γ-oryzanol of cold pressed organic jasmine rice bran oil was about 1.298%. As a result, it can be efficiently used for cooking purpose, health food and development of community products of Surin (OTOP).

Acknowledgments

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References


Anti-proliferative effect of extracts from Australian indigenous herbs against human cancer cells

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ABSTRACT

Over the last decade selected Australian indigenous edible plants have entered commercial production. A range of products derived from these plants are appearing in supermarkets, specialty shops and Australian restaurants. The objective of the present study was to generate information about the potential health-enhancing properties of selected herbs: Tasmanian pepper leaf (*Tasmannia lanceolata*), anise myrtle (*Syzygium anisatum*) and lemon myrtle (*Backhousia citriodora*). Within this study hydrophilic compounds were targeted. Purified and lyophilized ethanolic extracts of plant material were assessed for the presence of phenolic compounds (Folin-Ciocalteu assay and high performance liquid chromatography - diode array detector, HPLC-DAD) and antioxidant capacity [Ferric Reducing Activity Power (FRAP) assay and Oxygen Radical Absorbance Capacity (ORAC) assays]. Subsequently anti-proliferative effects of these extracts were evaluated against an array of human cancer cells: colorectal (HT-29) and gastric (AGS) adenocarcinomas, bladder carcinoma (BL-13). The extracts suppressed the growth of cells in a dose-dependent manner. Cell line – specific effect was observed.

**Keywords:** australian native herbs; antioxidant activity; FRAP; ORAC; total phenolics; anti-proliferative activity

Introduction

Australian native plants serve as a source of food and medicine to the Australian Aboriginal population for approximately 50 thousands years [1]. Some of the edible native plants have been reported to possess unique sensory properties [2], potentially indicating presence of a rich mixture of phytochemicals. At present these plants represent an enormous resource of attractive edible plants for the use by food industry.

Phenolic compounds represent a particularly rich family of phytochemicals, consisting of more than 10,000 compounds [3]. Many of these compounds play an important role as antioxidants which effectively reduce the oxidative stress and act as chemopreventive agents [4]. Consumption of polyphenol-rich diet arising from the consumption of fruits and vegetables have been associated with the decrease of cancer incidences [5].

Earlier reports have shown that a number of Australian native plants exhibit enhanced antioxidant capacities [6] and phenolic compounds were indicated as the major source of antioxidant activity. Among them are species which already have entered commercial production. Within this study selected native Australian species of an importance to the industry were evaluated for the presence of phenolic compounds. The effects of hydrophilic based extracts prepared from the fruits and spices on the proliferation of human cancer cells were investigated.
Materials and Methods

Materials

1. Plant material
   Commercial samples of Tasmannia Pepper Leaf (Tasmannia lanceolata, R. Br.), Anise myrtle (Syzygium anisatum, Vickery, Craven & Biffen) and Lemon myrtle (Backhousia citriodora, F. Muell) were obtained from Australian Native food Industry Ltd.

2. Chemicals
   AAPH (2,2-azobis (2-methylpropionamide) dihydrochloride), chlorogenic acid, Dimethyl sulfoxide (DMSO), fluorescein, gallic acid, quercetin, trolox, XAD-16, Eagle’s minimum essential medium (EMEM), Dulbecco’s Modified Eagle’s medium (DMEM), Folin-Ciocalteau reagent, acetonitrile, ethanol and TPTZ were purchased from Sigma-Aldrich, Inc. (Sydney, Australia). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent, fetal bovine serum (FBS), and culture media: McCoy’s 5a, RPMI and F12K, were purchased from Invitrogen Australia Pty Ltd. (Victoria, Australia).

