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# Antioxidant capacities of vitamin E (tocopherols) in purple rice (*Oryza sativa* L. *indica*), perilla (*Perilla frutescens* L.) and sesame (*Sesamum indicum*)

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**Abstract** Vitamin E is widely utilized as an antioxidant source for functional food products. In our report, antioxidant activities of vitamin E (tocopherol) were evaluated in purple rice, white rice, perilla, white sesame and black sesame. Crude fat contents were extracted from rice bran, and from seeds of sesame and perilla. Tocopherols (gamma-γ, beta-β, alpha-α, and delta-δ forms) were examined. Antioxidant activities were determined from scavenging of free radicals (DPPH). Results showed DPPH in perilla (14.51 µmol TE/g fat), black sesame (24.06 µmol TE/g fat), white sesame (33.22 µmol TE/g sample) were higher than DPPH in purple rice (13.62 µmol TE/g fat) and white rice (8.65 and 14.51 µmol TE/g fat for RD6 and KDML 105 varieties, respectively). Crude fat content in black sesame seeds was higher than in perilla and white sesame. Only γ-tocopherol was detected in perilla (276.78 µg/g dw), black sesame (248.55 µg/g dw) and white sesame (147.75 µg/g dw). Crude fat content in white rice bran (KDML 105 and RD6) was significantly higher than crude fat in purple rice bran. Total tocopherols content in purple rice varieties ranged from 254.07 to 87.90 µg/g dw while the content in KDML 105 and RD6 were 118.57 µg/g dw and 124.49 µg/g dw, respectively. Genetic variation in tocopherol was detected in purple rice. Three forms of tocopherol were detected in rice bran; the γ form (86.73 µg/g dw) was highest in proportion, followed by the β form (28.95 µg/g dw) and α form (23.15 µg/g dw). δ-Tocopherol was not detected.

**Key words:** Crude fat; gamma-γ, beta-β, alpha-α and delta-δ tocopherol; Radical scavenging activity; DPPH.

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## Introduction

Antioxidants are considered to be important nutraceuticals on account of their many health benefits (Sharma & Bhat, 2009). Many compounds, including polyphenolics, flavonoids, phytic acid, γ-oryzanol and vitamin E, are beneficial for their antioxidant capacities (Kong & Lee, 2010). Natural vitamin E has demonstrated better antioxidant activity than synthetic vitamin E )Eitenmiller, 2004; Gast et al., 2005(, and also is more effective at lowering cholesterol (Cham et al., 1998). Vitamin E is a fat-soluble vitamin, derived as a set of eight related fraction of tocopherol and tocotrienol (each having alpha-α, beta-β, gamma-γ, and delta-δ forms). Wheat, corn and soybeans are rich in tocopherol, while as much as 70% of the vitamin E in barley, oats and rice bran is in the form of tocotrienol (Minhajuddi et al., 2005). Sesame seeds are rich in γ-tocopherol (Yamashita et al., 1995). Of the four forms of tocopherol, α-tocopherol has been the most thoroughly studied. As a lipid-soluble antioxidant, it is capable of protecting membranes from oxidation by reacting with lipid-radicals produced in acylglycerol peroxidation to remove the free radicals intermediates, and prevents the propagation reaction from continuing (Herrera & Barbas, 2001; Traber & Atkinson, 2007). However, many reports have claimed that γ- and δ-tocopherols are more powerful antioxidants than α-tocopherol (Li et al., 2011; Velasco et al., 2004; Wagner et al., 2004). γ -Tocopherrol is a nucleophile that may react with electrophilic mutagens, and is the most common vitamin E dietary supplement in North America (Brigelius-Flohé & Traber, 1999). γ-Tocopherrol can be found in corn oil, soybean oil, margarine, and salad dressing (Bieri and Evarts,.1974; Brigelius-Flohé & Traber, 1999). In Asia, sesame seeds have long been used for human consumption and in many functional food products. Perilla, another natural source of vitamin E, is also widely used in Asia: the leaves as a potherb, for medicine and food coloring; and oil from the seeds as fuel drying oil, cooking oil, and flavoring (Brenner, 1993). Purple rice is riches in γ-aminobutyric acid (GABA) (Karladee & Suriyong, 2012) and natural anthocyanin compounds such as cyanidin 3-glucoside and peonidin 3-glucoside (Chung and Woo, 2001; Mazza and Miniati, 1993) and also possesses anti-oxidant and anti-inflammatory activities (Hu et al., 2003; Iqbal et al., 2005).

As scavenging of DPPH free radicals is the basis of a common antioxidant assay (Sharma and Bhat, 2009), in our study, the antioxidant capacities (DPPH) of vitamin E (tocopherol) were investigated. Tocopherol content were quantified in crude fat extracted from the bran of purple rice and white rice varieties, and from the seeds of perilla and sesame (black and white varieties). Result would explain biofortification of the nutrient which would be of benefit subjected to the utilization of these crops in developing a standard commercial health product, and for other advanced purposes in agricultural.

## Materials and methods

### *Rice, perilla and sesame varieties*

Of 24 varieties of landrace purple rice, 23 were collected in Thailand and 1 Vietnam. White rice varieties KDML 105 and RD6 were the checks. Twelve landrace perilla, two varieties of black sesame and one variety of white sesame were collected in northern Thailand.

