



## Chemical Characterization of Flour Fractions from Five Yam (*Dioscorea alata*) Cultivars in Indonesia

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**Abstract.** The purpose of this study was to investigate the influence of particle size on the chemical properties of yam flour in five cultivars, yellow/YY, orange/OY, light purple/LPY, purple/PY, and dark purple/DPY. With a mesh sieve, three flour fractions were separated according to particle size: small (128.6-139.7  $\mu\text{m}$ ), medium (228.7-257.9  $\mu\text{m}$ ), and large (475.4-596.3  $\mu\text{m}$ ). The content of moisture (6.81-11.26 %db) and lipids (4.48-9.85 %db) decreased with the increase of particle size, while proteins (4.48-9.85 %db) and carbohydrates (78.12-83.76 %db) were not influenced by particle size. Folin-Ciocalteu reagent and chlorogenic acid were used as standard to investigate the total phenolic compounds in the yam flour, and high-performance liquid chromatography (HPLC) was used to investigate the anthocyanin and carotene contents. It was found that there was no size influence on the content of phenolics (0.27-2.82 %db), anthocyanin (2.25-15.27 mg/100g db) in LPY, PY, DPY or carotene (23.75-132.12 mg/100g db) in YY, OY. The differences in chemical composition were due to differences in particle size and heat treatment, but may also have been caused by the different composition of the milling process.

**Keywords:** *anthocyanin; carotene; flour; particle size; phenolics; yam.*

### 1 Introduction

Flour is a common form best known for long-term storage of tubers. Making flour out of tubers is much easier and simpler with higher yields over a shorter time period than extracting the tuber starch. Depending on the processing, other advantages of flour are that the nutritional components of bulbs can still be maintained. In addition, flour is a form that is easy to apply and can serve a variety of food products. In this regard, the chemical character of yam flour is very valuable information. For common forms of tuber processing, the flour particle size used is  $< 212 \mu\text{m}$ , or the equivalent of sieve size  $> 65$  mesh [1].

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Yam tubers have a fairly high starch content (75-84 %db bulbs) [2]. Other components of yam flour are fat, protein, fibers, minerals and vitamins, which range from 16-25% [3,4], including phenolic compounds and dyes. Phenol, commonly found in plants, is an essential component that contributes to color, nutrition, and antioxidants [5-7]. Phenolic compounds and various other phenolic components have been reported in domestic *Dioscorea* tuber [6,7].

The polyphenol compound that gives color to the tuber of *Dioscorea alata* is anthocyanin. Farombi, *et al.* [7] reported that the brownish *Dioscorea* flour of the purple tuber contains anthocyanin. Anthocyanins are water-soluble, vacuolar pigments that appear red, purple or blue depending on the pH of the environment [7,8]. Anthocyanin is a derivative of the anthocyanidin that has sugar clusters mainly tied in the form of 3-glucosides and forms the anthocyanidin glucosides. Common cyanidins found in plants (from 30 compound groups) are delphinidin, petunidin, pelargonidin, peonidin and malvidin [8]. According to previous research, cyanidin 3.5-diglucoside is a major component of anthocyanin in the variety of *Dioscorea alata* purpurea, which also contains small amounts of cyanidin-3-glucoside and cyanidin-3-rhamnoglucoside [8,9].

Besides anthocyanin, other components that promote color in *Dioscorea* tubers are carotenoids. It has been reported that from this compound group there are also lutein, zeaxanthin, and *all-trans*- $\beta$  carotene, which can be found in *Dioscorea* tuber [10]. Two compounds, lutein and zeaxanthin, are very important as carotene nutrients of the eye, which can prevent eye retinal damage and cataracts [11]. As a source of nutrition and provitamin-A, they are required for food intake, which makes the existence of lutein, zeaxanthin and *all-trans*- $\beta$ -carotene in food materials very important.

Therefore, the focus of this research was to find the chemical characteristics of tuber flour separated into three particle-size fractions. Chemical analysis covered total phenolic compounds, anthocyanins and carotenoids in each of the flour fractions.

