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Antiradical activity and physico-chemical analysis of crackers from *Cucurbita moschata* and modified cassava flour

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Abstract

Pumpkin (*Cucurbita moschata*) is one of the food commodity plants that grow in Indonesia. There is an increasing demand for fibre rich food and food ingredients. This study aimed to evaluate the antioxidant activity, morphological structure, chemical and physicochemical properties of crackers (Indonesian name: Kerupuk) made from pumpkin flour and mocaf. In this study, pumpkin pulps were processed into flour and substituted at 14%, 28% and 42% (K1, K2 and K3, respectively) and combined with modified cassava (mocaf) flour in a composite flour crackers formulation. The control crackers comprised of 100% of mocaf flour (K4). The crackers were characterized using SEM, FTIR, XRD, Chromameter and UTM. The crackers extracts were obtained by the maceration method using ethanol as the solvent. The higher antioxidant activity measured by DPPH assays was found in K3 formula crackers which are 0.35%. The proximate analysis showed that the energy, protein and ash content of K2 formula crackers had the highest content of 382.89 Cal/100 g, 4.45% and 11.72%. K2 formula crackers also had the lowest water content as of 5.62%. The texture analysis showed that K1 to K4 formula crackers have F max value between 11.39-50.48 N. The colour analysis showed that the amount of pumpkin flour added affected the crackers' colour, while the SEM showed that pumpkin-mocaf crackers had a cellular porous structure. Meanwhile, the FTIR results showed that several functional groups from carbohydrate, carboxyl acid, ketone, amide and lipids were detected. Furthermore, the diffractogram of K1-K4 crackers showed an amorphous structure. The results in this study confirm the potential for industrial exploitation of modified cassava flour and pumpkin flour through processing into crackers which would be suitable for gluten intolerants.

1. Introduction

Oxidative damage in the human body can be caused by free radicals. Free radicals come from the environment such as air pollution, chemicals and also from several biochemical processes in the human body. The form of free radicals is hydroxyl radicals, superoxide radicals and peroxyl radicals (Poulson *et al.,* 1998). Free radicals are dangerous because it causes several chronic diseases such as diabetes, aging and cancer (Ani *et al*., 2006). Therefore, antioxidants are important, because they can delay the process of oxidation of compound and inhibit the formation of free radicals in the early stages. Antioxidant is usually used in the food industry as potential inhibitors from peroxidation reaction of lipid (Scherer and Godoy, 2009). Natural antioxidants from plants are highly recommended in drug and food applications, since it had therapeutic effects, high nutrition and more safety (Deng *et al*., 2011), while synthetic antioxidant can cause organ damage if it is accumulated in the human body. Spices, herbs, fruits and vegetable contain natural antioxidants such as terpenoids, vitamin, and phenolic compounds (Larson, 1988; Cotelle *et al*., 1996; Cai *et al*., 2003; Indrianingsih *et al*., 2015). Understanding of these health benefits, intensive research on natural antioxidants from plants has been conducted.

There is a wide variety of plants and seasonal crops in Indonesia, due to its tropical weather. Numerous plants are used in traditional medicine and also functional food. Pumpkin (*Cucurbita moschata*) is one of the plants that is widely cultivated in Indonesia. There is an increasing interest of *C. moschata* because of its high nutritional value, digestive effects and good sensory characteristics (Noelia *et al*., 2011). The pulp of *C.*

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moschata is used in pies, soups and pastry. Meanwhile, the seeds of *C. moschata* are used in pharmaceutical as well as a snack. Therefore, *C. moschata* is a promising plant for functional food, pharmaceutical and cosmetic and industries (Quintana *et al*., 2018). Due to the good acceptance and industrialization of the derived products from *C. moschata*, more study and evaluation about the physico-chemical properties of this fruit is necessary.

