

## Original Article

# Enhancing the oxidative stability of rice crackers by addition of the ethanolic extract of phytochemicals from *Cratoxylum formosum* Dyer

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*Cratoxylum formosum* Dyer is consumed throughout the year as food and medicine in Thailand. It contains large amounts of chlorogenic acid and quinic acid derivatives. The antioxidative activity of the extract was studied in refined soybean oil coating on rice crackers without any seasoning. They were stored in accelerated oxidation conditions at 40°C, 80% relative humidity (RH) in the dark for 18 days. The oxidative state of each sample was monitored by analyzing of the peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) as well as by odor analysis by quantitative descriptive analysis (QDA). The *C. formosum* extract was more effective than  $\alpha$ -tocopherol due to metal ions present in the crackers, which resulted in  $\alpha$ -tocopherol being less effective as an antioxidant. Sensory odor attributes of rice crackers were related more closely to TBARS than to PV values by linear regression analysis. The present study indicated that *C. formosum* extract was a promising source of a natural food antioxidant and was effective in inhibiting lipid oxidation in rice crackers.

**Key Words:** phytochemicals, Antioxidant, Thai plant, oxidative stability, rice, cracker, quantitative descriptive analysis

## Introduction

Rice cracker is one of the numerous Japanese snack foods made from rice. The crackers are susceptible to lipid oxidation.<sup>1</sup> Lipid oxidation is a major cause of food deterioration, but limited success has been achieved in preventing this group of reactions from occurring in dried foods.

Iron in cereals is known to catalyze the development of oxidative rancidity in polyunsaturated vegetable oils used with cereal food products. Oxidation of lipids not only produces rancid odors and flavors, but decreases the nutritional quality and safety by the formation of secondary products in foods.<sup>2</sup>

Conventional control methods are often ineffective or not practical for dried foods. Many manufacturers resort to more frequent rotation of retail stock to prevent consumers from purchasing rancid products.<sup>3</sup> Consumers generally perceive natural antioxidants as being better than synthetic additives. Phenolic compounds are one of the most important groups of natural antioxidants. Natural phenolic compounds added to crackers are widely used to retard lipid oxidation. The ways to add these compounds into products include direct addition of plants or extracts as ingredients.<sup>4,5</sup>

*Cratoxylum formosum* Dyer is an indigenous Thai plant which is traditionally consumed as fresh shoots and young leaves. The plant tastes sour and a little astringent due to phenolic components. Health benefits of *C. formosum* include applying the leaf to the skin to heal a wound and

consuming the flower to remedy a cough. *C. formosum* contains a concentration of chlorogenic acid (5-O-caffeoylquinic acid) at 60% of the extract.<sup>6</sup> The minor components present were identified as dicaffeoylquinic acid and ferulic acid derivatives. Chlorogenic acid is widely recognized to be active by free radical scavenging<sup>7</sup> and it inhibits peroxidation of linoleic acid,<sup>8</sup> and acts as a cancer chemopreventive agent.<sup>9</sup> A previous study demonstrated that the high radical scavenging activity of extracts from *C. formosum* was due to the high content of phenolic compounds especially chlorogenic acid.<sup>6</sup> Therefore, this report was designed to study the antioxidant activity of the plant extract and dried plant powder in rice crackers from glutinous rice.

## Materials and methods

### Chemicals

$\alpha$ -Tocopherol and butanol were purchased from Fluka Co. (Buchs, Switzerland). Ethanol and 2-propanol were purchased from Sigma (Milwaukee, USA). BHT was purchased from BDH (Poole, United Kingdom).

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The other chemicals and solvents used in this experiment were analytical grade purchased from Sigma-Aldrich Company Ltd. (Gillingham, UK).

### Plant

One batch of *C. formosum* (*Cratoxylum formosum* Dyer.) leaves was purchased from market place at Saraburi province during harvest season in April 2005. Immediately upon arrival after harvesting, *C. formosum* leaves were cleaned and sound leaves were selected to develop the sample preparation for further experiments.