## 2 Methods

### 2.1 Flour Samples

Yam tubers were obtained from yam plant cultivation in Kulon Progo, DIY, Central Java. There were five cultivars, called *uwi wayang* (yellow yam/YY), *uwi koneng* (orange yam/OY), *bang kulit* (light purple yam/LPY), *punuk banteng* (purple yam/PY), and *rondo seluku* (dark purple yam/DPY). The flour was obtained using the methods developed by Ige and Akintunde [12] and

Imanningsih [13] with modifications. The bulbs were peeled, cleaned, sliced and then soaked in a solution of citric acid (1%) for 30 minutes, followed by blanching the tuber slices on steam for 10 minutes and then drying in a cabinet dryer at a temperature of  $50\pm 5^{\circ}\text{C}$  for 24 hours (moisture content  $< 15\%$  is a safe level for storage) [12]. Next, the chips were smoothed with a grinder and sifted with 100- and 80-mesh sieves. The flour fractions obtained were packed in aluminium foil, sealed and stored at a temperature of  $-20\pm 5^{\circ}\text{C}$ .

## 2.2 Chemical Compound Analysis

The proximate analysis of the flour fractions covered moisture, fat, protein, and ashes [14]. The determination of total phenolic content was done with the method developed by Ishiguro, *et al.* [15], which Folin-Ciocalteu phenol reagent and chlorogenic acid as the standards. For analyzing the total anthocyanin content, the pH differential method developed by Giusti and Ronald [16] was used and for analyzing the anthocyanin components the HPLC method developed by Ishiguro, *et al.* [15] with modification. The carotenoid content was analyzed by the HPLC method developed by Ishiguro, *et al.* [17] with modification.

Analysis of variance was conducted to determine the influence of the cultivars on the size and chemical properties at 95% confidence level, with a completely randomized design. Data analysis was performed with Microsoft SPSS Statistics 20.

## 3 Results

### 3.1 Flour Size Distribution

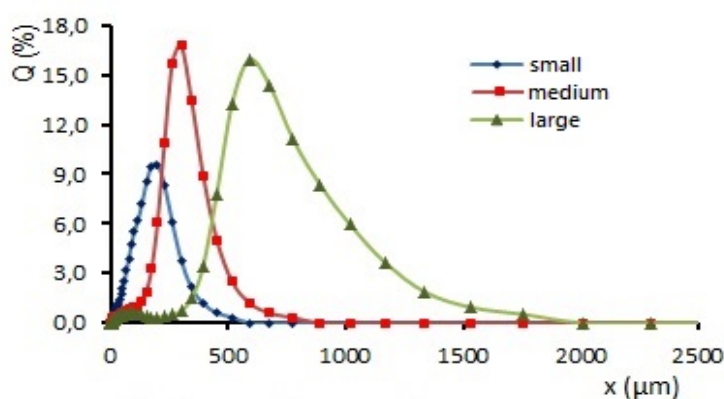
Based on the mesh size used, the fractions of flour obtained were categorized as small (under 100 mesh or particle size  $< 150\ \mu\text{m}$ ), medium (between 100 mesh and 80 mesh or particle size  $150 < x < 180\ \mu\text{m}$ ), and large (residu on 80 mesh or particle size  $> 180\ \mu\text{m}$ ). The Codex Standard for flour size is  $< 212\ \mu\text{m}$ , or the equivalent of sieve  $> 65$  mesh [1].

The particle size analysis (PSA) observations acquired a fairly wide range of sizes, from 7.50 to 1531.91  $\mu\text{m}$  (Table 1), with the average flour particle size ranging from 128.59 to 763.45  $\mu\text{m}$ . Size distribution was not symmetric.

The flour particle distribution was unimodal and contained a high proportion of the larger size particles (Figure 1). The average value of the flour particle size for each cultivar of yam, from small to large, was LPY, DPY, YY, PY and OY.