24 varieties of purple rice

| Varieties of purple rice | | |
|---|---|---|
| 1.Kum Phayao | 9.Kum 5153 | 17.Kum 88069 |
| 2.Kum Wiengsa | 10.Kum 7677 | 18.Kum 88083 |
| 3.Kum Na | 11.Kum 87061 | 19.Kum 99151 |
| 4.Kum Nan | 12.Kum 87090 | 20.Kum 11875 |
| 5.Kum Doi SaKet | 13.Kum 87046 | 21.Kum 19104 |
| 6.Kum Hoksalee | 14.Kum 89038 | 22.Kum 19959 |
| 7.Kum Doi Moseur | 15.Kum 89057 | 23.Kum Supan |
| 8.Kum Fang | 16.Kum 88061 | 24.Kum Vietnam |

12 varieties of perilla, 2 of black sesame and 1 of white sesame

| Perilla varieties | Perilla varieties | Black sesame varieties |
|---|---|---|
| 1.Thawangpha | 7. Nanoi 3 | 1.Chaiburi |
| 2. Wiengsa 1 | 8. Nanoi 4 | 2.Wiengsa |
| 3. Wiengsa 2 | 9. Nanoi 5 | |
| 4. Wiengsa 3 | 10. Nanoi 6 | White sesame vareity |
| 5. Nanoi 1 | 11. Nanoi 7 | 1.Chaiburi |
| 6. Nanoi 2 | 12. Nanoi 8 | |

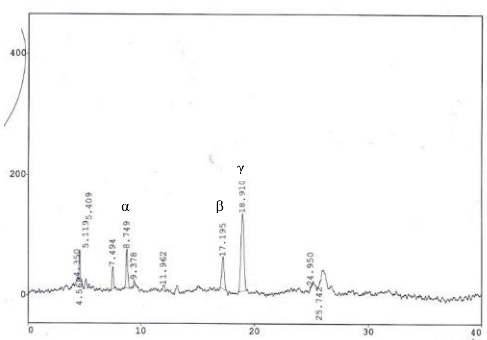
### *Analysis of crude fat content*

Crude fat content was determined by Soxhlet extraction according to method of Pathak et al. (1996). The extracted flask were filled with 2 or 3 tablets of boiling stones, dried at 105 °C for 1 h. cooled down in a desiccators and then weighed (X). The sample (2.5 g) was placed into the thimble which was then connected to a Soxhlet apparatus, and 250 ml of dichloromethane was added. The extraction was performed for 8 h. After boiling, the solvent was reduced to less than 5 ml. The extraction flasks were dried at 105$^{o}$C for 1 h., then cooled down to room temperature in a desiccators and weighed (Y). The percent of crude fat (CF) was calculated using the following equations:

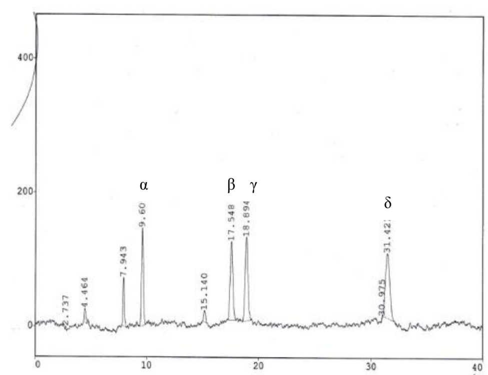
% CF = [(Y-X)/2.5)] x100

### *Quantification of tocopherols*

The α, β, γ and δ tocopherols were determined according to AOCS; method Ce 8-89 (AOCS ,1997). The HPLC system consisted of LC10AD pumps (Shimadzu, Japan), manure injector (Rheodyne), and RF10 AX fluorescence detectors. Chromatograms were recorded and processed using LC10 chromatography software. Normal phase chromatographic separation was performed using a Pinnacle® DB silica 5 µm column, 250 × 46 mm (Restek, Bellefonte PA, USA). The column was preceded by a 5 cm × 4.6 mm i.d. guard column packed with 40-µm silica. The mobile phase consisted of 99.5% hexane and 0.5% isopropanol at a flow rate of 1.0 mL/min. The fluorescence detector was set at 290 nm excitation and 330 emissions to monitor α, β, γ and δ tocopherols. Ten µL of the solution was injected into the HPLC system. All compounds were confirmed by chromatographic comparisons with their respective authentic standards. The α, β, γ and δ tocopherols in samples were identified by retention time and quantified by the external standard calibration curve (Fig. 1).