Methods

1. Preparation of lyophilized mixtures of bioactive compounds isolated from plant sources. The lyophilized extracts were prepared as described earlier [7]. Briefly, the raw plant material of the native fruits and herbs was initially weighed and ground into a pulp using a heavy duty blender (Waring Laboratory Science, Torrington, CT, USA). A 2-fold volume of acidified methanol (80% methanol, 19% H₂O and 1% Acetic acid, v/v/v) was then added, stirred for 2 hours at cool temperature (4°C) and centrifuged for 20 minutes at 10,000 rpm at 4°C (Sorvall RC-5B; DuPont, Wilmington, DE, USA) with the supernatant collected. The extraction was repeated twice. The third extraction was carried out overnight. The supernatants from the consecutive extractions were combined and the solvent evaporated under reduced pressure at 40°C using a rotary evaporator (Rotavapor R-205; Buchi, Switzerland).

   The concentrated alcoholic extract was further purified using an XAD-16 resin column (300 x 60 mm i.d.). The extract was dissolved with acidified water (99% H₂O, 1% acetic acid, v/v), applied to the column, washed with acidified water and eluted with 80% ethanol (80% ethanol, 19.9% H₂O, 0.1% trifluoroacetic acid, v/v/v). The eluate was collected and evaporated under reduced pressure at 37°C using a rotary evaporator. The purification was repeated. The resulting fraction was dissolved in purified water and freeze-dried under vacuum to obtain a fine lyophilized powder representing a polyphenolic-rich fraction of the fruit.

2. Total phenolics
   The total phenolic content of the extracted Australian herbs and fruits was investigated using the Folin-Ciocalteau assay with some modifications [8]. The experiment was performed in 96-well microplate (Sarstedt Australia, Technology Park, SA, Australia). Extracted samples were diluted (1:20) in distilled water, followed by
adding Folin-Ciocalteau reagent diluted (1:10) in distilled water. The 6% of Na₂CO₃ was then added with 8 minutes shaking. The absorbance was measured at 595 nm using a spectrophotometer (Labsystems Multiskan MS; Thermo Fisher Scientific, Waltham, MA, USA). The total phenolic content of the extracts was calculated and expressed as gallic acid equivalents per gram of dry weight (µmol GAE/gDW) based on gallic acid standard curve.

3. Antioxidant activity

3.1 Ferric Reducing Activity Power (FRAP) assay

Total reducing capacity was determined using FRAP assay [9]. The FRAP reagent was initially prepared consisting of 10 ml of 300 mmol/l acetate buffer, 10 ml of 20 mmol/l FeCl₃ and 1 ml of 10 mmol /TPTZ solution. The acetate buffer (pH 3.6) consisted of 3.1 g of sodium acetate and 16 ml acetic acid per liter of water. The TPTZ solution consisted of 31.2 mg of TPTZ in 10 ml HCl. Initially, the extracts diluted in water were added to FRAP reagent in 96-well microplate (Sarstedt Australia) and followed by 15 seconds shaking. After incubation for 8 minutes, the absorbance was read at 595 nm using a spectrophotometer (Labsystems Multiskan MS; Thermo Fisher Scientific). The reducing capacity of the extracts was expressed as umol of Iron (II) per gram of dry weight (µmol Fe²⁺/gDW) based on an Iron (II) sulphate standard curve.

3.2 Oxygen Radical Absorbance Capacity (ORAC) assay

Oxygen radical scavenging capacity of hydrophilic compounds was determined using the ORAC assay according to Prior [10]. Initially, preparations of fluorescein (120 nM) and AAPH (360 mM) were formulated in phosphate buffered saline (PBS; 75 mM, pH 7.0). Fluorescein and diluted sample was added to quartz cuvettes (Starna Pty. Ltd., Baulkham Hills), NSW, Australia) and inserted into a fluorescence spectrophotometer (Cary Eclipse; Varian, Inc., Palo Alto, CA, USA) and allowed to equilibrate at 37°C under rigorous stirring. Following this, AAPH was added to each cuvette and fluorescence measurements initiated immediately. Fluorescence (excitation wavelength 495 nm, emission wavelength 515nm, 37°C) was recorded every 5 seconds until the fluorescence reached zero and a kinetic curve generated. The area under the curve (AUC) was integrated and standardized against a blank control. The measurements were carried out in duplicate. The antioxidant capacity of the samples was expressed as µmol of Trolox equivalent per gram of dry weight (µmol Trolox E./gDW).

