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Physicochemical and Bioactives Characteristics of Purple and YellowWaterYam(Dioscorea alata) Tubers

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Abstract: This study aimed to characterize the physico-chemical properties and identify the bioactive compounds including diosgenin, water soluble polysaccharides, andwatersoluble proteins containing dioscorinfrom purple water yam(*Dioscorea alata*var*purpurea*) and yellowwater yam(*Dioscorea alata*L.). Both varieties of water yamcontainwater soluble polysaccharides that had CH_3, CH_2, OH, NH,C=O, acetyl (C-O), carboxyl (COOH), and C-O-C groups. WSP hydrolyzate contained more glucose. Mannose, arabinose, glucoronic acid and galacturonic acid in small quantities. Purple and yellow water yam contained 25.94 and 25.45% dioscorin from water soluble protein. The analysisof aminoacid profileshowed that the water soluble proteinofwater yam comprisesofaspartate, glutamate, serine, histidine, glycine, arginine, alanine, tyrosine, valine, phenylalanine, ileusin, leucine, andlysine. Both tubers also had diosgenin, a steroidal saponin of yam family.
Keywords: water yam, diosgenin, water solublepolysaccharides, watersoluble proteins, dioscorin.

Introduction

Dioscorea family or yam tuber consists of about 600 species which are around 50-60 species cultivated or food and medicine. The well known yam family in Indonesia is *D.alata* L, *D.hispida*, *D. bulbilfera* L, and *D.esculenta* that have productivity of 60-70 ton/ ha, but these tubers have not yet been used intensively. *Dioscorea alata* Lor water yam is acreep and shrub plant, generally cultivated in between forest plants[1].Water yam tuber is differentiated by its fleshcolor, namely purple, yellow, and white. It is reported that *Dioscorea* contains several bioactive compounds such as diosgenin, water soluble polysaccharides, and dioscorin[2,3].

Diosgenin is a phytochemical compoundto preventcolon cancerand decreasecholesterol absorption[4,5]. Water soluble polysaccharides of yam could be used to reduce the blood glucoseand cholesterol levels (especially the low density lipo protein cholesterol)[6,7,8].Dioscorin of *Dioscorea* tubers is aproteins that bind strongly to water soluble polysaccharides[9]. *Dioscorea alata* cv.Tainong number 1 fromTaiwan contains dioscorin up to 90% of water soluble protein. Dioscorin is a storage proteinin some species of yam that has function as trypsin inhibitor, carbonic anhydrase, antioxidant, immunomodulator, and antihypertension [10,11,3,12].

The purposeof this study is tocharacterize the physico-chemical properties and identify bioactive compounds of purple and yellow water yams, including diosgenin, water soluble polysaccharides, and dioscorin.

Materials And Methods

Materials

One of purple (*Dioscorea alata* var *Purpurea*) and yellow water yam (*Dioscorea alata* Linn.) were obtained from a local market inTuban, East Java, Indonesia. Reagents used for analysis and bioactive compound extraction were 96% ethanol (pa), distilled water, sulfuric acid, chloroform, trichloroacetic acid10%, KBr, $Ba(OH)_2$, glucose (sigma), mannose (sigma), galactose (sigma), arabinose (sigma), rhamnose (sigma), glucoronic acid (sigma), galacturonic acid (sigma), acetonitrile, water for HPLC, Tris, HCl, acrylamide, bis-acrylamide, glysine, TEMED (N-tetramethyl-ethylenediamine), protein marker (sigma, St Louis, MO, USA), coomassie blue R-250, Na_2 EDTA, KCl, PVP (polyvinylpyrrolidone), DTT (dithiothreitol), APS (ammonium persulfat), acetate acid glacial, methanol, HCl (pa), NaOH, sodium acetate, THF (tetra hidrofuran), OPA (orthophalaldehid), and H_2SO_4 0.005 M.