**Quantified solution in rice (Kum Doisaket)**




**Stock solution in rice**

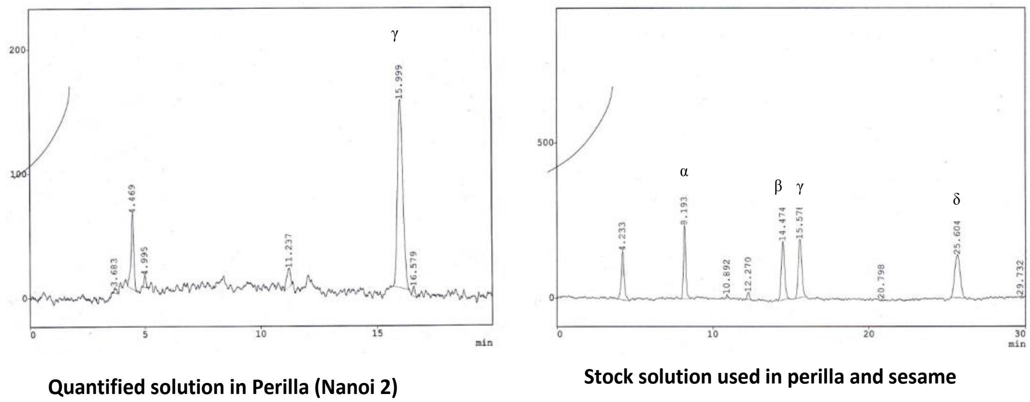



**Fig. 1.** Chromatograms of α, β, γ and δ-tocopherols (tocopherol standard stock solution)

### *Determination of DPPH radical scavenging activity*

The DPPH radical scavenging activity assay procedure followed the method of Brand-Williams et al. (1995). 1,1-Diphenyl-1-picrylhydrazyl radical (DPPH) solution was prepared in 100% ethanol. This solution was mixed with Tris buffer (pH 7.4) and 80 % ethanol in equal amounts (0.6:0.6:0.6 ml). Then, 0.6 ml of crude fat solution was added. After incubating in the dark at 25 $^{o}$C for 30 min, measurements were taken with a spectrophotometer (Shimadzu, Japan) at 525 nm. The crude fat solution and the Trolox solution were defined as the Trolox equivalent antioxidant activity. This was then converted into µmol of Trolox equivalent (TE) per g of crude fat.

## Results

### *Crude fat and vitamin E (tocopherol) contents in bran of purple rice and white rice*

The highest crude fat extracted from purple rice bran (var. Kum 1187) was 20.59 g%, which was significantly lower ($P\leq0.05$) than the crude fat extracted from white rice bran: KDML 105 (24.68 g%) and RD6 (23.00 g%).

In contrast to crude fat content, the total tocopherol content of purple rice varieties on average was higher than the total tocopherol content of KDML105 and RD6 (Fig.2). The differences in total tocopherol content among rice varieties were significant ($P\leq0.05$), with a mean of 137.82 µg/g dw. The highest content was 254.07 µg/g dw in the purple rice variety Kum Na. As many as 10 purple rice varieties exhibited total tocopherol contents higher ($P\leq0.05$) than KDML 105 (118.57 µg/g dw) and RD6 (124.49 µg/g dw) (Table 1). Three forms of tocopherol (α, β and γ) were detected 62.92% of the total was the γ form (86.73 µg/g dw(, a significantly higher proportion (*P*≤0.05) than the 20.28% of the β form (27.95 µg/g dw) and the 16.80% of the α form (23.15 µg/g dw).Delta (δ form) tocopherol was not detected in the present investigation (Table 1).

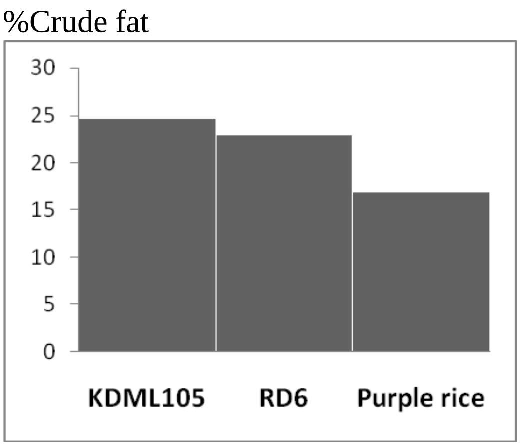
**Table 1.** Crude fat and vitamin E (Tocopherols) contents in rice bran

| **Purple rice genotype** | **Crude fat (% of seed mass)** | **Antioxidant content (µg/g dw)** | | | |
|---|---|---|---|---|---|
| | | **Tocopherols** | | | |
| | | α | β | γ | Total |
| Kum Phayao | 6.23 hijklm | 37.57 | 49.55 | 119.58 | 206.69 b |
| Kum Wiengsa | 19.58 cd | 22.75 | 24.10 | 55.59 | 102.44 lk |
| Kum Na | 17.44 fg | 59.88 | 50.12 | 144.07 | 254.07 a |
| Kum Nan | 17.2 fgh | 14.63 | 26.09 | 65.16 | 105.88 klm |
| Kum DoiSaKet | 19.82 cd | 26.68 | 30.84 | 83.82 | 141.38 efghi |
| Kum Hoksalee | 17.72 ef | 13.62 | 23.34 | 88.36 | 125.31 ghijkl |
| Kum DoiMoseur | 20.06 c | 20.59 | 29.43 | 57.18 | 107.21 jklm |
| Kum Fang | 15.75 klmn | 22.73 | 39.22 | 87.05 | 149.0 defg |
| Kum 5153 | 16.16 hijklm | 24.85 | 29.32 | 117.70 | 171.86 cd |
| Kum 7677 | 14.10 o | 16.98 | 36.54 | 83.67 | 137.19 efghi |
| Kum 87061 | 14.81 no | 20.40 | 29.53 | 93.67 | 142.96 efgh |
| Kum 87090 | 18.85 de | 29.84 | 26.82 | 105.23 | 161.89 de |
| Kum 87046 | 16.08 hijklm | 19.63 | 25.15 | 84.27 | 129.05 fghijk |
| Kum 89038 | 15.57 lmn | 20.19 | 25.28 | 92.99 | 138.47 efght |
| Kum 89057 | 14.36 o | 17.94 | 28.70 | 69.74 | 116.38 ijkl |
| Kum 88061 | 16.30 hijklm | 17.49 | 30.88 | 83.05 | 131.42 fghij |
| Kum 88069 | 16.72 hijklm | 31.63 | 24.02 | 75.35 | 130.99 fghij |
| Kum 88083 | 16.05 ijklm | 15.08 | 19.62 | 72.57 | 107.27 jklm |
| Kum 99151 | 16.42 ghijkl | 20.63 | 24.28 | 63.61 | 108.53 jklm |
| Kum 11875 | 20.59 c | 9.67 | 19.18 | 59.05 | 87.90 m |
| Kum 19104 | 16.96 fghij | 37.44 | 48.87 | 106.03 | 192.33 bc |
| Kum 19959 | 17.09 fghi | 18.62 | 22.86 | 69.62 | 111.10 jklm |
| Kum Supan | 15.19 mno | 18.17 | 32.21 | 76.67 | 127.06 ghijkl |
| Kum Vietnam | 15.86 jklmn | 27.70 | 29.13 | 97.11 | 153.93 def |
| (White rice) | | | | | |
| RD6 | 23.00 b | 19.01 | 0.0 | 105.48 | 124.49 ghijkl |
| KDML105 | 24.68 a | 18.13 | 1.54 | 98.90 | 118.57 hijkl |