### Preparation of plant extract

The fresh plant leaves (80 g) were blended for 1 min with ethanol at  $-20^{\circ}\text{C}$  and the containers were then flushed with nitrogen and shaken for 4.5 hours in the dark at  $25^{\circ}\text{C}$ . The supernatant, after filtration through cheesecloth and Whatman No 4 filter paper, was evaporated under vacuum. Sample was dried in a freeze dryer and stored in aluminum foil after flushing with nitrogen at  $-20^{\circ}\text{C}$ . The acute toxicity of *C. formosum* leaf extract was reported in a previous study ( $>32\text{ g}\cdot\text{kg}^{-1}$ ).<sup>6</sup>

### Preparation of dried plant powder

*C. formosum* leaves were dried by air drying at room temperature  $25^{\circ}\text{C}$  for 12 h with air velocity about  $3.2\text{ m}\cdot\text{s}^{-1}$  provided by an electric fan. Dried *C. formosum* leaves were blended to be a powder before mixing in oil to coat onto a rice cracker.

### Rice cracker from glutinous rice

The baked rice cracker used in the study was donated by manufacturer in Thailand. In this study, we used fresh rice cracker product to study the effect of antioxidant in a soybean oil coating on the rice product. The production process for rice crackers is complicated. Briefly, milled rice is washed and soaked for 16–20 hr, then drained and crushed by rollers into a fine powder. After steaming for 15–30 min, the resulting rice dough or cake is kneaded and cooled to  $2\text{--}5^{\circ}\text{C}$  for two to three days for hardening. The hard cake is cut, dried, and baked to produce rice crackers. The nutrition values obtained from the manufacturer of the product are shown in Table 1.

**Table 1** Chemical composition of studied rice crackers

	Nutrient composition g per 100 g edible portion (db)
Fat	$1.8 \pm 0.3$
Cholesterol	$0.0 \pm 0.0$
Saturated fat	$0.0 \pm 0.0$
Total carbohydrate	$79.3 \pm 0.2$
Protein	$10.2 \pm 0.2$
Iron (mg)	$4.3 \pm 0.1$

Note: db means dry weight basis of the sample. Results are expressed as mean  $\pm$  SD (n=3).

### Preparation of rice cracker coated with antioxidants

Antioxidants were added to refined soybean oil, which contained natural tocopherols and no added antioxidants, in the following quantities:  $100\text{ mg}\cdot\text{kg}^{-1}$  of crude *C. formosum* extract,  $\alpha$ -tocopherol, and BHT. Oil containing  $2.44\text{ g}\cdot\text{kg}^{-1}$  dried *C. formosum* leaf powder, giving the same concentration of *C. formosum* extract as  $100\text{ mg}\cdot\text{kg}^{-1}$  added extract, was included for comparison.

The rice cracker was coated with 10% oil (W/W) containing antioxidants without any seasoning. The control was rice cracker coated with refined soybean oil without added antioxidant. The cracker was coated with oil without subsequent drying because the cracker was able to absorb the oil. After that, 3 pieces ( $\sim 10\text{ g}$ ) of the rice cracker were packed into plastic containers covered with OPP/PE/LLDPE film,  $80\text{ }\mu\text{m}$  thickness with packaging size  $11 \times 13\text{ cm}^2$ . The plastic film contains three layers of oriented polypropylene (OPP), polyethylene (PE) and linear low density polyethylene (LLDPE). The samples were stored in an accelerated oxidation condition at  $40^{\circ}\text{C}$ , 80% relative humidity in the dark for 18 days. Samples were removed every 3 days for analysis. The oxidative state of each sample was monitored by analysis of the peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) as well as by quantitative descriptive analysis (QDA). The water activity was analyzed every 6 days.