**Table 1** Particle Size of Flour Fractions.

Size Category		Particle size of flour sample ( $\mu\text{m}$ )				
		YY	OY	LPY	PY	DPY
Small ( $<35 \mu\text{m}$ )	range	7-517	9-517	7-451	7-517	9-517
	mean	138.9 $\pm$ 85.9	139.7 $\pm$ 86.0	128.6 $\pm$ 81.7	131.5 $\pm$ 85.0	139.5 $\pm$ 86.5
	diversity	510	508	444	510	508
Medium ( $35 < x < 53 \mu\text{m}$ )	range	26-678	34-777	13-678	13-777	34-678
	mean	253.9 $\pm$ 109.8	257.9 $\pm$ 117.1	228.7 $\pm$ 125.3	233.5 $\pm$ 129.5	255.3 $\pm$ 110.7
	diversity	652	743	665	764	644
Large ( $>53 \mu\text{m}$ )	range	39-1531	51-1754	39-1337	39-1531	59-1532
	mean	486.3 $\pm$ 0.25	596.3 $\pm$ 283.4	475.40 $\pm$ 217.8	480.9 $\pm$ 230.6	510.2 $\pm$ 222.2
	diversity	1492	1703	1298	1494	1473

**Figure 1** Size distribution of DPY flour particle, Q = quantity, x = particle size.

### 3.2 Chemical Character of the Flour

The proximate analysis of the flour fractions from the five yam cultivars showed that the chemical composition was quite varied, except for the level of ashes. The flour fractions had a moisture content ranging from 6.81 to 11.26%dw of flour, which decreased with increasing flour particle size for all cultivars (Table 2). The ash level of the flour fractions ranged from 1.33 to 3.75 %dw of flour. The ash levels showed that the minerals in the yam flour were almost the same in all fractions and flours YY and DPY, while flour PY had a relatively higher ash level than flours OY and LPY.

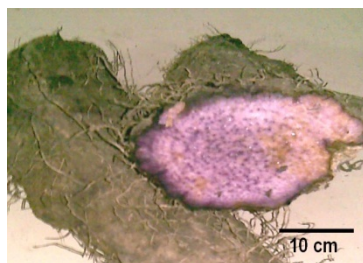
The existence of fat for all fractions ranged from 0.01 to 0.77%dw of flour, with higher concentrations in the small fractions of flour, while DPY had higher levels than the other tubers. Protein levels in all fractions ranged from 4.48 to 9.85%dw of flour with a higher concentration in LPY and a lower concentration in DPY compared to the other flours. Besides starch, the presence of fat and

protein in the flour is very important, especially for the establishment of its paste, which influences the texture of the food product [18]. The levels of protein and fat in yam flour is higher than in tapioca and potatoes, while half of it is found in wheat flour [19,20].