### *Crude fat and vitamin E (tocopherol) contents in seeds of perilla, black sesame and white sesame*

Crude fat contents extracted from seeds of perilla, black sesame and white sesame differed significantly (*P*≤0.05) (Table 2). On average, crude fat content in black sesame was higher than in perilla and white sesame (Fig. 2).

The higher content, 50g% was in black sesame (var. Chaiburi). The maximum crude fat content in perilla was 44.26 g% (var. Wiengsa 3), and in white sesame 33.77 g% (var. Chaiburi). As these are oil seed crops, their average crude fat content was much higher than that extracted from rice bran.



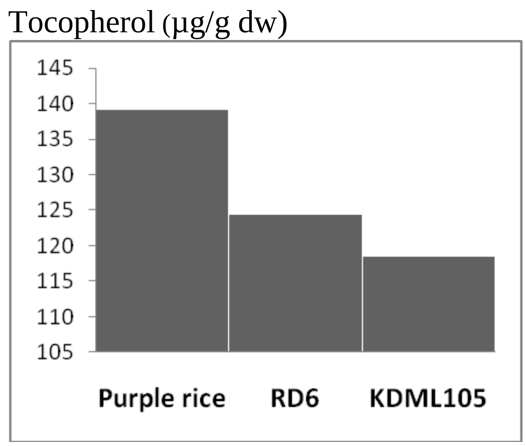




**Fig. 2.** Comparison in crude fat and vitamin E (total tocopherol) between the white rice improved varieties (KDML 105 and RD6) and mean of the purple rice landrace

**Table 2.** Crude fat and vitamin E (tocopherols) contents in sesame and perilla seeds

| Species | Genotype | Crude fat (% of seed mass) | γ-Tocopherols (µg/g dw) |
|---|---|---|---|
| Perilla | | | |
| | Thawangpha | 36.18 d | 241.23 bc |
| | Wiengsa 1 | 31.93 g | 312.00 a |
| | Wiengsa 2 | 32.01 fg | 292.18 ab |
| | Wiengsa 3 | 44.26 b | 325.66 a |
| | Nanoi 1 | 34.05 ef | 278.70 ab |
| | Nanoi 2 | 33.71 efg | 322.70 a |
| | Nanoi 3 | 32.00 fg | 283.41 ab |
| | Nanoi 4 | 33.76 efg | 269.26 ab |
| | Nanoi 5 | 33.13 efg | 316.68 a |
| | Nanoi 6 | 34.13 de | 242.56 bc |
| | Nanoi 7 | 34.25 de | 235.70 bc |
| | Nanoi 8 | 34.44 de | 201.29 cd |
| Black sesame | | | |
| | Chaiburi | 50.26 a | 300.37 a |
| | Wiengsa | 40.26 c | 196.73 cd |
| White sesame | | | |
| | Chaiburi | 33.77 efg | 147.75 d |

Total tocopherol contents in perilla, black sesame and white sesame were significantly different (*P*≤0.05). The highest content in perilla was found in the varieties Wiengsa 3, Nanoi 2, Nanoi 5 and Wiengsa 1(325.66, 322.70, 316.68 and 312.00 µg/g dw, respectively). For black sesame, var. Chaiburi had the highest total tocopherol content (300.37 µg/g dw) (Table 2). Unlike most oil seeds; which mainly accumulate γ-tocopherol and δ-tocopherol (Padley et al. 1994), only γ-tocopherol was detected in perilla. Perilla varieties showed the highest content of γ-tocopherol (Fig.3). Furthermore, the mean total γ-tocopherol content (264.41 µg/g dw) found in perilla, black sesame and white sesame was higher than the average total content (137.82 µg/g dw) of the three forms detected in the rice varieties (Tables 1 and 2).