4. Quantification and identification of phenolic compounds: high performance liquid chromatography – diode array detector (HPLC-DAD) and liquid chromatography-photodiode array-mass spectrometry (LC-PDA-MS/MS).

Quantification of phenolic compounds in the purified polyphenolics extracts was conducted using HPLC-DAD as described earlier [11]. Briefly, the HPLC system consisted of two LC-10AD pumps, SPD-M10A diode array detector (DAD), CTO-10AS column oven, DGU-12A degasser, SIL-10AD autoinjector, and SCL-10A system controller (Shimadzu Corporation, Kyoto, Japan) equipped with a 150 x 4.6 mm i.d., 5 µm Luna C18(2) column (Phenomenex, Sydney, Australia). The solvents, prepared in water (solvent A: 0.5% trifluoroacetic acid (TFA) and solvent B: 95% acetonitrile, 0.5% TFA) were applied at a flow rate of 1.0 ml.min⁻¹. The elution profile was a linear gradient elution for solvent B of 0 to 10% over 10 minutes, to 55% over 45 min, to 80% over 5
min, to 100% over 15 minutes. Analytical HPLC was run at 25°C and monitored at 280 nm for phenolic acids and flavan-3-ols, 326 nm for cinnamic acids and 370 nm for flavonones and at 520 nm anthocyanins. Calibration curves of gallic acid (280 nm), chlorogenic acid (326 nm), rutin (370 nm) and cyanidin 3-glucoside (520 nm) were prepared to quantify the compounds detected at the respected wavelengths. Identification of compounds was conducted as described earlier [11].

5. Anti-proliferative activity against cancer cell lines
All cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and were maintained at 37°C in a humidified 5% CO₂ atmosphere in media containing 10% foetal bovine serum (FBS), 100 µg/ml streptomycin and 100 units/ml penicillin unless otherwise stated. HT-29 (colorectal adenocarcinoma) cells were grown in McCoy’s 5a; AGS (gastric adenocarcinoma) was cultured in F12-K Ham’s medium; Bladder cancer cells (BL-13) were grown in RPMI medium. The assay was conducted as described previously [7]. Initially, cells (5x10⁵/ml) were incubated for 24 hours at 37°C in 96-well clear-walled microplates (Thermo fisher Scientific, Sydney, Australia). Subsequently, a range of concentrations of purified extracts were applied over 24 hours. Next, the medium and samples were removed from each cell and the wells were gently washed with PBS. A 100 µl of PBS and 10 µl of 5 mg/ml MTT solution were then added to each well and the cultures were further incubated for four hours. The MTT formazan product was dissolved with dimethyl sulfoxide (DMSO). The plate was shaken for 10 minutes and the absorbance was measured at 595 nm using a spectrophotometer. The results were expressed as the optical density ratio of the treatment to control. At least 6 measurements were conducted for each treatment.

Results and Discussion

Total phenolics and antioxidant capacity

Hydrophilic extracts obtained from the selected commercially grown native Australian herbs were evaluated for the presence of phenolic compounds. The samples were evaluated using the Folin-Ciocalteu (F-C) assay, which was suggested as a fast and reliable method to quantify phenolics in foods [10] and with the help of HPLC. The results are presented in Table 1.