On the other hand, the consumption of food from wheat such as bread, biscuits, cakes, noodles and other processed products has greatly increased in Indonesia. This is probably due to the influence of the culture of western countries and the expansion of urban culture that affects the consumption patterns of the Indonesian people. In fact, wheat or flour needs to be imported from abroad because climate and land conditions in Indonesia are not suitable for growing wheat. This gives an important task to replace wheat flour with other ingredients from local ingredients that have similar properties and functions (Hsu *et al*., 2004).

As an agricultural country, one of the many local foods produced in Indonesia is cassava (*Manihot esculenta* Crantz). Nowadays, it has been widely used mocaf flour which is a modification of cassava flour through a fermentation process so that it has better quality. The advantage of mocaf flour is that it does not contain gluten so it is can be safely consumed by toddlers and autistic sufferers, as well as diabetics (Firdaus *et al*., 2014). Mocaf flour also contains carbohydrates, low fat, high calcium and high in fiber. This fiber is needed by the body to help absorption in the digestive process, prevent constipation and control cholesterol levels in the body.

Recently, Aly and Saleem (2015) developed gluten-free bread and biscuits from cassava flour and pumpkin powder. However, in the study, rice flour and extruded soy protein were added for its functional properties. Another study, also evaluated the crackers from cassava flour and sweet potato flour (Owusu *et al*., 2011). However, to date there is no study about the using of 100% of mocaf as crackers, and usually, another flour, such as cassava flour, rice flour or wheat flour still be used as one of the ingredients. To the best of our knowledge, this study is the first to evaluate the usage of modified cassava flour and pumpkin flour only, without any other ingredients in making crackers. From the above explanation, the aim of this study was to evaluate the antioxidant activity, morphological structure, chemical and physicochemical properties of crackers made from pumpkin flour and mocaf. This work is important in the development of new products especially crackers from *C. moschata* and mocaf flour for industrial exploitation which would be suitable for gluten intolerants.

2. Materials and methods

2.1. Materials

The pumpkin (*C. moschata*) was collected from Yogyakarta and the mocaf flour was purchased in Gunungkidul Regency, Yogyakarta, Indonesia. 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, methanol, and ethanol were obtained from Sigma-Aldrich (Japan). Egg, baking powder, salt, and garlic were obtained from local market in Yogyakarta, Indonesia.

2.2 Flour milling process and preparation of ethanol extracts of crackers

The pumpkin was cut into pieces, peeled the rinds and separated the seeds. The pulp was dried using the oven for 3 days at 50°C and it was made into flour with particle size around 80 mesh. The ethanol extracts of crackers were obtained by macerating crackers powders with ethanol (1:8 w/w) for 2 days at room temperature.

2.3. Preparation of crackers

Crackers were prepared according to Purwanti (2011). This procedure involved mixing the mocaf flour and *C. moschata* flour (three formulations of *C. moschata* flour and mocaf flour, i.e. 14:86% (K1); 28:72% (K2); 42:58% (K3)) and 100% of mocaf flour (K4-control) with egg, garlic, salt, baking powder and water. Ingredients were mixed into a cohesive dough, steamed at 100°C for 45 mins, cooled for 12 hrs and cut into circular pieces. They were then dried under sunlight for 3 days. After drying, they were fried using vegetable oil and stored in sealed bags at room temperature.

2.4. Characterization

SEM of the surface of dried pumpkin flour was performed using a Hitachi SU-3500 instrument operating at 4 kV. FTIR analysis of the pumpkin flour was performed on a wavelength from 500 cm^{-1} to 4000 cm^{-1}. The proximate analysis procedure follows the method of SNI 01-2891-1992. Colour instrumental parameters were determined using colour reader (CR 20 Color Reader Konica Minolta). Results were expressed in L*, a* and b*, with L (how light or dark the sample is), ranging from black (0) to white (100), a* ranging from green (-60) to red (60) and b* ranging from blue (-60) to yellow (+60). Colour instrumental analyses were performed in three replicates. The texture analysis of the pumpkin-mocaf crackers was tested using Universal Testing Machine (UTM) with a preloaded force of 0.02N, preload speed of 50 mm/min and test speed of 10 mm/

min. Texture analysis was performed in three replicates.