**Table 2** Proximate, Phenol, Anthocyanin and Carotene Content of Flour Fractions.

Flour sample	Moisture (%)	Concentration based on dry weight						
		Ash (%)	Fat (%)	Protein (%)	CH (%)	Phenol (%)	Anthocyanin (mg/100g)	Carotenoid ( $\mu$ g/100g)
Yys	11.23 $\pm$ 0.15 <sup>a</sup>	3.35 $\pm$ 0.52 <sup>a</sup>	0.60 $\pm$ 0.07 <sup>a</sup>	6.70 $\pm$ 0.08 <sup>a</sup>	78.12 $\pm$ 0.38 <sup>a</sup>	1.17 $\pm$ 0.02 <sup>a</sup>	nd	132.12
Yym	8.55 $\pm$ 0.28 <sup>b</sup>	3.55 $\pm$ 0.01 <sup>a</sup>	0.32 $\pm$ 0.05 <sup>b</sup>	5.89 $\pm$ 0.52 <sup>b</sup>	81.69 $\pm$ 0.37 <sup>b</sup>	1.09 $\pm$ 0.09 <sup>a</sup>	nd	129.79
Yyl	7.92 $\pm$ 0.39 <sup>c</sup>	3.75 $\pm$ 0.05 <sup>a</sup>	0.34 $\pm$ 0.00 <sup>b</sup>	6.46 $\pm$ 0.08 <sup>ab</sup>	81.53 $\pm$ 0.81 <sup>b</sup>	0.73 $\pm$ 0.05 <sup>b</sup>	nd	123.14
Oys	10.64 $\pm$ 0.11 <sup>a</sup>	1.50 $\pm$ 0.01 <sup>a</sup>	0.26 $\pm$ 0.01 <sup>a</sup>	7.46 $\pm$ 0.22 <sup>a</sup>	80.14 $\pm$ 0.13 <sup>a</sup>	1.39 $\pm$ 0.02 <sup>a</sup>	nd	23.75
Oym	10.47 $\pm$ 0.11 <sup>a</sup>	3.02 $\pm$ 0.01 <sup>b</sup>	0.01 $\pm$ 0.00 <sup>b</sup>	6.52 $\pm$ 0.12 <sup>b</sup>	79.78 $\pm$ 0.22 <sup>a</sup>	2.82 $\pm$ 0.03 <sup>b</sup>	nd	110.64
Oyl	6.81 $\pm$ 0.42 <sup>b</sup>	3.26 $\pm$ 0.08 <sup>b</sup>	0.04 $\pm$ 0.02 <sup>b</sup>	6.13 $\pm$ 0.22 <sup>b</sup>	83.76 $\pm$ 0.29 <sup>b</sup>	2.20 $\pm$ 0.06 <sup>c</sup>	nd	99.40
Lps	11.26 $\pm$ 0.66 <sup>a</sup>	1.33 $\pm$ 0.02 <sup>a</sup>	0.53 $\pm$ 0.03 <sup>a</sup>	5.30 $\pm$ 0.05 <sup>a</sup>	81.58 $\pm$ 0.70 <sup>a</sup>	0.27 $\pm$ 0.02 <sup>a</sup>	3.47 $\pm$ 0.06 <sup>a</sup>	nd
Lpm	8.90 $\pm$ 0.10 <sup>b</sup>	2.43 $\pm$ 0.11 <sup>b</sup>	0.59 $\pm$ 0.02 <sup>a</sup>	9.85 $\pm$ 0.19 <sup>b</sup>	78.23 $\pm$ 0.27 <sup>ab</sup>	0.52 $\pm$ 0.01 <sup>b</sup>	4.23 $\pm$ 0.06 <sup>b</sup>	nd
Lpl	7.14 $\pm$ 0.13 <sup>c</sup>	2.55 $\pm$ 0.02 <sup>b</sup>	0.06 $\pm$ 0.02 <sup>b</sup>	7.20 $\pm$ 0.23 <sup>c</sup>	83.05 $\pm$ 0.06 <sup>b</sup>	0.48 $\pm$ 0.01 <sup>b</sup>	2.25 $\pm$ 0.09 <sup>c</sup>	nd
Pys	10.91 $\pm$ 0.29 <sup>a</sup>	3.20 $\pm$ 0.04 <sup>a</sup>	0.23 $\pm$ 0.00 <sup>a</sup>	5.92 $\pm$ 0.42 <sup>a</sup>	79.74 $\pm$ 0.19 <sup>a</sup>	1.09 $\pm$ 0.03 <sup>a</sup>	12.70 $\pm$ 0.52 <sup>a</sup>	nd
Pym	7.57 $\pm$ 0.26 <sup>b</sup>	3.68 $\pm$ 0.01 <sup>b</sup>	0.17 $\pm$ 0.02 <sup>b</sup>	5.90 $\pm$ 0.07 <sup>a</sup>	82.68 $\pm$ 0.24 <sup>b</sup>	0.80 $\pm$ 0.02 <sup>b</sup>	10.27 $\pm$ 0.23 <sup>b</sup>	nd
Pyl	7.19 $\pm$ 0.14 <sup>b</sup>	3.70 $\pm$ 0.02 <sup>b</sup>	0.08 $\pm$ 0.01 <sup>c</sup>	6.81 $\pm$ 0.09 <sup>b</sup>	82.22 $\pm$ 0.64 <sup>b</sup>	0.87 $\pm$ 0.10 <sup>c</sup>	7.47 $\pm$ 0.40 <sup>c</sup>	nd
Dpys	11.26 $\pm$ 0.41 <sup>a</sup>	3.33 $\pm$ 0.12 <sup>a</sup>	0.77 $\pm$ 0.09 <sup>a</sup>	4.48 $\pm$ 0.29 <sup>a</sup>	80.16 $\pm$ 0.91 <sup>a</sup>	0.81 $\pm$ 0.10 <sup>a</sup>	14.20 $\pm$ 0.17 <sup>a</sup>	nd
Dpym	8.56 $\pm$ 0.16 <sup>a</sup>	3.35 $\pm$ 0.06 <sup>a</sup>	0.58 $\pm$ 0.04 <sup>b</sup>	6.65 $\pm$ 0.03 <sup>b</sup>	81.86 $\pm$ 0.16 <sup>b</sup>	1.09 $\pm$ 0.03 <sup>b</sup>	15.27 $\pm$ 0.06 <sup>b</sup>	nd
Dpyl	8.10 $\pm$ 0.05 <sup>b</sup>	3.42 $\pm$ 0.17 <sup>a</sup>	0.53 $\pm$ 0.04 <sup>b</sup>	6.49 $\pm$ 0.29 <sup>b</sup>	81.46 $\pm$ 0.06 <sup>b</sup>	0.73 $\pm$ 0.02 <sup>a</sup>	13.07 $\pm$ 0.12 <sup>c</sup>	nd