The highest F-C values were obtained for the Tasmannia pepper leaf extract (911.9 mg GA E/gDW) followed by the anise myrtle and lemon myrtle extracts (728.9 and 660.5 mg GA E/gDW, respectively) (Table 1). However, the HPLC quantification indicates that the levels of phenolic compounds in extracts were lower than identified with the help of F-C assay. The levels of phenolic compounds as quantified by the HPLC for lemon myrtle, anise myrtle and Tasmannia pepper leaf were respectively 65%, 55% and 60% lower than indicated by the F-C values (Table 1). The Folin-Ciocalteu values can be affected by other compounds present in the extracts: sugars, ascorbic acid, aromatic amines and unanticipated phenols [8]. The above evaluation indicates that beside phenolics, extracts used in this study may contain other unknown compounds.
Table 1 Total phenolic (TP) content in extracts of native Australian herbs.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total Phenolics (F-C*) (mg GA E*/g DW)</th>
<th>Phenolic compounds (HPLC**) (mg/gDW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>280 nm (GA E¹)</td>
<td>326 nm (CHA E²)</td>
</tr>
<tr>
<td>Lemon myrtle</td>
<td>660.5 ± 15.7</td>
<td>35.3 ± 3.4</td>
</tr>
<tr>
<td>Anise myrtle</td>
<td>728.9 ± 74.2</td>
<td>25.7 ± 1.5</td>
</tr>
<tr>
<td>Tasmannia pepper leaf</td>
<td>911.9 ND</td>
<td>359.2 ± 18.1</td>
</tr>
</tbody>
</table>

*F-C: Folic –Ciocalteu assay; **HPLC: high performance liquid chromatography
¹ GA E: Gallic acid equivalent; ² CHA E: Chlorogenic acid equivalent; ³ Rutin E: Rutin hydrate equivalent; ⁴ C₃-G: Cyanidin 3-glucoside equivalent; ND: not detected

FRAP assay measures the total level of redox-active compounds in a solution [9]. In this assay the highest activity displayed anise myrtle extract (Table 2), which was 81% higher than the antioxidant capacity of Tasmannia pepper leaf extract.

Oxygen radical absorbance capacity (ORAC) assay measures an ability of phytochemicals to scavenge oxygen free radicals. This assay is considered to be the closest to human physiology [10]. Tasmannia pepper leaf extract exhibited superior oxygen radicals scavenging capacity to all other extracts and its activity was 3-fold that of lemon myrtle and 1.7-fold that of anise myrtle Table 2).

Table 2 Total phenolics and antioxidant capacity (FRAP and ORAC assay) of native Australian herbs.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total reducing capacity (FRAP*) (µmol Fe²⁺/100 gDW)</th>
<th>ORAC** (µmol T Eq/gDW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lemon myrtle</td>
<td>502.5 ± 10.9</td>
<td>41.4 ± 5.9</td>
</tr>
<tr>
<td>Anise myrtle</td>
<td>805.4 ± 15.2</td>
<td>75.6 ± 12.7</td>
</tr>
<tr>
<td>Tasmannia pepper leaf</td>
<td>444.4 ± 12.2</td>
<td>127.9 ± 9.9</td>
</tr>
</tbody>
</table>

*FRAP: Ferric Reducing Antioxidant Power; ***ORAC: Oxygen Radical Absorbance Capacity