Methods

Extraction of diosgenin

The extraction of diosgenin[13],10 grams of water yam tuber were cut into small pieces. Then, they were added 100mL of ethanol-sulfuric acid 2.5M.The mixture were refluxed for 4 hours at 80^{o}C. The solution were cooled and filtered through a Whatman filter No. 1. The filtrate were diluted to 200 mL with distilled water. The solution were extracted with chloroform10 mL for 3 times. Chloroform extract was evaporated under 60^{o}C.The evaporated wereredissolved in chloroform to 10 mL.

Extractionof water soluble polysaccharides (WSP)

Fresh water yam tubers were peeled, cut, weighed and added distilled water with a ratio 1:3 (w/v) then they were blended and filtered. Water yam juicewerecentrifuged at 4500 rpm for 20 minutes. After that, supernatant were taken and precipitated with 96%ethanol (1:4) for 36 hours[14].Wet water soluble polysaccharide extracts is dried at 50^{o}C for 18 hours.

Extractionof water soluble protein

Supernatantof water yam juice were added with a ratio 1:1 of 10% trichloroacetic acid, mixed evenly, and centrifuged at 3500 rpm for 20 minutes. The obtained precipitate waswashed by distilled water until neutral pH. This is modification from protein extract methode biuret[15].

Physico-chemical properties

Purple and yellow water yam were characterized for their physical properties by descriptive test with scoring[16]. Physical analysis were based on the test scoring of 20 panelists on tuber peel color, tuber shape, tuber peel texture, the abundance of mucilage, the intensity hair bulbs, color of the flesh, and texture of the flesh.The chemical analysis were moisture, ash, protein, and fat, crude fiber[16], soluble fiber, insoluble fiber[17],and antioxidant activity[18].The analysis of soluble and insoluble fiber usedthe multi enzyme method.

Identificationof bioactive compound

The diosgenin extract wasdissolved in 1 mL of 96% ethanol and then injected on HPLC (Shimadzu), a Sunfire®C18 column (150 mL x 4.6 mm, 5 µm), the experimental conditions were an isocratic binary system of acetonitrile: water (90:10), a flow rate of 1 mL/ min and a temperature of 35°C. Detection was performed at 194 nm[19].

Functional groups of dry water soluble polysaccharide were analyzedby Fourier Transformation Infra Red Spectrometer (Shimadzu, model 8400S). The analysis of sugar constituent were performed by HPLC. Before

HPLC analysis, water soluble polysaccharides was hydrolyzed by strong acid[20]. HPLC (KNAUER) with Refractive Index S2300 detector, Aminex HPX-87H column (300 x 7.8 mm), and H_2SO_4 0.005 Mas eluent.

Soluble protein extracts containing dioscorin were analyzed in Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (Bio-Rad, Richmond, USA) to identify the composition of protein based on molecular weight, and gel documentation (Chemidoc XRS, Bio rad Laboratories) were used forquantitative analysis of separated protein. Amino acids composition of water soluble protein were analyzed by HPLC (ShimadzuLC10). Licrospher®100 RP 18column (5mm), length 125 mL x 4 mm with mobile phase A were CH_3OH: 50 mM sodium acetate: THF (2:96:2) pH 6.8 and mobile phase B 65% CH_3OH (modification from AOAC, 2005), and detector of Fluorescence (Shimadzu RF-138).

Results and Discussion

Physical-chemical properties

There are variations of tuber shape regularity on various sizes of purple water yam tubers, while the yellow one was almost similar. Purple water yam peel texture more rough than yellow one. The leather peel texture of purple yam were more rough on a larger tuber. Mucus from purple water yam tuber were influenced by the tuber size, where bigger tuber has more mucus. The mucus of yellow water yams were vary and did not depend on tuber size. Yellow water yams were more hairy than purple yam. The hair were influenced by the tuber size, where the bigger tuber has more hair. The yellow water yam texture not be affected by tuber size while purple yam affected. The larger size of the tuber had more delicate texture (Table 1), which may related to the size of the tuber starch granules.