Values with the same letter in one column showed no significant difference ( $p < 0.05$ ), CH=carbohydrate, s=small, m=medium, l=large, nd=not detected



**Figure 2** Cross section of DPY tuber.

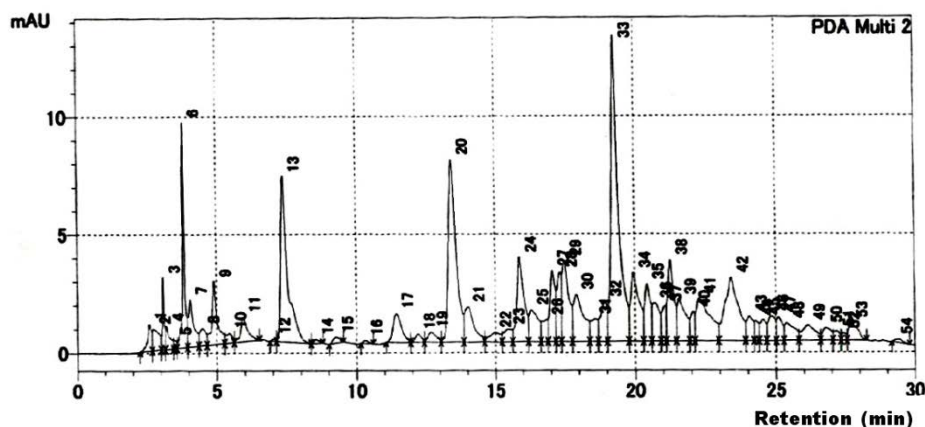
The higher lipid content in the small flour fraction can be ascribed to the different fat content in the tuber, which may result from the process of making the yam flour. The degradation of the bulb color (Figure 2), which becomes paler towards the edges, indicates a difference in chemical content between the central part and the tuber edges. This is similar to wheat and corn, which have a

difference in composition between the endosperm (middle) and the pericarp (edge) [21].

Flour lipids existed in the form of polar and non-polar lipids. The polar lipids are in the form of a phospholipid, which is derived from the cell membrane. The non-polar lipids are in the form of triglycerides that are trapped between the components in the flour particles and form a complex [22]. It has been reported that lipids in grain can be separated into three groups, namely non-starch lipids, granule surface lipid (triglycerides, free fatty acids, glycolipids and phospholipids) and internal lipids (lipid monoacyl mainly with major components of free fatty acids and lysophospholipids), which form a complex with amylose [23].

Proteins in the flour fractions existed especially in the form of dioscorin. The protein in flour is in complex form with lipids or carbohydrates. The protein complex in yam commonly exists in the form of complex glycoproteins that are concentrated in the mucus of the yam tuber, which is called mannan-dioscorin [24]. This lipoprotein complex has an influence on the formation of the flour paste, and can lower the viscosity and increase the temperature of the paste [25].

The levels of phenols obtained were higher than reported previously by Ozo, *et al.* [5], and Bhandari and Kawabata [26]. It was found that the phenols were concentrated in the small flour particles of YY and PY, in the medium flour particles of LPY and DPY, and in the large flour particles of OY. Anthocyanin was more concentrated in the small flour particles of PY and in the medium flour particles of LPY and DPY. Carotenoids were more concentrated in the small flour particles of YY and in the medium flour particles of OY.