Major phenolic compounds present in extracts

Antioxidant capacity of phenolic extracts depends on the level of phenolic compounds in a mixture and compound’s identity. Therefore detailed study towards identification of major phenolic compounds was conducted. The results are presented in Table 3. Chlorogenic acid was found to be the main component of Tasmannia pepper leaf extract present at a level of 289.3 mg/gDW. It was accompanied by p-coumaric acid present at a level of 51.5 mg/gDW. Another compound identified was quercetin rutinoside (rutin). Traces of anthocyanin cyanidin 3-glucoside were also detected (Table 3). Quercetin was the main component of the anise myrtle and lemon myrtle extracts, and was present at the level of 29.1 ± 4.9 and 31.7 ± 4.4 mg/gDW, respectively. Other interesting compounds found in lemon myrtle were myricetin (35.3 ± 6.8 mg/gDW). Hesperetin was the main compound of lemon myrtle and was present at a level of 160.6 ± 35.8 mg/gDW (Table 3).
**Table 3** Major phenolic compounds in extract of native Australian herbs tentatively identified with LC/MS.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Compound</th>
<th>MS/MS</th>
<th>Amount (mg/g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lemon myrtle</td>
<td>Hesperetin rhamnoside</td>
<td>449/-</td>
<td>160.6±35.8*</td>
</tr>
<tr>
<td></td>
<td>Hesperetin pentoside</td>
<td>449/-</td>
<td>35.3±6.8</td>
</tr>
<tr>
<td></td>
<td>Myricetin</td>
<td>319/317</td>
<td>31.7±4.4</td>
</tr>
<tr>
<td></td>
<td>Hesperetin hexoside</td>
<td>465/-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quercetin</td>
<td>303/-</td>
<td></td>
</tr>
<tr>
<td>Anise myrtle</td>
<td>Quercetin hexoside</td>
<td>465/-</td>
<td>29.1±4.9**</td>
</tr>
<tr>
<td></td>
<td>Quercetin pentoside</td>
<td>449/-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Myricetin</td>
<td>319/317</td>
<td>17.6±3.6</td>
</tr>
<tr>
<td></td>
<td>Chlorogenic acid, unknown</td>
<td>-353/-</td>
<td></td>
</tr>
<tr>
<td>Tasmannia pepper leaf</td>
<td>Cyanidin 3-glucoside</td>
<td>449/-</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td></td>
<td>Cyanidin 3-rutinoside</td>
<td>-353/-</td>
<td>0.02±0.001</td>
</tr>
<tr>
<td></td>
<td>Chlorogenic acid</td>
<td>-353/-</td>
<td>289.3±14.6</td>
</tr>
<tr>
<td></td>
<td>Rutin</td>
<td>611/609</td>
<td>119.4±8.6</td>
</tr>
<tr>
<td></td>
<td>Quercetin</td>
<td>303/-</td>
<td>33.5±2.2</td>
</tr>
<tr>
<td></td>
<td>p-coumaric acid</td>
<td>163/-</td>
<td>51.5±7.4</td>
</tr>
</tbody>
</table>

* Hesperetin – total; ** Quercetin – total

Anti-proliferative activity against cancer cell lines

Anti-proliferative activity of hydrophilic extracts obtained from the native Australian herbs was evaluated against cancerous cell lines that are directly associated with the digestive system: colorectal adenocarcinoma (HT-29, colon cancer), gastric adenocarcinoma (AGS, stomach cancer) and bladder cancer cell (BL13).
Figure 1 Effect of herbs extracts on proliferation of: A - bladder cancer (BL13), B – gastric adenocarcinoma (AGS), C - colorectal adenocarcinoma (HT-29).

The lyophilized powders from herbs applied at concentrations from 0.0–2.0 mg/ml inhibited the proliferation of cancer cells in a dose dependent manner. The growth inhibition varied with different degree of potency between the cell lines. The anti-proliferative effects of the extracts were more pronounced against bladder cancer and stomach cancer cells than colon cancer cells. Anise myrtle extract consistently displayed the highest anti-proliferative activity among the evaluated samples. The IC50 value (amount of the extract necessary to reduce the proliferation of cancer cells by 50%) of anise myrtle extract was approximately 600 µg/ml for BL-13 and AGS cells and 1250 µg/ml for HT-29 cells. Tasmania pepper leaf extract was equally effective to anise myrtle against the BL-13 cells (IC50 = 0.6mg/ml). Further studies are in progress to understand the mechanism of the anti-proliferative activities of these extracts.

Conclusions

The selected Australian native herbs have been identified as a novel source of phenolic compounds. Phenolic-rich extracts prepared from these herbs inhibited proliferation of human cancer cells associated with the digestion system. The results indicate possible application of these native Australian herbs in a health-promoting food.
Acknowledgments

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References


SP4-04
The effect of storage temperatures on antioxidant activity, total phenolic and flavonoid content of selected indigenous vegetables
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ABSTRACT