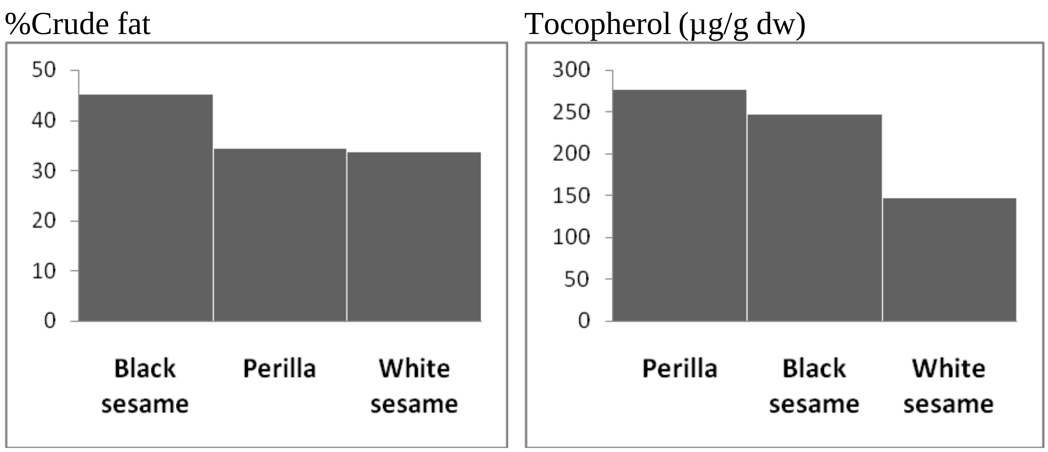



**Fig. 3.** Comparison in crude fat and vitamin E (total tocopherol) between white sesame and mean of black sesame and mean of perilla landrace varieties

### *Antioxidant capacity of vitamin E (tocopherol)*

Antioxidant capacity of tocopherol (vitamin E), measured as free radical scavenging activity (DPPH) (Trolox value: µmol TE/g sample) showed that the average DPPH of vitamin E detected in purple rice bran was 13.62 µmol TE/g fat, and varied from 5.68 to 21.59 µmol TE/g fat. The white rice DPPH (14.54 µmol TE/g fat for KDML 105, and 8.65 µmol TE/g fat for RD6) were lower than that of purple rice. The highest DPPH among rice varieties was in purple rice variety Kum 5153 (2.55 µmol TE/g fat).

Tocopherol DPPH determined in the white sesame variety was 33.22 µmol TE/g fat higher than in the two black sesame varieties (28.24 and 19.89 µmol TE/g fat) and perilla (14.51 µmol TE/g fat on average). Genetic variation of DPPH in perilla varied from 36.68 to 11.76 µmol TE/g fat, with a mean of 14.51 µmol TE/g fat; var. Wiengsa 1 exhibited the highest DPPH (36.68 µmol

TE/g fat). Six varieties of perilla showed antioxidant capacities superior to that of black sesame.

The antioxidant capacities determined in the rice varieties were lower than those in perilla, black sesame and white sesame (Table 3.). Genetic variation DPPH was identified in both perilla (SD = 0.80) and rice (SD = 0.91) Correlations among α-, β-, and γ-tocopherol in rice were significantly positive, but the three forms had no relationship to crude fat and DPPH. Crude fat also had no relationship to DPPH (Table 4). Similar to rice, in perilla and sesame the relationships among γ-tocopherol, crude fat and DPPH were not significant (Table 5). This is in accordance with Velasco et al. (2002), who also found that in sunflowers the tocopherol content was not correlated with seed oil or seed yield.

**Table 3.** DPPH (Trolox: μmol TE / g sample) of rice bran, perilla and sesame seed

| **Species** | **Genotype** | **Trolox (μmol TE / g fat)** |
|---|---|---|
| Purple rice | Kum Phayao | 14.77 |
| | Kum Wiengsa | 5.68 |
| | Kum Na | 15.86 |
| | Kum Nan | 5.65 |
| | Kum DoiSaKet | 16.37 |
| | Kum Hoksalee | 8.17 |
| | Kum DoiMoseur | 16.23 |
| | Kum Fang | 17.57 |
| | Kum 5153 | 21.26 |
| | Kum 7677 | 11.71 |
| | Kum 87061 | 13.25 |
| | Kum 87090 | 21.59 |
| | Kum 87046 | 13.96 |
| | Kum 89038 | 11.18 |
| | Kum 89057 | 18.23 |
| | Kum 88061 | 11.95 |
| | Kum 88069 | 17.39 |
| | Kum 88083 | 6.44 |
| | Kum 99151 | 7.25 |
| | Kum 11875 | 6.78 |
| | Kum 19104 | 15.10 |
| | Kum 19959 | 14.72 |
| | Kum Supan | 12.24 |
| | Kum Vietnam | 23.37 |
| White rice | RD 6 | 8.65 |
| | KDML105 | 14.54 |
| Perilla | Thawangpha | 29.86 |
| | Wiengsa 1 | 36.68 |

| | | |
|---|---|---|
| | Wiengsa 2 | 31.16 |
| | Wiengsa 3 | 30.92 |
| | Nanoi 1 | 23.29 |
| | Nanoi 2 | 30.12 |
| | Nanoi 3 | 32.65 |
| | Nanoi 4 | 21.17 |
| | Nanoi 5 | 11.76 |
| | Nanoi 6 | 15.45 |
| | Nanoi 7 | 16.11 |
| | Nanoi 8 | 15.01 |
| Black sesame | Chaiburi | 19.89 |
| | Wiengsa | 28.24 |
| White sesame | Chaiburi | 33.22 |