**Figure 3** HPLC profile at  $\lambda= 326$  nm of large flour particles of DPY flour.

In the HPLC analysis of phenolic compounds, a number of peaks was obtained at  $\lambda = 326$  nm (Figure 3), which is the maximum  $\lambda$  for flavonon. There were 22 and 28 peaks for the small and medium flour fractions of LPY respectively; 52 and 34 peaks for the small and medium flour fractions of PY respectively; and 55, 50 and 54 peaks for the small, medium and large flour fractions of DPY respectively. The HPLC profile shows that many other compounds besides anthocyanin compounds belong to the phenolics in the yam flour, which together cause the purple color of flour (LPY, PY and DPY). As consequence, these compounds contribute to higher levels of phenols. As observed by previous researchers, tropical tubers have a very diverse phenolic composition [5,26,27].

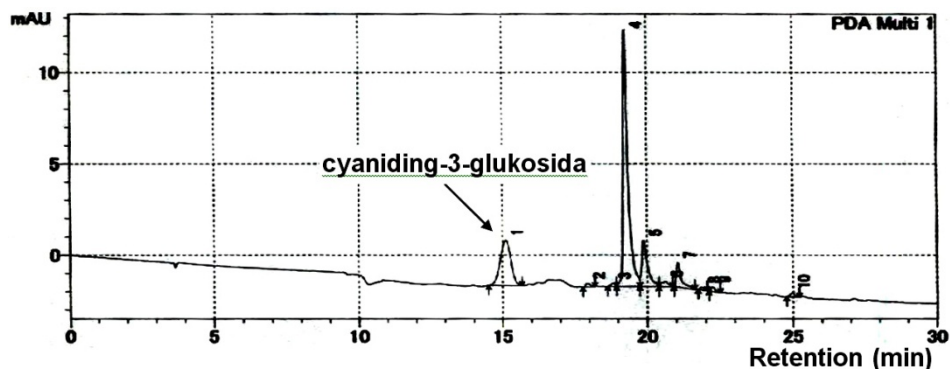
Considering the rather high levels of phenols obtained (0.27 to 1.09 % in 100 g dw of flour), the anthocyanin levels in these observations were quite low (3.47 to 15.27 mg in 100 g dw of flour). This level is much lower than that reported by Champagne, *et al.* [27]. This could be due to the extraction of anthocyanin not being maximal. The methanol extraction method with a slightly acidic solution (HCl) could have led to methanol evaporation and caused the acyl bonds in the anthocyanin compounds to be hydrolyzed, which could reduce the amount of anthocyanins produced. In addition, low levels of anthocyanins also may be due to the steam blanching and chip drying methods, which may reduce the anthocyanin level. As Lahacoompol [28] found, high temperatures can reduce the anthocyanin level up to 53%.

Anthocyanins are water-soluble pigments, therefore the low levels of anthocyanin in the large flour particles could be due to the low levels of moisture, while being more concentrated in the small flour particles of PY. The high levels of anthocyanin in the medium particles of LPY and DPY was due to the small surface contact of the particles, which can reduce anthocyanin damage during blanching and drying. The anthocyanin levels, for all size fractions, from the lowest to highest, were shown by LPY, PY and DPY.

The obtained anthocyanin profile had similar peaks as standard cyaniding-3-glucoside for the small fraction of DPY flour (Figure 4), as also found by Rasper and Coursey [29] and Ozo, *et al.* [5]. However, petunidin was not found in all flour fractions. In addition, four other peaks were obtained, of which the sharpest and highest one was obtained at 19 minutes of retention time.

According Jordheim [8], there are six natural aglycones of anthocyanin, sequentially ordered according to retention time as follows: delphinidin < cyaniding < pelargonidin < petunidin < peonidin < malvidin. Champagne, *et al.* [27] found that petunidin appears earlier than pelargonidin (petunidin < pelargonidin). In the obtained profiles, the highest peak was at a much greater

retention time for petunidin. Thus, it is assumed that the peak was located between the aglycon of anthocyanin pelargonidin and peonidin.