### Determination of peroxide and TBARS values

Rice cracker samples (200 g) were blended for 1 min and extracted with hexane (400 mL) three times by gently swirling for about 1 min each time. The combined hexane of the three extractions was evaporated from the oil extracted from the rice cracker samples by using a rotary evaporator at 100 mm Hg pressure and  $40^{\circ}\text{C}$ . The peroxide value was determined using a method based on AOCS official method Cd 8 – 53.<sup>10</sup> The TBARS value was analyzed according to McDonald and Hultin (1987).<sup>11</sup>

### Evaluation by QDA

A total of 10 panellists participated in the study. Prior to being allowed to participate in the study, panellists were screened by an odor recognition test (ASTM manual series: MNL 13-Manual on descriptive analysis testing for sensory evaluation) and trained to assess samples on an anchor scale. In addition, those who passed the screening test had to commit themselves to the full time required for the study. They were required to attend one session per day. Each session was one hour to two hours in length, at a specific time. The sensory quality was assessed by the odor since this avoided any possible concerns about the toxicity of the antioxidants, and panellists were trained with reference materials to evaluate the intensity of the attributes.

The first week of the study was devoted to panel training. One training session was conducted approximately 30 min in length. The first part of the panel training was generation of the terms to describe the odor related to the quality of the rice cracker samples. Approximately 10 grams of each sample was presented in 60 mL odor-free plastic cups with lids and labelled with three digit random numbers and equilibrated at  $30^{\circ}\text{C}$  for a minimum 30 min

prior to serving. Samples presented were fresh rice crackers, and 7 as well as 14 days stored crackers. At the end of term generation for odor attributes, a total of 6 terms were collected. The next part of the panel training consisted of reduction in the number of terms so that only the terms that were significantly different were used to assess the samples. After the sessions were conducted, 3 attributes were selected from the results. These terms consisted of baked rice odor, vegetable oil odor and rancid odor. The consensus definitions and references were determined from the panellists. The final terms used to describe the attributes and their definitions are listed in Table 2. The reference samples used in this experiment are listed in Table 3, and once the lists of terms and reference samples were finalized, panellists started rating the intensity of the reference samples for each attribute. There were three references for each attribute. To obtain a well represented average for the intensity of the reference sample values, the references were rated a total of twice. The panellists also practiced rating the intensity using a 15-point scale with increments of 0.5, each with anchors of 0 (none) and 15 (very much) and were also trained by using control sample which was the cracker stored at 40°C for 10 days. The PV and TBARS values of the control sample were  $19.25 \pm 0.28$  meq peroxide·kg<sup>-1</sup> oil and  $9.37 \pm 0.05$  mmol·kg<sup>-1</sup> oil, respectively.

**Table 2.** The attributes and their definitions used in QDA of the different antioxidant samples

Attribute	Definition
Baked rice odor	The amount of baked odor identified in the sample.
Rancid odor	The amount of odor associated with old or oxidized oil.
Vegetable oil odor	The amount of typical vegetable oil odor

**Table 3.** The reference samples used throughout QDA experiments

Attribute	Reference samples	Quantity (g)	Quality <sup>§</sup>	Intensity
Baked rice odor	Baked rice cracker brand name; Shinmai	5.0 ± 0.1		3
	Baked rice cracker brand name; Dozo	5.0 ± 0.1		6
	Baked rice cracker; Kanom sakol Co., Ltd.	5.0 ± 0.1		11.5
Rancid odor	Refined soybean oil heated at 110 °C 5 h.	20.0 ± 0.1	PV=21.20±0.21 TBARS=9.36±0.05	5.5
	Refined soybean oil heated at 110 °C 15 h.	20.0 ± 0.1	PV=55.60±0.05 TBARS=29.88±0.10	7.5
	Refined soybean oil heated at 110 °C 24 h.	20.0 ± 0.1	PV=115.74±0.58 TBARS=95.33±0.03	14
Vegetable oil odor	Soybean oil brand name; Kesorn	20.0 ± 0.1	PV=0.49±0.00 TBARS=0.49±0.00	2.5
	Soybean oil brand name; Aa-ngun	20.0 ± 0.1	PV=0.74±0.02 TBARS=2.12±0.00	4
	Soybean oil brand name; Dok-Poy-Sien	20.0 ± 0.1	PV=1.29±0.32 TBARS=3.23±0.06	6

<sup>§</sup> Values are expressed as mean ± SD (n=3). Peroxide value is shown in term of meq peroxide·kg<sup>-1</sup> oil. TBARS value is shown in term of mmol·kg<sup>-1</sup> oil.