Chemical characteristics of purple water yam were similar with yellow water yam tuber (Table 2), except the water content and carbohydrate. Yellow water yam had a higher moisture content. Yellow water yam did not have economic value because of the high moisture content.Mean while, there were several sub-types of *Dioscorea alata* which had low moisture content and high level of starch[21]. Protein of water yam tuber were lower than expectation. Dioscorin was a reserve protein in Dioscorea group that medicinal properties.Likewise, the fat content were quite low, so the most contribution to energy of water yam tuber comes from carbohydrates.Crude fiber content of water yams were lower than fruits and vegetables. Soluble fiber of water yam were almost similar. Dietary fiber were often underestimated, compared with protein, vitamins, and other nutrients. Although not classified as nutrients, dietary fiber proved to be very beneficial for health. From the analysis of insoluble fiber also indicates that the water yams contain high insoluble fiber.

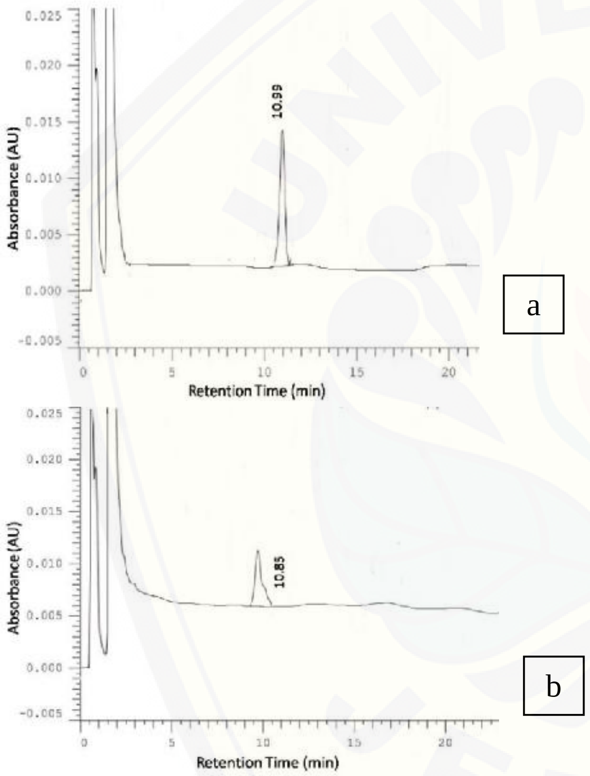
Table 1: The difference in physical properties of purple and yellow water yam tubers

Physical Property	Purple water yam	Yellow water yam
Color of the peel	dark brown	light brown
Tuber shape	Slightlyirregular (oval and round)	Irregular (oval, round, and fingered)
Texture of the peel	rough	rather smooth
Manylittle mucus	many mucus	rather mucus
Presence or absence ofhair	little hair	rather hair
Color of the flesh	purple	light yellow
Textureof the flesh	smooth	rather smooth

Table 2: Chemical composition of purple and yellow water yam

Component (%)	Purple water yam	Yellow water yam
Water	78.12	87.75
Ash	0.69	0.81
Protein	1.69	1.58
Fat	0.97	0.47
Carbohydrat by difference	18.53	9.39
Crude fiber	1.46	1.17
Soluble fiber	3.23	3.28
Insolublefiber	36.06	51.45

Figure 1: Chromatogram of extract diosgenin from purple (a) and yellow water yams (b)