2.5. Antiradical assay using DPPH

Crackers extracts were dissolved in methanol and reacted with DPPH (1.01 mM) to obtain concentration of 800 µg/ml at room temperature. It was left to stand in the dark condition for 30 min. Absorbance was recorded at 517 nm using a UV-Vis spectrophotometer (Yen and Chen, 1995). The scavenging activity was calculated using the equation:

$$DPPH\ Savenging\ Activity\ (\%) = \frac{A_o - A_1}{A_o} \times 100$$

Where A_0 is absorbance of the control and A_1 is absorbance of the sample. The DPPH radical scavenging assays were carried out in three replicates.

2.6. Statistical analysis

All assays were conducted in triplicate. Statistical analyses were performed with SPSS 16.0 for an analysis of variance (ANOVA) followed by Duncan's test. Differences at P<0.05 were considered to be significant.

3. Results and discussion

3.1. Proximate compositions of crackers

Proximate compositions of K1-K3 (pumpkin-mocaf crackers) and K4 (control) are shown in Table 1. The data indicated that K2 contained the highest percentage of protein and ash, 4.45% and 11.72%, respectively. In the case of water of carbohydrate content, K4 had the highest value as of 8.65 and 68.72%. Meanwhile, for the fat and energy content, K1 has the highest value as of 13.41% and 395.78 Cal/100 g. Usually, the fat of pumpkin pulp is low, around 0.16% to 1.15% (Kuchtová *et al*., 2016; Indrianingsih *et al*., 2018). The higher fat content of the crackers made from the pumpkin-mocaf composite flour was possibly from vegetable oils and the egg. It should be noted that there was no gluten in the pumpkin flour (Quintana *et al.,* 2018)

3.2 Texture analysis

The hardness of the pumpkin-mocaf crackers was tested using Universal Testing Machine (UTM) with a preload force 0.02N. The texture analysis data is shown in Table 2. The texture is an important factor of physical characteristics of food product that influences consumer (Durgadevi and Shetty, 2012). The results showed that K1 to K4 have F max values between 11.39-50.48 N. F max indicates the compressive force given to the sample until the shape changes and broken. From the results, K1 has the lowest score of F max, which means it was not so hard to chew. The highest result of F max was from K3 which meant that K3 tend not to break easily compared to other crackers. Depends on this texture, sensory evaluation was required to be conducted to know the consumer perceptive (Kobayashi and Kawamura, 2016). Another study of rice crackers revealed that a certain level of moisture affected the hardness and peculiar viscosity in such samples which made them difficult to crunch (Wada and Higo, 2007).

3.3. Colour analysis

Table 3 shows the result of colour analysis of pumpkin-mocaf crackers. The b* values are related to the yellowish colour of the pumpkin-mocaf crackers. The b* values ranged from 17.03 to 31.40. It can be seen that the K1-K3 have higher b* values than K4. The highest b* value as of 31.40 was achieved from K3 which contained the highest pumpkin flour as of 42%. The a* values are correlated with the reddish colour of the pumpkin-mocaf crackers. The a* values ranged from 4.87 to 10.53. The L* values are related to the lightness. In this study, the L* values ranged from 44.10 to 53.93. Generally, the amount of pumpkin flour added affected

Table 1. Proximate analysis of crackers

Parameter	K1	K2	K3	K4
Water (%)	6.48±0.3^{b}	5.62±0.2^{a}	8.46±0.5^{c}	8.65±0.4^{c}
Ash (%)	9.34±0.4^{b}	11.72±0.8^{c}	9.45±0.4^{b}	8.78±0.3^{a}
Protein (%)	4.35±0.1^{b}	4.45±0.1^{b}	4.32±0.1^{b}	3.76±0.1^{a}
Fat (%)	13.41±0.8^{d}	12.09±0.9^{c}	9.46±0.3^{a}	10.06±0.7^{b}
Carbohydrate (%)	66.34±1.4^{a}	65.66±1.3^{a}	68.28±1.4^{b}	68.72±1.2^{b}
Energy (Cal/100g)	395.78±3.5^{d}	382.89±3.4^{c}	366.8±3.3^{a}	371.57±3.7^{b}