#### Determination of water activity

Water activity of samples was determining using a thermoconstanter (Novasina, Zurich, Switzerland) at 25°C during the storage period.<sup>12</sup>

#### Statistical analysis

Each experiment, from sample preparation to analysis, was repeated in triplicate, and the data were then analyzed by SPSS software (SPSS Inc., Chicago, IL, USA). The general linear model procedure was applied and Duncan's multiple range test was used to compare the mean values at  $p < 0.05$ . Mean values and pooled standard error of the mean (SEM) were calculated.

**Table 4.** *p*-values of PV, TBARS, baked rice odor, rancid odor and vegetable oil odor for the tests of each antioxidant and storage time differences

	Factors	<i>p</i> -values
PV	days	< 0.001
	antioxidants	< 0.001
TBARS	days	< 0.001
	antioxidants	< 0.001
Baked rice odor	days	< 0.001
	antioxidants	< 0.001
	panel	0.112
	replications	0.965
Rancid odor	days	< 0.001
	antioxidants	< 0.001
	panel	0.272
	replications	0.841
Vegetable oil odor	days	< 0.001
	antioxidants	< 0.001
	panel	0.118
	replications	0.711

Note: a low *p*-value means a highly significant difference.

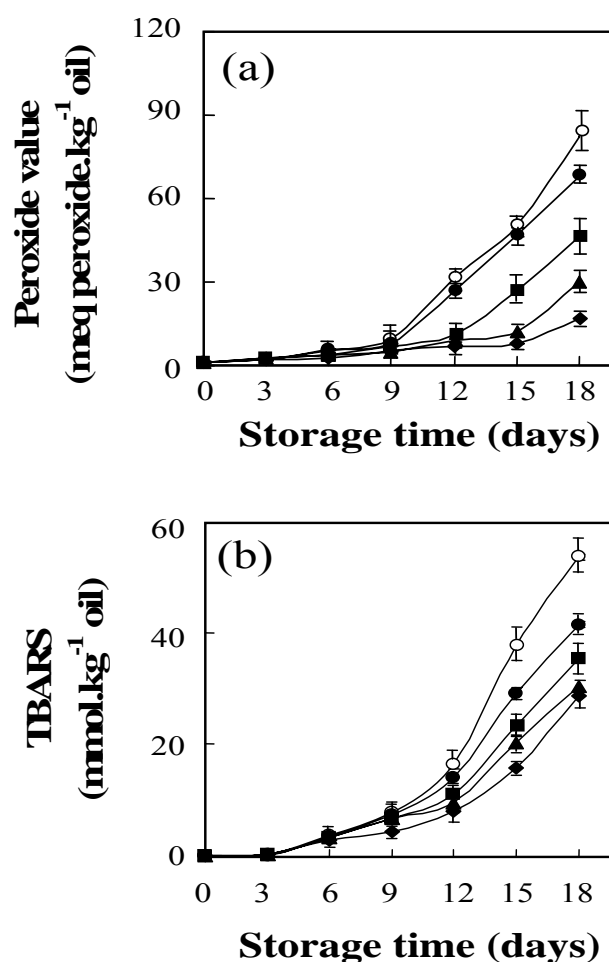
## Results and discussion

Consistency of data obtained from the two assessment years was checked by measuring total phenolic content, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activities of the extracts according to Maisuthisakul *et al.* (2007),<sup>6</sup> before referring the previous studied data. It was found that the standard deviations of the previous and present set data were less than 0.1. The data obtained from three replications. Hence the data had good reproducibility over the two years. This may affect that plant materials were obtained from the same period of each year.