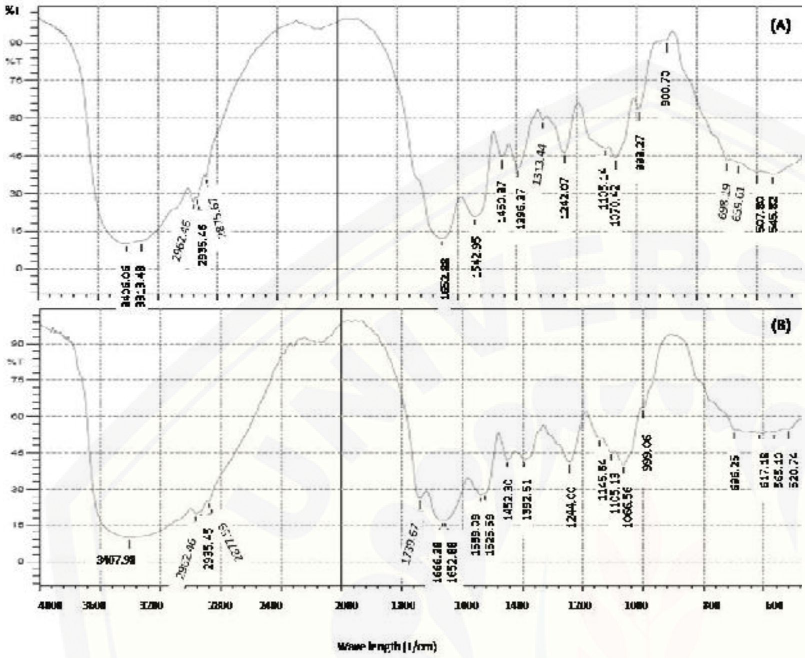


Diosgenin

Diosgenin was a steroidal sapogenin from acid, base, or enzyme hydrolysis of saponins which has chemical formulaof $C_{27}H_{42}O_3$. The purple and yellow water yams contained diosgenin of 0.015 g/ kg and 0.006 g/ kg. *Dioscorea alata* var *Purpurea* from Mumbai contained 0.78 g/kg of diosgenin[2]. The highest content of diosgenin glycoside were in subterranean organs such as rhizomes or tubersand the contents werevary which depends on the individual and age of the plant. Diosgenin content of yam family were vary depending on the species and extraction methode.

According Behera et al.[22], edible yams did not contribute for steroid production and the present study revealed that diosgenin content were very low in right twining species as compared to left twining species.The highest amount of diosgenin was observed in *D. bulbifera* (13.83 g/ kg) followed by *D. hispida* (8.25 g/ kg) and *D. pentaphylla* (8.18 g/ kg). Among right twining species *D.oppositifolia* contained the highest amount of diosgenin 6.58 g/ kgand among others *D.alata* had the lowest amount of diosgenin 0.95 g/ kgascended by *D.belophylla* (1.20 g/ kg), *D.wallichii* (1.29 g/ kg).

Figure 2: Spectrogram of water soluble polysaccharides (WSP) from purple (a) and yellow (b) water yams



Water Soluble Polysaccharides (WSP)

Figure 2 showed a similar pattern of WSP purple and yellow water yams based on FTIR spectrum. It means that purple and yellow water yams had almost identical functional groups. Purple water yams were marked C-O-C functional groups at 1070.42 and 1105.14 cm^{-1} while the yellow was at 1066.56, 1105.13, and 1145.65 cm^{-1}. C-O-C functional groups showed that a water soluble polysaccharides in purple and yellow water yams were β-1, 4 mannosidic that connecting mannose foreach other. Functional group C=O stretch was shown in wave numbers 1652 and 1739.67 cm^{-1}. C=O group showed the aldehyde group making up a simple sugar mannose. It was supported Yu *et al.*[23]that the existence of a bond β-1, 4 glucosidic and β-1,4 mannosidic of glucomannan were marked with C-O-C stretch vibration at 1027.02 and 1244 cm^{-1}.