K1 = pumpkin:mocaf (86:14%); K2 = pumpkin:mocaf (72:28%); K3 =pumpkin:mocaf (58:42%); K4 = mocaf (100%). The different letter superscript in the table shows a significant difference at p<0.05.

Table 2. Texture analysis of crackers

Parameter	K1	K2	K3	K4
Fmax (N)	11.39±0.9^{a}	43.79±1.6^{c}	50.48±1.7^{d}	26.98±1.3^{b}
Emax (mm)	0.69±0.02^{a}	0.89±0.01^{c}	0.74±0.01^{b}	1.26±0.06^{d}

K1 = pumpkin:mocaf (86:14%); K2 = pumpkin:mocaf (72:28%); K3 =pumpkin:mocaf (58:42%); K4 = mocaf (100%). The different letter superscript in the table shows a significant difference at p<0.05.

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Table 3. Colour analysis of crackers

Sample	L*	a*	b*
K1	53.93 ± 1.9^{d}	5.37 ± 0.1^{b}	19.67 ± 0.9^{b}
K2	49.63 ± 1.4^{b}	8.23 ± 0.2^{c}	24.73 ± 1.1^{c}
K3	52.07 ± 1.5^{c}	10.53 ± 0.3^{d}	31.40 ± 1.3^{d}
K4	44.10 ± 1.2^{a}	4.87 ± 0.1^{a}	17.03 ± 0.8^{a}

K1 = pumpkin:mocaf (86:14%); K2 = pumpkin:mocaf (72:28%); K3 =pumpkin:mocaf (58:42%); K4 = mocaf (100%). The different letter superscript in the table shows a significant difference at $p<0.05$.

the colour of the cracker.

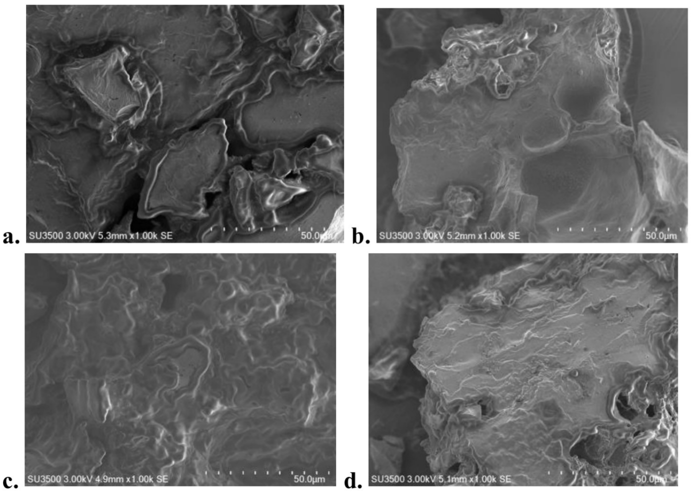

Figure 1. Morphology structure of crackers of a. K1; b. K2; c. K3; and d. K4. K1 = pumpkin:mocaf (86:14%); K2 = pumpkin:mocaf (72:28%); K3 = pumpkin:mocaf (58:42%); K4 = mocaf (100%).