Crackers are susceptible to lipid oxidation because the surface area in contact with the air is high, and metal ions in the product can catalyse the formation of carbonyl compounds and hydroperoxides at the aging stage<sup>13</sup> from the fat (Table 1) in the cracker. In addition, autoxidation occurs rapidly at the range of water activity between 0.01-0.15. From our study, the water activity of the product is 0.08. This can indicate that oxidation in the studied rice crackers is rapid unless retarded by antioxidants. The product contains iron (Table 1), a catalyst for oxidation. It was shown in a previous study<sup>14</sup> that *C. formosum* extract contained chlorogenic acid which acts as free radical scavenger and metal chelator. Hence, rice cracker was selected to study the antioxidant effectiveness of crude *C. formosum* extract compared with commercial antioxidants including  $\alpha$ -tocopherol and BHT.

### Changes in chemical properties

The progression of chemical changes in rice crackers with added antioxidants was monitored in rice crackers during storage at 40°C for 18 days. The total extractable lipid in samples averaged about 11 % of the mass of samples (data not shown). Water activity of the rice cracker samples was about 0.08-0.20 during storage (data not shown). Oxidative changes in crackers were initiated by the formation of free radicals, the precursors for the hydroperoxides, which were the primary oxidation products. Free radicals may be formed enzymatically, or by transition metal catalyzed reactions.<sup>15</sup> Storage time was a significant factor ( $p < 0.001$ ) for changes in PV and TBARS values (Table 4). Each antioxidant including freeze-dried *C. formosum* extract, dried *C. formosum* leaf powder,  $\alpha$ -tocopherol and BHT was also significant in inhibiting lipid oxidation in term of PV and TBARS (Fig 1). PV and TBARS values increased markedly in samples without antioxidants. The PV value increased slowly during the early storage period and then increased more rapidly at longer storage times. The more rapid increase in PV is an indication that later stages of lipid oxidation have been reached.<sup>16</sup> The sample containing BHT had the lowest PV and TBARS values. *C. formosum* extract appeared to possess stronger antioxidant activity than  $\alpha$ -tocopherol and dried *C. formosum* leaf powder. The iron in rice crackers was 4.3 mg per 100 g edible portion (db). In a previous study, we found that the *C. formosum* extract contained 0.60 g chlorogenic acid equivalent per gram (g of CAE/g) as well as other caffeic acid derivatives. Chlorogenic acid can function both as a radical scavenger and as a metal chelator. Consequently, the antioxidant effect of *C. formosum* extract in rice cracker at 100 mg·kg<sup>-1</sup> was higher