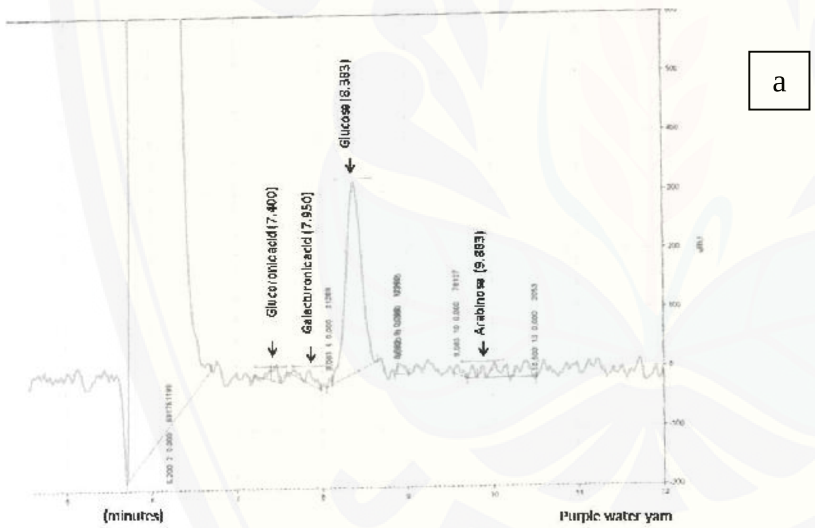
Results of this analysis indicated the presence of an acetyl group (CH3-CO), respectively the number frequency 1242.07 cm^{-1} and 1244 cm^{-1} of WSP purple and yellow water yam, which was the side groups of glucomannan, hence it were suggested that the water soluble polysaccharide of water yam were glucomannan. According to Parry[24], glucomannan had an acetyl group by 10-19 units of carbon clusters at C2, C3, and C6 positions. Numbers wave frequency 2875.67; 2935.46, and 2962.46 cm^{-1} in purple water yam whereas 2877.59; 2935.45, and 2962.46 cm^{-1} in yellow water yam were a carboxylic acid functional group (RCOOH). Presence of carboxylic acid showed a WSP had the result of oxidation glucose or galactose. Simple sugars had primary alcohol group (carbon atom, C number 6) were changed to carboxyl, such as glucoronic acidand acid galacturonic.

The wave number of 3406.06 cm^{-1} in purple water yam and 3407.98 cm^{-1} in yellow water yam were functional group of N-H (secondary amide, CONHR), that indicated the WSP was bound to proteins. This finding was supported by the result of previous research[9]that dioscorin (a type of protein in *Dioscorea*) was strongly bound to the WSP from *Dioscorea hispida*. Dennst and Lu[25]revealed that dioscorin protein which was bound to the WSP had secondary structure.

The analysis using HPLC (KNAUER) and Aminex HPX 87H column.The column could be elute with standard sugars glucose, mannose, galactose, arabinose, rhamnose, glucoronic acid, and galacturonic acid. But, the type of sugar glucose and mannose could not be separate well as retention time almost the same. Figure 3, 4, and Table 3showedthe sugar analysis of WSP hydrolyzate from purple and yellow water yam. Hydrolyzate were obtained from dried WSP of purple or yellow water yam as much as 0.1 grams then were hydrolyzed with H_2SO_4 80% and 20%, neutralized with Ba $(OH)_2$ to pH 7 with final volume of 175 mL. WSPhydrolyzate contained more glucose. Mannose, arabinose, glucoronic acid and galacturonic acid in small quantities. Galactose and rhamnose werenot detected in the hydrolyzate. Sugar analysis were affected polysaccharide hydrolysis process and the purity of WSP. $Ba(OH)_2$ could be reduce mannoselevel. Aman and Westerlund[26] explained the glucomannan and mannan could be precipitate by $Ba(OH)_2$ group likely due to the reaction of 2,3-cis-hydroxyl on mannose residues.

Dioscorea opposita contains mucus from mannan and proteins[3]. It is also supported by Liu *etal*.[27]that the major sugar composition of *Dioscorea batatas* is mannose, in addition to a small proportion of glucose and galactose in a ratio of 76.7, 16.9, and 6.3%, respectively. The sugar linkage of the polysaccharides was mainly 1,4-mannosidic. In addition, the acetyl groups are found in the polysaccharides, and the ratio of *O*-acetylation is estimated as 28% and shows the *O*-acetylation of C-2 and C-3 positions.