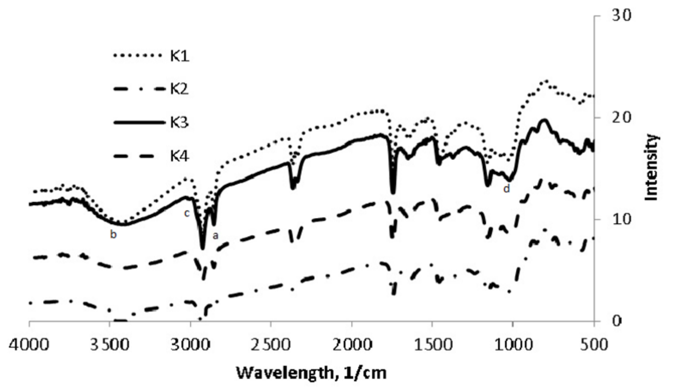

Figure 2. Spectra of crackers of a. K1; b. K2; c. K3; and d. K4. K1 = pumpkin:mocaf (86:14%); K2 = pumpkin:mocaf (72:28%); K3 = pumpkin:mocaf (58:42%); K4 = mocaf (100%).

3.4. SEM analysis

Figure 2 shows the SEM image of the surface of the pumpkin-mocaf crackers. The SEM of pumpkin-mocaf crackers is shown in Figures 1a–d. The micrographs of the pumpkin-mocaf crackers showed that the surface structure is very compact. The compact structure might be affected by protein-lipid complexes (Nakhon *et al.,* 2017). All the pumpkin-mocaf crackers showed a cellular porous structure, which may be depended on the pumpkin concentration. Although the significant difference among the formulation in relation to the pattern was very clearly observed, it can be seen that K3 has the firmer structure compared to others. Meanwhile, K4 showed the most porous structure. A similar result was obtained by Park *et al.* (2012) in which the addition of rice flour in cupcakes influenced the texture that was detected by electron microscopy analysis. The frying process can cause the sudden vaporization of water and causing samples to puff (Campbell and Mougeot, 1999). It also caused the porous structure and low density. The lipid content in cracker might play a role in the texture properties (Kaewmanee *et al*., 2015).

3.5. FTIR analysis

FTIR spectroscopy is a technique for evaluating the functional groups as part of a compound in chemical analysis. It was also used as a quality control monitoring in food industries because of its rapid screening and quantification of chemical components in samples (Rodriguez-Saona and Allendorf, 2011). Figure 2 shows the spectra of pumpkin-mocaf crackers. The FTIR spectra of pumpkins-mocaf crackers were evaluated at the frequency of 4000–500 cm^{-1}. The frequency around 2948–2851 cm^{-1} is related to CH absorptions (a). Band absorption at 3550–3255 cm^{-1} is hydroxyl groups (O-H) from carbohydrate or other compounds such as carboxyl acid and ketone (b). The frequency around 3121– 3032 cm^{-1} is related to amide (N-H groups) (c). The bands at 1108–1015 cm^{-1} is associated with lipids (d). These spectra were in accordance Quintana *et al*. (2018) who revealed that pulps of *C. moschata* from Colombia showed several bands corresponding to carboxyl groups, aldehydes, ketones of carbohydrates, amino acids and esters of lipids. From the spectra it was observed that the intensity absorption bands of K4, especially in OH bands absorption was the lowest compared to K1-K3. This was due to the absence of active compounds in it as pumpkin usually contained active compounds in flavonoid or phenol forms which contain many hydroxyl groups (Rocha-Guzman *et al*., 2012).

3.6. XRD analysis

The XRD diffractogram of pumpkin-mocaf crackers is shown in Figure 3. The diffractogram pattern of pumpkin-mocaf crackers (Figure 3) showed different patterns of the four crackers formulation. In general, the diffractogram showed K1-K4 had amorphous structure and that K1-K3 has lower intensities than K4. A study revealed that starch from pumpkin has a diffraction angel around 15°, 18°, 20° and 23° (Wani *et al*., 2015). Furthermore, the crystallinity level of plant starch varies according to amylopectin content and crystal size (Jan *et al*., 2016). The difference of the peak intensity on starches could possibly be induced by the increase of shorter and longer branch chains and the arrangement of