TE = Trolox Equivalent

**Table 4.** Correlation between antioxidant fraction, DPPH and crude fat in rice

| Variable | Crude fat | α-tocopherol | β-tocopherol | γ-tocopherol | DPPH |
|---|---|---|---|---|---|
| | | | Correlation coefficient | | |
| Crude fat | | 0.002ns | -0.0438ns | -0.0522ns | -0.0232ns |
| α-tocopherol | | | 0.6025** | 0.7147** | 0.1960ns |
| β-tocopherol | | | | 0.3385* | 0.1005ns |
| γ-tocopherol | | | | | 0.2302ns |

Significance of the correlation coefficients at the 5 % or 1 % indicated by * or **, respectively

**Table 5.** Correlation between antioxidant fraction, DPPH and crude fat in perilla and sesame

| Variable | Crude fat | γ-tocopherol | DPPH |
|---|---|---|---|
| Crude fat | | 0.0215ns | 0.0233ns |
| γ-tocopherol | | | 0.2269ns |

## Discussion

Vitamin E is a fat-soluble antioxidant compound that eliminates reactive oxygen species, such as lipid peroxide and superoxide anion radicals, and also lowers cholesterol content (Herrera&Barbas.2001; Nam *et al*., 2008; Packer et al., 2001). Wheat germ oil is rich in vitamin E. Rice bran oil has also been reviewed for its active constituents, which were found to improve blood cholesterol and triglyceride concentrations (Cicero & Gaddi, 2001). Significant medicinal components responsible for the activity of rice bran oil are **γ-**oryzanol, which composes around 2% of its crude fat content, and a relatively high fraction of tocopherol and tocotrienol which are the constituents of vitamin E (Orthoefer, 2005).

In our results, crude fat content in white rice bran RD6 (23.00%) and KDML 105 (24.68%) were comparatively higher than the content in purple rice bran (16.75% average). Boonsit et al. (2010) also reported a difference in crude fat content of KDML unpolished brown rice (3.01%) and purple rice unpolished brown rice (2.52%). Various forms of vitamin E (α-, β-, and γ-tocopherol) in purple rice bran oil confirmed its association with genetic variation, and therefore it is a significant resource of genetic diversity for a breeding program to improve rice bran oil. Genetic variation of tocopherol has also been identified in soybean (Carrão-Panizzi & Erhan, 2007) and in *Brassica napus* L. (Goffman & Becker, 2002). The higher tocopherol content found in purple rice bran oil compared with that of white rice signified that purple rice bran is a better natural source of vitamin E. The significantly higher content of the γ-form compared with the β and α forms differed slightly the results of Kong & Lee (2010), who also studied black rice bran of Korean varieties; they found a higher content of the α form. However, races of rice may have played a role in the differences in tocopherol content; Kong & Lee (2010), used japonica rice varieties, while in the present study *indica* was used. Furthermore, genotype and environment have a significant effect on tocopherol content and composition (Carrão-Panizzi & Erhan, 2002; Velasco et al., 2002). For α-, β- and tocopherol contents, the effect of genotypes was shown to be larger than the environmental effect, whereas the environment had a greater effect on γ-tocopherol; but, genotype × environment interaction was significant for α-and γ- and total tocopherol contents (Velasco et al., 2002).

A high content of crude fat and γ-tocopherol has been detected in many varieties of black sesame and perilla. The suggestion could be made here that sesame varieties with extraordinarily high contents of crude fat and vitamin E would therefore be a better potential source for manufacturing sesame cooking oil with added antioxidant benefits. Unlike most oil seeds, which mainly accumulate γ-tocopherol and δ-tocopherol (Padley et al., 1994), in the present study only γ-tocopherol was identified in perilla and the sesame varieties. However, the high content of γ-tocopherol identified could compensate for the absence of δ-tocopherol. Fukuda et al. (1986) claim that the main antioxidative constituent in fresh sesame oil extracted from roasted sesame seed is γ-tocopherol. Furthermore, both γ- and δ-tocopherol are effective antioxidants; for example, they have been shown to inhibit colon and lung carcinogenesis and the growth of transplanted lung-cancer cells (Li et al. (2011). Yamashita et al. (1992) also mentioned that the major tocopherol in sesame seed is γ-tocopherol.

α-Tocopherol is the predominant form of vitamin E dietary supplement (in America, for instance), while γ-tocopherol has been largely neglected. But recent studies have suggested that γ-tocophero may be important to human health and advice as vitamin E supplement (Dietrich et al., 2006; Jiang et al., 2001). Tocopherol appears to be a more effective trap for lipophilic electrophiles than α-tocopherol, it is also well absorbed, and accumulates to a significant degree in some human tissues (Jiang et al., 2001). The present findings show that γ-tocopherol is a major form of vitamin E in rice bran and sesame seed crude fat. These crops therefore warrant utilization, together with other major crops, as a supplementary source of vitamin E in the diet. Perilla, nutritious and aromatic could prove to be another interesting eligible oil for a number of purposes: for its 'ancient wisdom' medicinal benefits in countries where perilla is a familiar herb; in food flavorings and also as an industrial crop for vitamin E antioxidant production and cosmetics (e.g. anti-aging).