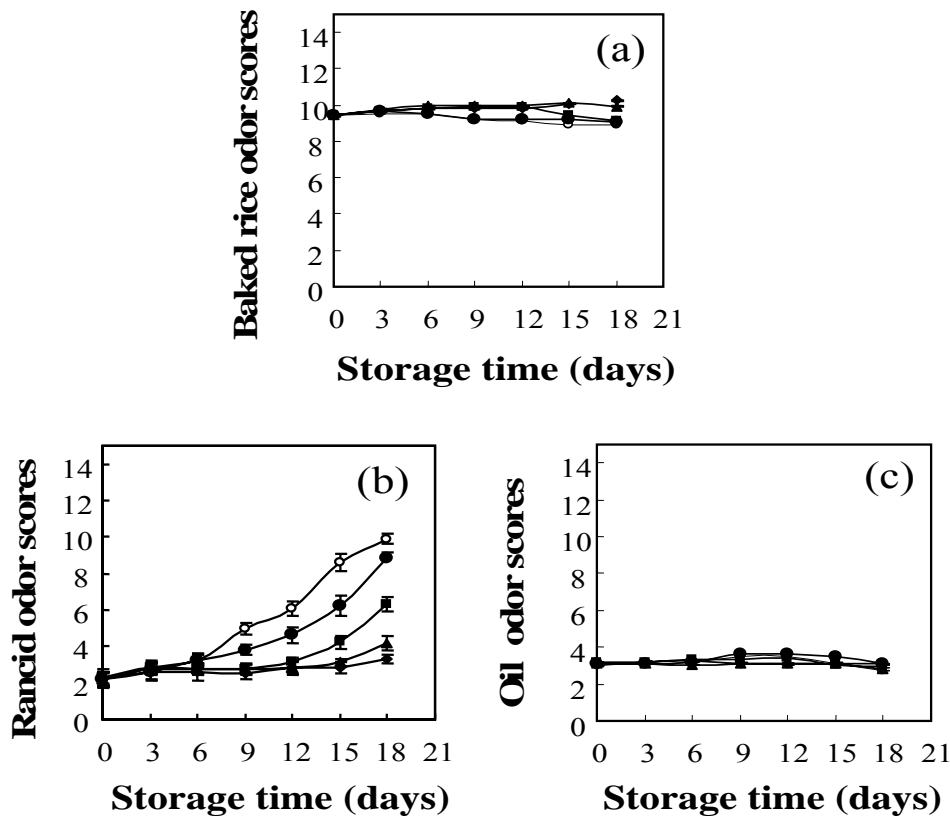


**Figure 1.** Effect of studied antioxidants on the oxidative stability of rice crackers at 40°C, assessed by determination of (a) PV and (b) TBARS. The concentration of each antioxidant was 100 mg·kg<sup>-1</sup> except dried *C. formosum* leaf powder (2.44 g·kg<sup>-1</sup>). (○= Control, ▲= *C. formosum* extract, ■=  $\alpha$ -Tocopherol, ◆= BHT and ●= dried *C. formosum* leaf powder). Data points represent mean  $\pm$  standard deviation (n=3).

than that of  $\alpha$ -tocopherol. The TBARS values (Fig 1b) confirmed this finding. The TBARS values of sample containing *C. formosum* extract were lower than those of samples containing  $\alpha$ -tocopherol.

### Changes in sensory properties

Changes in the odors of rice crackers, namely, baked rice, rancid, and vegetable oil odor after storage for 18 days at 40°C are shown in Figure 2. It was concluded that each panel and the repetitions from sensory analysis did not differ significantly (Table 4). Normally, the replicates were used to check panellists' accuracy and reliability. This result showed that calibration with warm-up sample, balancing serving with random three digit codes could reduce classical psychology errors. Storage time and antioxidants significantly affected baked rice, rancid, and oil odors (Table 4). A significant storage time affected rancid odor more than baked rice odor and oil odor (Fig 2). The sample containing BHT had significantly lower rancid odor scores from sensory analysis than the other samples. The samples containing BHT showed the highest baked rice odor. The intensity of baked rice odor for the control (no antioxidant) and samples containing dried *C. formosum* leaf powder increased in the first period (6 days) and



**Figure 2.** Sensory (a) baked rice, (b) rancid and (c) vegetable oil odor characterization of rice cracker with different antioxidants at 40°C, 80% RH for 18 days, assessed by quantitative descriptive analysis (QDA) using 10 trained panellists with two replications. (○ = Control, ▲ = *C. formosum* extract, ■ =  $\alpha$ -Tocopherol, ◆ = BHT and ● = dried *C. formosum* leaf powder). Data points represent mean  $\pm$  standard deviation (n=20).

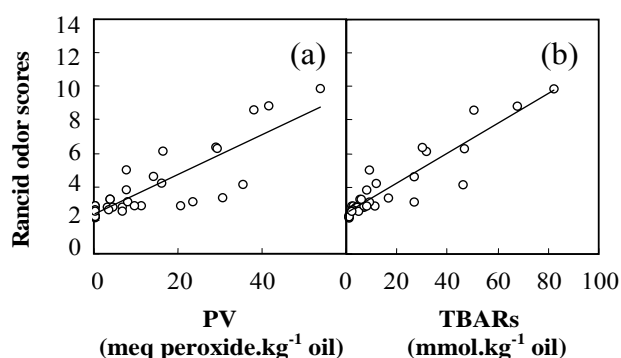
then decreased with the rapid development of rancidity (Fig 2). The baked rice odor was slightly increased for the samples containing *C. formosum* extract. These samples had low rancid odor scores compared to the control. These results implied that samples which had higher rancid odor score, showed lower baked rice scores. Increasing rancid or oxidation-related warmed-over odor is often accompanied by decrease of the other flavours,<sup>17</sup> and this was observed in the present study as well. The decrease might be caused by a loss of components contributing to these desirable attributes or by masking due to increasing amount of lipid oxidation products. The changes in oil odor during storage showed the same scores in the low range between 2.8-3.5. This implied that natural vegetable oil had a small amount of odor. Typically, the odor of oil comes from products of numerous reactions between fat and other food components such as proteins and carbohydrates as well as oxidation products. Consequently, the oxidized oil had a higher odor than the fresh one. Moreover, it was difficult for humans to detect the odor at a low intensity so the odor scores were slightly different.