Figure 3: Chromatogram of glucoronic acid, galacturonic acid, glucose, arabinose (a) and mannose (b) from hydrolyzate WSP of purple water yams by AMINEX HPX 87H Column



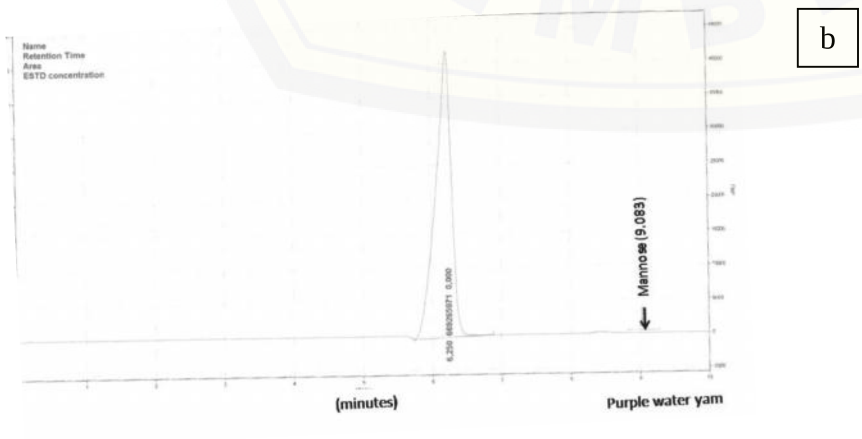


Table 3: The results of sugar analysis from WSP hydrolyzate of purple and yellow water yam

Types of sugar	Purplewater yam		Yellowwater yam	
	Concentration (%)	% Relative	Concentration (%)	% Relative
Glucoronic Acid	0	0.00	0.002	5.71
Galacturonic Acid	0.001	2.56	0.001	2.86
Glucose	0.031	79.49	0.027	77.14
Galactose	0	0.00	0	0.00
Mannose	0.004	10.26	0.003	8.57
Rhamnose	0	0.00	0	0.00
Arabinose	0.003	7.69	0.002	5.71
Totale	0.039	100.00	0.035	100.00

* Concentration of glucoronicacidinpurple water yamwere0.0003% but rounded to0%

Figure 4: Chromatogram of glucoronic acid, galacturonic acid, glucose, arabinose (a) and mannose (b) from hydrolyzate WSP of yellow water yams by AMINEX HPX 87H Column

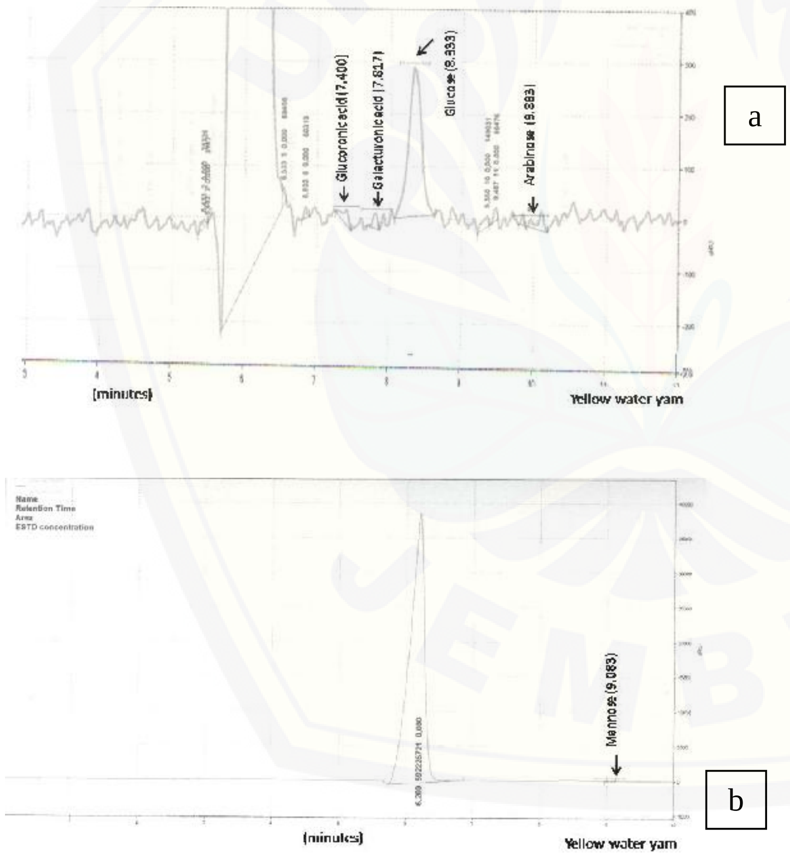


Figure 5: SDS PAGE of water yam (M:marker; A: purple water yam; and B: yellow water yam).