Scavenging of free radicals (DPPH) proved that perilla expressed on average a better antioxidant capacity than the two sesame and the two white rice varieties. The higher tocopherol content in perilla was consequently the reason for its higher antioxidant activity. Having high **γ**-tocopherol and DPPH, perilla could prove to be a better source of cooking oil among the crops of the order Lamiales.

Choi et al. (2007) claimed that pigmented rice has a greater antioxidant capacity than white rice. We also found that purple rice varieties exhibited a higher DPPH than the white rice varieties. Purple rice varieties high in α-, β- and γ-tocopherol content should be important in future breeding efforts toward improving high-vitamin-E rice varieties. As α- and γ-tocopherols are the most efficient of the four forms (although α-tocopherol has the lowest antioxidant effect in vitro) (Pongracz et al., 1995), the ratio of the content of α- to γ-tocopherols could be used to better describe the composition of the rice bran. Moreover, purple rice bran which apart from vitamin E also contains high levels of other antioxidant compounds such as **γ** oryzanol and C3G should be considered as a better material for producing a functional rice product with high antioxidant capacities, and also as a potential source of a natural vitamin E to supplement the countries where rice is consumed as a staple food. Similar to vitamin E, the genetic variation found in DPPH values signified that the trait is under genetic control, and that genotypic improvement through breeding is possible.

Since α-, β- and γ-tocopherol derivatives (determined by the number of methyl groups on the chromanol ring) differ in the methylation of the tocol head group (Pongracz et al., 1995), this may explain the strong significant coefficients o positive correlation among the three forms found in our results. Improvements to enhance the content of α-, β- and γ-tocopherol could concentrate only on one of these forms. Crude fat and vitamin E had no correlation with DPPH activity, indicating the possibility of selecting for this trait without affecting the performance of the genotype.

In conclusion, the present research as identified γ-tocopherol as a major form vitamin E in rice, perilla and sesame. In rice varieties α β and γ forms were indentified, while in perilla and sesame only the γ form was identified. The δ form was not detected in rice, perilla and sesame.

Black sesame seed, with potentially high crude fat and vitamin E contents, is suggested for manufacturing sesame oil production. Perilla oil, which is high in γ-tocopherol possibly should be the determination in tocopherol composition by combining it with the oil of other oil seed crops which are rice in α- and δ- tocopherol.

Purple rice varieties with strong radical scavenging activity can be considered as good sources of natural antioxidants for medicinal and commercial uses, particularly in communities where rice is a stable food. Furthermore, these varieties could be potential genetic sources for breeding programs aimed towards enhancing the quantity and quality of rice bran oil. Purple rice bran, which contains three forms of tocopherol, could be used for its medicinal benefits in a “mixed” product containing other tocopherols a part form a single α-tocopherol. Evidence of the immunoregulatory properties of purple rice bran suggests that further attention should be directed toward purple rice research. Estimates of the genetic variance and heritability of tocopherol accumulation would provide useful guidelines for future improvement of breeding programs.

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## References

AOCS (1997). Official methods and recommended practices of the AOCS 5th ed. Washington, DC: American Oil Chemists’ Society Press.

Bieri, J.G. and Evarts, R.P. (1974). Gamma-tocopherol: metabolism, biological activity and significance in human vitamin E nutrition. American Journal of Clinical Nutrition 27:980–986.

Boonsit, P., Pongpiachan, P., Julsrijival, S. and Karladee, D. (2010). Gamma oryzanol content in glutinous purple rice landrace varieties. CMU Journal of Natural Science 9:151–157.

Brand-Williams, W., Cuvelier, M.E. and Berset, C. (1995). Use of a free radical method to evaluate antioxidant capacity. LWT – Food Science and Technology 28:25–30.

Brenner, D.M. (1993). Perilla: botany, uses and genetic resources. In J. Janick & J. E. Simon, New crops New York: Wiley. pp. 322–328.