To determine the effect of storage conditions on antioxidant activity, total phenolics, and total flavonoids of selected indigenous vegetables. Selected vegetables; lettuce, Thai holy basil, sweet basil and mint, were used to determine an antioxidant activity and also studied about the effect of storage temperatures on their antioxidant activity. They were packed in the typical packaging system which was referred to “polymeric bags” and stored at refrigerated temperature (4°C) and at room temperature (28°C). Antioxidant activities, total phenolics and total flavonoids have been determined every 2 days until the vegetables were rotten (about 8 days). Antioxidant activity was affected by storage conditions. It has increased in the first two days then it was decreased in both storage temperatures. For flavonoid, the outcome was similar. During storage, the content of total phenolics was rather stable. Normally, vegetables which were stored at room temperature visually spoiled before any significant antioxidant activity and flavonoid loss had occurred. Vegetables storage did not show negative relationship with antioxidant capacity of vegetables. In most cases, vegetables stored in refrigerator had higher antioxidant capacity (p<0.05).

Keywords: antioxidant activity, total phenolics, flavonoids, indigenous vegetables

Introduction

Vegetables are not only providing nutritional values but also being an important source of dietary antioxidants. Several Thai vegetables contain high phenolic content and also antioxidant capacity (Nanasombat and Teckchuen, 2009, Tangkananukul et al., 2006, Thalang and Trakoontivakorn, 2001). Phenolic contents such as flavonoids and phenolic acids are mainly noticeable of their high antioxidative activity (Pilarski et al., 2006). The dietary antioxidant including flavonoids and phenolics may prevent degenerative disease and lower the risk of chronic diseases (Hertog et al., 1993, Knekt et al., 2002, Kwon et al., 2008, Schramm and German, 1998).

Vegetables on sell in the fresh markets in Thailand are normally displayed at room temperature. If they are not sold out within one day, they are generally stored in the refrigerator or at room temperature until visual spoilage is showed. In general, after purchase, consumers can not cook all those vegetables within one day, it is normal practiced to store them in refrigerator. Therefore, it should be interesting to look at how the quantity of antioxidant in vegetables can be affected by storage temperature. From literature there were studies which reported that in the days after purchase fruits and vegetables, antioxidant capacity did not lose but it even increased in some cases (Gil et al., 2006, Kevers et al., 2007). The main purpose of the present study is to determine the effect of storage temperature on antioxidant capacity, total phenolics and flavonoids of selected vegetables stored at room temperature and in the refrigerator.
Materials and Methods

Materials

Four vegetables used in this study were Thai holy basil (*Ocimum sanctum* Linn.), sweet basil (*Ocimum basilicum* Linn.), mint (*Mentha cordifolia* Opiz), and lettuce (*Lactuca sativa* Linn.). All vegetables were obtained from the fresh market in A.Muang, Makasarakham province. The materials were instantaneously taken out and analyzed directly at time zero. Some were packed in the typical packaging system or polymeric bags and stored at room temperature (28°C) and others at refrigerated temperature (4°C). When vegetables showed visual spoilage, the storage was stopped.

Methods

1. Sample preparation: Following the method of Kevers *et al.* (2007), 75g of fresh materials were blended with 150mL of solvent (70% acetone, 28% water, 2% acetic acid). The mixture was shaken at 4°C for 1 hour and centrifuged at 6000rpm for 15 min. The supernatant was kept. The same solvent was added and incubated for 15 min. The mixture was centrifuged using the same procedure. The supernatants were pooled and evaporated at 30°C until 70% of the supernatant remained. Water was added until the volume reached 300mL.

2. Determination of Total Phenolics. Total phenolic content was determined by the Folin-Ciocalteu method (Caboni *et al.*, 1997). Sample extracts (1mL) were mixed with 0.5mL of Folin-Ciocalteu reagent, and 30 min later, were added with 3mL of sodium carbonate (20%W/V). 15 min later, 10mL of distillated water were added. The absorbance at 725nm was measured. Gallic acid (Fluka) was used as standard. Results were reported as milligrams of gallic acid equivalents (GAE) per 100g-1 of FW.