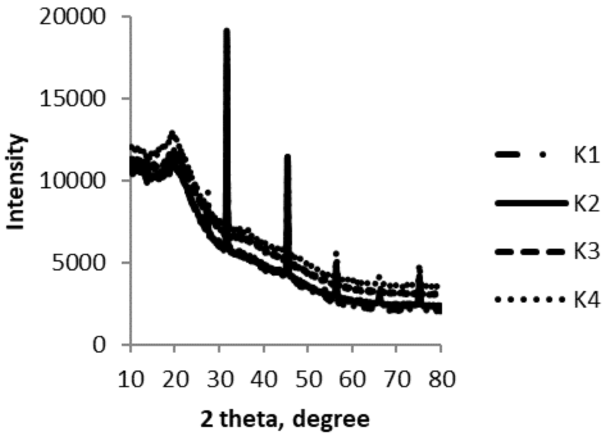

Figure 3. Diffractogram of crackers of a. K1; b. K2; c. K3; and d. K4. K1 = pumpkin:mocaf (86:14%); K2 = pumpkin:mocaf (72:28%); K3 = pumpkin:mocaf (58:42%); K4 = mocaf (100%).

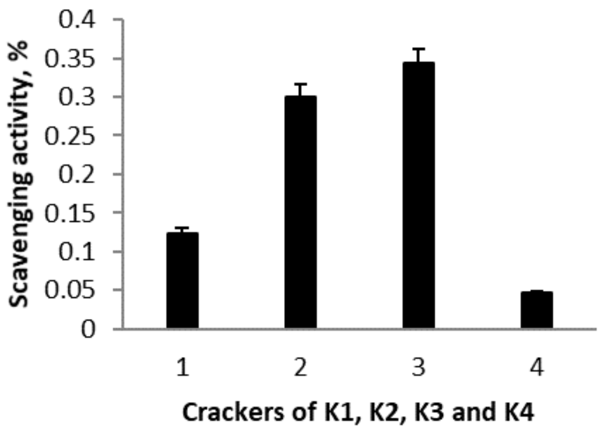

Figure 4. Antioxidant activity of crackers of a. K1; b. K2; c. K3; and d. K4 at concentration extract of 800 µg/mL. K1 = pumpkin:mocaf (86:14%); K2 = pumpkin:mocaf (72:28%); K3 = pumpkin:mocaf (58:42%); K4 = mocaf (100%).

amylopectin which lead to a decrease in the amorphous region (Kim *et al*., 2012).

3.7. Antioxidant activity using DPPH assay

The antioxidant activity of the extract of pumpkin-mocaf crackers was evaluated using DPPH assay as shown in Figure 4. From Figure 4, it can be observed that the scavenging activities of pumpkin-mocaf crackers extract ranged from 0.047 to 0.34% at 800 µg/mL of extract. Among the pumpkin-mocaf crackers extract, K3 showed the highest scavenging activity of 0.35% at the concentration extract of 800 µg/ml. It is followed by K2, K1 and K4 where 0.30, 0.12, and 0.047% at the concentration extract of 800 µg/ml, respectively. The extract of pumpkin-mocaf crackers has different activities in DPPH assay which might be associated with the different amount of the content of the active compounds in the extract. Morin and catechin are some bioactive compounds that were detected in pumpkin (Rocha-Guzman *et al*., 2012).

4. Conclusion

The antioxidant activities of pumpkin-mocaf crackers with the highest pumpkin flour (K3) were the highest compared to K1 and K2 crackers. This study showed that pumpkin-mocaf flour is a potential comestible that contained high antioxidants. The results of other physico-chemical studies also showed that crackers of pumpkin-mocaf composite have better properties than 100% mocaf flour. The results in this study confirmed the potential of mixture of pumpkin flour with modified cassava flour in the production of crackers suitable for gluten intolerants.

Conflict of Interest

The authors declare no conflicts of interest.

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