Brigelius-Flohé, R. and Trab, M.G. (1999). Vitamin E function and metabolism. The FASEB Journal 13:1145–1155.
Carrão-Panizzi, M.C. and Erhan, S.Z. (2007). Environmental and genetic variation of soybean tocopherol content under Brazilian growing conditions. Journal of the American Oil Chemists Society 84:921–928.
Cham, B.E., Smith, J.L. and Colquhoun, D.M. (1998). Correlations between, vitamin E, and vitamin K in serum paradoxical relationships to established epidemiological risk factors for cardiovascular disease. Clinical chemistry 44:1753–1758.
Choi, Y., Jeong, H.J. and Lee, J. (2007). Antioxidant activity of methanolic extracts from some grains consumed in Korea. Food Chemistry 103:130–138.
Chung, H.S. and Woo, W.S. (2001). A quinolone alkaloid with antioxidant activity from the aleurone layer of anthocyanin-pigmented rice. Journal of Natural Products 64:1579–1580.
Cicero, A.F. and Gaddi, A. (2001). Rice bran oil and gamma oryzanol in the treatment of hyperlipoproteinaemias and other conditions. Phytotherapy Res 15:277–286.
Dietrich, M., Traber, M.G., Jacques, P.F., Cross, C.E., Hu, Y. and Block, G. (2008). Does **γ-**tocopherol play a role in the primary prevention of heart disease and cancer? A review. Journal of the American College of Nutrition 25:292–295.
Fukuda, Y., Nagata, M., Osawa, T. and Namiki, M. (1986). Chemical aspects of antioxidant activity of roasted sesame seed oil, and the effect of using the oil for flying. Agricultural and Biological Chemistry 50:857–862.
Goffman, F.D. and Becker, H.C. (2002). Genetic variation of tocopherol content in a germplasm collection of *Brassica napus* L. *Euphytica* 125:189–196.
Herrera, E. and Barbas, C. (2001). Vitamin E: action, metabolism and perspectives. Journal of Physiology and Biochemistry 57:43–50.
Hu, C., Zawistowski, J., Ling, W. and Kitts, D.D. (2003). Black rice (*Oryza sativa* L. *indica*) pigmented fraction suppresses both reactive oxygen species and nitric oxide in chemical and biological model systems. Journal of Agricultural and food Chemistry 51:5271–5277.
Iqbal, S., Bhanger, M.I. and Anwar, F. (2005). Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. Food Chemistry 93:265–272.
Jiang, Q., Chirsten, S., Shigenaga, M.K. and Ames, B.N. (2001). **γ-**Tocopherol the major form of vitamin E in the US diet, deserves more attention. American Journal of Clinical Nutrition 74:714–722.
Kannel, W.B. (1988). Cholesterol and risk of coronary heart disease and mortality in men. Clinical Chemistry 34(8):53–59.
Karladee, D. and Suriyong, S. (2012). **γ-**Aminoburyric acid (GABA) content in different varieties of brown rice during germination. Science Asia 38:13–17.
Kong, S. and Lee, J. (2010). Antioxidants in milling fractions of black rice cultivars. Food Chemistry 120:278–281.
Li, G.X., Lee, M.J., Liu, A.B., Yang, Z., Lin, Y., Shih, W.J. and Yang, C.S. (2011). δ-tocopherol is more active than α- or γ-tocopherol in inhibiting lung tumorigenesis *in vivo*. Cancer Prevention Research 4404–413.
Mazza, G. and Miniati, E. (1993). Introduction. *Anthocyanins in fruits, vegetables, and grains*. Boca Raton, FL. CRC Press.
Minhajuddin, M., Beg, Z.H. and Iqbal, J. (2005). Hypolipidemic and antioxidant properties of tocotrienol rich fraction isolated from rice bran oil in experimentally induced hyperlipidemic rats. Food and Chmical Toxicology 43:747–753.
Nam, Y.J., Nam, S.H. and Kang, M.Y. (2008). Cholesterol-lowering efficacy of unrefined bran oil from the pigmented black rice (*Oryza sativa* L. cv. Suwon 415). in hypercholesterolemic rats. Food Science and Biotechnology 17:457–463.

Orthoefer, F.T. (2005). Rice bran oil. In F. Shahidi, *Bailey's industrial oil and fat products*, 6$^{th}$ ed. Chapter 2.10: p. 465). New York: John Wiley & Sons.

Packer, L., Weber, S.U. and Rimbach, G. (2001). Molecular aspects of alpha-tocotrienol antioxidant action and cell signaling. Journal of Nutrition 131:3698–3738.

Padley, F.B., Gunstone, F.B. and Harwood, J.L. (1994). Occurrence and characteristics of oils and fats. In F. D. Gunstone, J. L. Harwood, & F. B. Padley, The lipid handbook, London: Chapman & Hall. pp. 47–223.

Pathak, N.N., Kamra, D.N., Agarwal, N. and Jakhmola, R.C. (1996). Analytical techniques in animal nutrition research. Luknow, India: International Book Distributing Co.

Pongracz, G., Weiser, H. and Matzinger, D. (1995). Tocopherol – antioxidantien der nature. Fat Science Technology 97:90–104.

Sharma, O.P. and Bhat, T.K. (2009). DPPH antioxidant assay revisited. Food Chemistry 113:1202–1205.

Traber, M.G. and Atkinson, J. (2007). Vitamin E, antioxidant and nothing more. Free Radical Biology and Medicine 43:4–15.

Velasco, L., Fernández-Martínez, J.M., García-Ruíz, R. and Domínguez, J. (2002). Genetic and environmental variation for tocopherol content and composition in sunflower commercial hybrids. Journal of Agricultural Science 139:425–429.

Velasco, L., Pérez-Vich, B. and Fernández-Martínez, J.M. (2004). Development of sunflower germplasm with high delta-tocopherol content. *Helia* 27:99–106.

Wagner, K.H., Isnardy, B. and Elmadfa, I. (2004). γ- and δ-Tocopherols are more effective than α-tocopherol on the autoxidation of a 10% rapeseed oil triacylglycerol-in-water emulsion with and without a radical initiator. European Journal of Lipid Science and Technology 106:44–51.

Yamashita, K., Iizuka, Y., Imai, T. and Namiki, M. (1995). Sesame seed and its lignans produce marked enhancement of vitamin E activity in rats fed a low alpha-tocopherol diets. *Lipids* 30:1019–1028.

Yamashita, K., Nohara, Y., Katayama, K. and Namiki, M. (1992). Sesame seed lignans and gamma-tocopherol act synergistically to produce vitamin E activity in rats. Journal of Nutrition 122:2440–2446.

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