3. Determination of antioxidant capacity. Antioxidant capacity was determined by scavenging of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Dasgupta and De, 2007). Extracted mixture was added to a 0.004% MeOH solution of DPPH. Absorbance at 517 nm was determined after 30 min, and the percentage inhibition activity was calculated. 4. Determination of total flavonoids. Total flavonoid content was determined by using the AlCl₃ method (Lamaison and Carnat, 1991). Diluted extracts (1mL) were appropriately mixed with 1mL of reagent (2% AlCl₃·6H₂O in methanol). After 10 min, the sample was measured at 430nm. Quercetin (Sigma) was used as standard, and results were reported as milligrams of quercetin equivalents (QE) per 100g-1 of FW. For each sample from (2) (3) and (4), analyses were performed in duplicate.

Results and Discussion

1. Antioxidant capacity and contents in selected vegetables

The antioxidant capacity of 4 vegetables was presented in Figure 1. The percentage of inhibition ranged from 98.72-98.96. However, there was no significant different (p≥0.05) in antioxidant capacity in selected vegetables.
Figure 1 Antioxidant capacity in selected vegetables

2. Antioxidant content and capacity during storage

2.1 Antioxidant capacity. The result from this study depicted that the storage did not have negative effect on the antioxidant capacity (Figure 2). At 4°C, the percentage of inhibition had increased in the next day after purchase. Then they were rather stable until day 6 and started to decrease in all vegetables accept lettuce. At room temperature, lettuce and mint visually spoiled after 2 days and the basils were spoiled after 4 days of storage. However, the results of both storage temperatures were similar. Similar observations were concluded on vegetables stored in the fridge or at room temperature (Jimenez et al., 2003, Kevers et al., 2007). Storage temperature and time affected antioxidant capacity of lettuce and Thai holy basil. Vegetables stored in refrigerator had higher antioxidant capacity than those stored at room temperature ($p<0.05$)

Figure 2 Antioxidant capacity (DPPH, %inhibition) of lettuce, Thai holy basil, sweet basil and mint stored at 4°C (●) and 28°C (■). An asterisk indicates significant difference from average value at time 0 by ANOVA ($p<0.05$)
2.2 Total phenolic compounds. Total phenolic compounds of lettuce stored in refrigerator decreased after few days, subsequently, they increased and quite stable during storage (Figure 3). However, total phenolic content transitory rose after day 2 then decreased after day 6 at refrigerated temperature. Vegetables stored at room temperature had visual spoilage after 2 days (lettuce and mint) and 4 days (basils). Storage temperature affected total phenolics of basils ($p<0.05$), both basils kept at room temperature developed higher phenolic compounds.

![Figure 3](image)

**Figure 3** Total phenolic content (mg of GAE 100g-1 of FW) of lettuce, Thai holy basil, sweet basil and mint stored at 4°C (♦) and 28°C (■). An asterisk indicates significant difference from average value at time 0 by ANOVA ($p<0.05$)

2.3 Total flavonoids. Total flavonoid content of lettuce was quite stable. There were no significant different in both temperature conditions from the time 0 to entire stored time (8 days). Similar conclusions can be drawn from other vegetables. Total flavonoid content was increased during 2 days of storage then lowered on day 4 during storage at 4°C. Lettuce and mint stored at room temperature had visual spoilage after 2 days and after 4 days for basils. Storage temperature affected total flavonoids of basils ($p<0.05$), both basils kept at room temperature had lower flavonoid content.
Figure 4 Total flavonoid content (mg of QE 100g⁻¹ of FW) of lettuce, Thai holy basil, sweet basil and mint stored at 4°C (♦) and 28°C (■). An asterisk indicates significant difference from average value at time 0 by ANOVA (p<0.05)

Conclusions

Antioxidant activity, total phenolics and flavonoids were affected by storage temperature. Vegetables stored in refrigerator had higher antioxidant capacity, total phenolics and flavonoids. In the first two days, antioxidant capacity and flavonoids increased, subsequently, they decreased in both storage temperatures. Total phenolic and flavonoid contents of lettuce were rather stable during storage. In general, it can be concluded that vegetables stored at room temperature visually spoiled before any significant antioxidant activity and flavonoid loss occurred. Vegetables storage did not show negative relationship to antioxidant capacity of vegetables.

Acknowledgments

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