The rancid odor scores of samples were in agreement with the degree of rancidity measured by PV and TBARS values. After 6 days of storage at 40°C, the cracker samples had developed a relatively high degree of rancidity as shown not only by rancid odor scores from sensory analysis but also by high PV and TBARS values from chemical analysis. Lipid oxidation leads to formation of volatile aldehydes and other low molecular weight compounds with low sensory thresholds. Such components can give

odors and flavors typically associated with rancidity. Lipid oxidation products and compounds from other degradation reactions might also contribute to increased scores of other non-desirable sensory attributes.<sup>17</sup> Sensory rancid odor descriptors such as painty, grassy were found indicating production of lipid degradation products such as hexanal, heptanol, 2-hexanol, 2-octenal and (E, E)-2,4-decadienal. However, the susceptibility of various fatty acids toward oxidation is highly dependent on the number of doubly allylic hydrogen atoms present. Long chain fatty acids with more double bonds are therefore expected to be major contributors of lipid oxidation products in the matrix where they are present. Soybean oil contained unsaturated acid about 80.7% which are palmitoleic acid (<0.5%), oleic acid (20-50%), linoleic acid (35-60%), linolenic acid (2-13%) and eicosenoic acid (<1.0%).<sup>18</sup> Normally, the type of fatty acid is related to volatile oxidation products, such as hexanal, which is derived from the oxidation of omega-6-fatty acids such as linoleic acid, and propanal, which is derived from the oxidation of omega-3-fatty acids such as linolenic acid. For oleic acid rich oils, nonanal may be used as a marker of oxidation.<sup>19</sup> These results indicated that there was a relationship between sensory and chemical analysis.

#### **Relationship between chemical and sensory properties**

The PV and TBARS values determined by chemical analysis were significantly positively correlated with rancid odor sensory terms. However, TBARS values correlated better with sensory scores than PV values (Fig 3).



**Figure 3.** Regression analysis between (a) PV and (b) TBARS values with sensory rancid odor attributes of rice crackers stored at 40°C.

**Table 5.** Relationship between PV or TBARS values and rancid odor scores of rice cracker containing various antioxidants

Relationship	Regression equation	$r^2$
PV and rancid odor scores	linear	0.72
	Exponential	0.71
TBARS and rancid odor scores	linear	0.85
	Exponential	0.81

The TBARS method is widely used as an indicator of lipid oxidation and has repeatedly been demonstrated to correlate with sensory assessment, for example the exponential relationship of sensory assessment and log TBARS in meat.<sup>20</sup> In these results, we found that a linear relation between PV or TBARS values and rancid odor was better than the exponential relationship (Table 5). This difference could be explained by the fact that the ingredients in meat and crackers are different. The presence of protein in meat affects the flavor release from foods. Lipid oxidation products might thus be differently formed and perceived in food systems because of the influence of other compounds more than lipids.<sup>16</sup>

The present study indicated that dried *C. formosum* leaf powder possessed antioxidant effectiveness in inhibiting lipid oxidation but activity was less than that of *C. formosum* extract in stored rice crackers. In addition, *C. formosum* extract was more effective than  $\alpha$ -tocopherol due to metal ions present in the crackers, which made  $\alpha$ -tocopherol less effective as an antioxidant. Sensory odor attributes of rice cracker were related to TBARS more than PV values in the form of a linear regression.

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