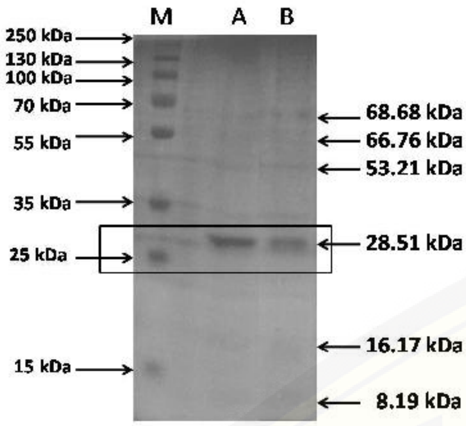


Figure 6: Chromatogram of amino acid purple water yam

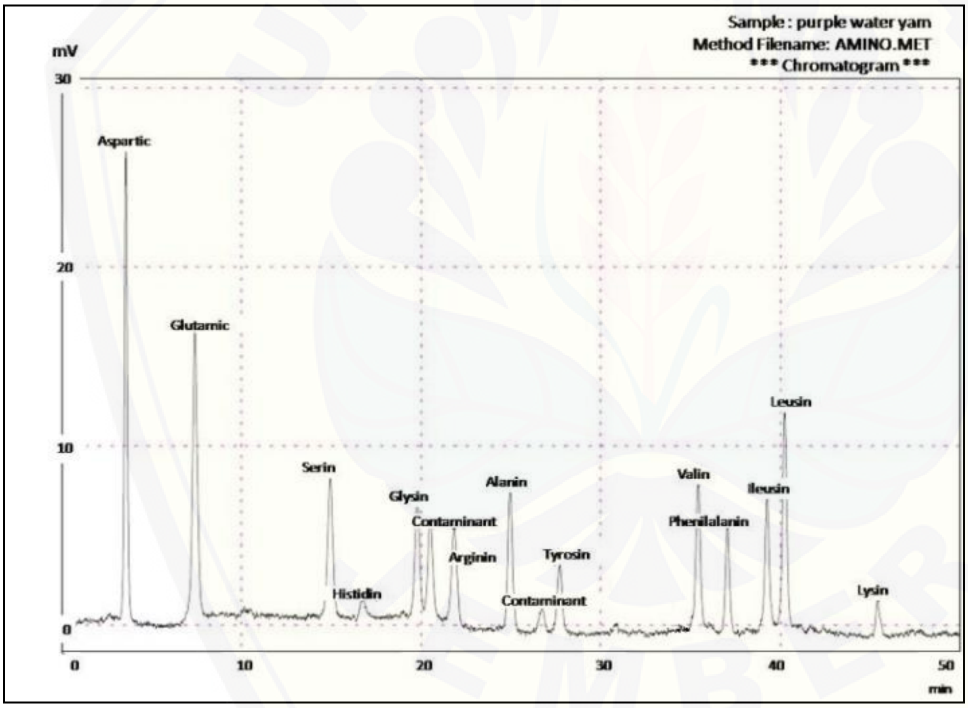
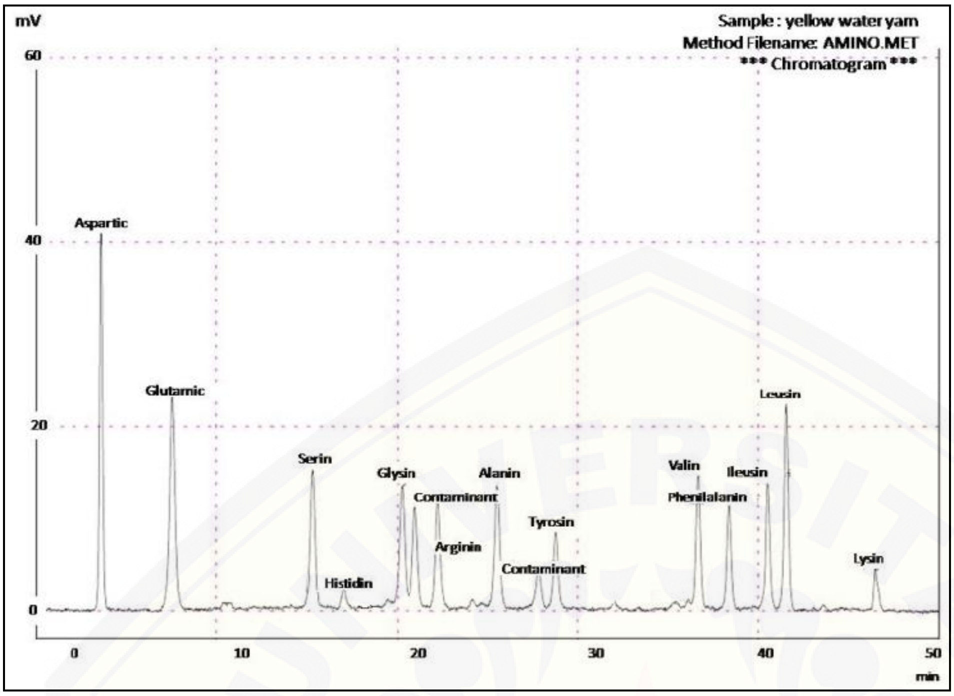