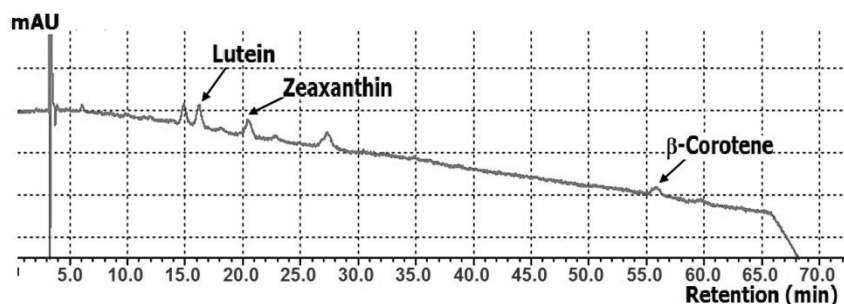
**Figure 4** HPLC profile of anthocyanin at  $\lambda= 520$  nm for small particles of DPY flour.

The carotenoid content in the YY and OY flour had fairly low levels (24 to 132  $\mu\text{g}$  towards  $\beta$ -carotene in 100 g dw of flour). This carotenoid level is higher than that reported by Champagne, *et al.* [10] for *Dioscorea alata*. The low level of carotenoids could have occurred due to the steam blanching and oven drying of the chips. The loss of carotenoids was reported by Bechoff [30], which can reach up to 57% on hot drying of potato chips.

The carotenoid components obtained were more concentrated in the small fraction of YY flour, while more fat was concentrated in the medium particles of OY flour. This could occur because the surface areas of the small particles and the large particles are different: small particles have a larger surface area than larger particles. A larger surface area of the particles leads to the higher carotene damage in the small particles on hot drying. It has been reported that carotenoids was fat-soluble pigments, which causes high levels of the carotenoids found in the small particles of YY flour.

The carotenoid HPLC graph shows that there were at most five major peaks in the large particles of the YY flour (Figure 5). Compared with the standard carotenoid compounds, three peaks were obtained that were similar to the standard, i.e. lutein, zeaxanthin and  $\beta$ -carotene. Another peak was found in the position under lutein and another one between zeaxanthin and  $\beta$ -cryptoxanthin. Only four peaks were obtained in the OY flour. Two peaks were found in the large particles of the OY flour indicating lutein and  $\beta$ -carotene. Two other peaks were under lutein and between zeaxanthin and  $\beta$ -cryptoxanthin.





**Figure 5** HPLC graph of carotenoids in the large fraction of YY flour.

These findings are consistent with previous findings that yam has a carotenoid in the form of lutein, zeaxanthin and  $\beta$ -carotene [10]. In addition, Champagne, *et al.* [10] also reported that there are unknown carotenoid compounds with a retention time under lutein and between zeaxanthin and  $\beta$ -carotene. These three carotenoid components are eye nutrition and provitamin-A, which are needed for food intake [11]. The content of carotenoids in the yam flour indicates that it has potential as a provitamin-A supply.

#### 4 Conclusions

Overall, it was found that the flour particles had sizes ranging from 7.50 to 1531.91  $\mu\text{m}$ . The yam particle size distribution was unimodal and non-symmetrical, with a higher proportion of the larger sizes. Size fractions were separated as follows: small particles (128.6 to 139.7  $\mu\text{m}$ ), medium particles (228.7 to 257.9  $\mu\text{m}$ ), and large particles (475.4 to 596.3  $\mu\text{m}$ ). Moisture content (6.81 to 7 %dw) and fat (0.01 to 3.19 %db) increased with the increase of the flour size. No influence was found of size toward protein content (4.48 to 9.85 %db), phenol content (0.27 to 2.82 %db), anthocyanin content (2.25 to 15.27 mg/100 g db) of the LPY, PY, DPY flours and the carotenoids (23.75 to 132.12 mg/100 g db) of the YY and OY flours. The polyphenol, carotenoids, and anthocyanin pigments tended to be higher in the medium flour fraction, which could be due to the lower level of damage of the hot drying process and a difference in composition between the center and the edges of the tuber, which results from the milling process.

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