Figure 7: Chromatogram of amino acid yellow water yam



Dioscorin

The water soluble protein in purple and yellow water yams contained dioscorin with molecular weight of 28.51 kDa. This finding was in accordance to the result that reported by Myoda *et al.*[3]who indicated that molecular weight of *Dioscorea opposita* dioscorin were 23 and 32 kDa, whileGaidamashvili[28] revealed that molecular weight of dioscorin of *Dioscorea batatas* was 31 kDa.

The dioscorin content of purple and yellow water yams were 28.94% and 25.45% from total water soluble protein. The other types of proteins such as maltose binding lectin (68.68 kDa and 66.76 kDa), alcohol dehydro genase (53.21 kDa), arabinogalaktan (16.17 kDa), and mannosebinding lectin (8.19 kDa) were found in *Dioscorea alata*. Gaidamashvili[27] found four major proteins of *Dioscorea batatas*, namely mannosebinding lectin (10 kDa), maltose binding lectin (66 kDa), dioscorin (31 kDa) and acidic chitinase homologous to chitinase from *Dioscorea japonica* with a ratio of 20:50:20:10. Protein band with a molecular weight of 16.7 kDa were arabinogalactan protein[29].

The protein of yellow and purple water yams were composed of aspartate, glutamate, serine, histidine, glycine, arginine, alanine, tyrosine, valine, phenylalanine, ileusin, leucine, and lysine by analysis HPLC and acid hydrolysis methode. Myoda *et al*.[3]showed that dioscorin sequence consists of VEDEFSYIEGNPNGPENWGN. The results of chromatogram showed that the peak of amino acids aspartate, glutamate, serine, glycine, tyrosine, valine, phenylalanine, and ileusin, supports the existence of protein dioscorin on yellow and purple water yams. However asparagine, tryptophan, and proline which were constituent of dioscorin, but it were not detected. Acid hydrolysis in sample preparation caused the damage of several other amino acids.

Conclusion

Purple water yam (*Dioscorea alata* var *Purpurea* and yellow water yam (*Dioscorea alata* L.) had different physical and chemical composition.The two tubers also contain bioactive compounds of diosgenin, water soluble polysaccharides, and dioscorin. Water soluble polysaccharides and soluble proteins from purple and yellow water yamhad similar characteristics, although their levels were slightly different. Water soluble polysaccharide hadmethyl group (-CH3), methylene (-CH2), OH, NH, C=O, acetyl (C-O), carboxyl (COOH), and C-O-C. WSP hydrolyzate contained more glucose. Mannose, arabinose, glucoronic acid and galacturonic acid in small quantities. Soluble protein of purple and yellow water yam contains dioscorin as one of bioactive compounds of yam family.

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