Compositional, spectroscopic and rheological analyses of mucilage isolated from taro (*Colocasia esculenta* L. Schott) corms

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Revised: 1 September 2011 / Accepted: 17 October 2011 / Published online: 24 November 2011
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Abstract Tropical roots and tubers generally contain mucilage. These mucilages exhibit unique rheological properties with considerable potential as a food thickener and stabilizer. A one-step extraction procedure was used to isolate starch free mucilage and associated proteins from a number of taro (*Colocasia esculenta*) varieties. The monosaccharide and amino acid composition, the structural and flow properties were investigated. The results showed that yield of mucilage fraction varied from 30 to 190 g.kg⁻¹. A negative correlation ($r=-0.87$; $p<0.05$) was observed

between the crude protein level and the yield. The monosaccharide profiles revealed that galactose, mannose and arabinose were the main monosaccharides in the hydrolysate of the mucilage. From the 17 amino acids analyzed, aspartic acid/asparagine (14.4–17.2%) and glutamic acid/glutamine (10.3–13.6%) were prominent in the mucilage as well as the flour. No significant differences were observed in the FT-IR spectra and in the viscosity behavior of the mucilage dispersions. The greatest difference in the mucilage is based on its monosaccharide profile while the protein composition, which reflects that of the flour, is relatively stable.

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Keywords Taro · Mucilages · Sugar composition · Amino acid composition

Introduction

Taro, *Colocasia esculenta*, is a plant of the Araceae family that is widely cultivated in the tropical areas of the world for its leaves and underground tubers (corms and cormels). It is found in South East Asia, the Pacific Islands, the Mediterranean, Africa and in the North America (Jane et al. 1992; Tagodoe and Nip 1994). The corms are low in fat, proteins and vitamins, but are a good source of carbohydrates and minerals, especially potassium, magnesium and calcium (Kaur et al. 2011; Nip 1997). Taro contains high levels of gum, which has also been shown to play a role in the reduction of high blood pressure, in hypercholesterolemia and in the management of diabetes (Habashy and Radwan 1997; Nip 1997). Taro flour has been used as a source of food thickeners (Onyeike et al. 1995). The use of crude taro gums as binding and emulsifying agents has also

been reported (Nip 1997). Due to its mucilage, the stabilization effect of precooked taro flour on fruit juice separation has been shown to be advantageous as compared to that of commercial stabilizers, such as locust bean gum, carboxymethylcellulose and carrageenan gum (Hong and Nip 1990). Recent studies initiated in our laboratories, aimed at producing and using precooked taro flour also pointed out the role of mucilage in the texture of the paste (Njintang et al. 2008). Lin and Huang (1993) have attributed the slimy texture of taro corms and taro products to the presence of water soluble gum or mucilage. In fact, it has been shown that starch/mucilage interaction lead to reduced viscosity and reduces pasting (Jiang et al. 1997) (Table 1).

Taro mucilage composition and texture have been previously studied by a number of research groups. Its sugar composition has established arabinose and galactose as the major sugars, but few or none studies have been carried out on the protein associated with the mucilage (Amin 1956; Lin and Huang 1993). Recently, Jiang and Ramsden (1999) reported the level of proteins associated with the mucilage, but there is no information on the amino acid composition of the proteins.

This study investigated the yields, the monosaccharide and amino acid compositions of the mucilage from five varieties of taro corms which could serve as baseline information related to the food technology of this species.

Materials and methods

Production of taro flour and extraction

Six varieties of taro corms were harvested after 9 months in 3 agro-ecological zones of Central Africa: Country Ekona (CE) and Ibo Ekona (RIE) were harvested from Ekona (forest zone, Cameroon), Country Ngaoundere (CN) and Ibo Ngaoundere (RIN) were harvested from Ngaoundere (savannah zone, Cameroon) while Kwanfre (KW) and

Sosso (WSC) were harvested from Ngaoundere and Chad (steppe, Chad) respectively. The corms were washed, peeled, sliced, and dried in a convection oven at 40 °C for 12 h. The dried slices were ground in a harmer grinder to pass through a sieve of 500 µm mesh. Flours were sealed in polyethylene bags and transported via airfreight to France for extractions and analyses.

Taro mucilage was extracted from taro flour in a saline buffer consisting of 50 mM Tris pH 8, supplemented with 10 mM EDTA, 0.1% v/v Triton X-100, 0.1% v/v β-mercaptoethanol. The mixture was incubated overnight at 4 °C with agitation followed by centrifugation at 3,000 × g for 20 min. The supernatant was collected and the mucilage precipitated by the addition of 4 volumes of 95% ethanol at 4 °C for 24 h. The precipitated polysaccharides were collected by centrifugation at 4,000 × g for 20 min. The pellets were washed twice with 80% ethanol and solubilised in deionised water followed by starch removal at 80 °C using a thermostable α-amylase and amyloglucosidase (EC 3.2.1.1; Megazyme International) as described by Li et al. (2006). The mucilage extract of each taro variety was then freeze-dried to powder which was stored in polyethylene bags and kept in a refrigerator at 4 °C prior to use. The mucilage yield was expressed as weight of the mucilage powder to the dry weight of the taro flour initially used.

Total protein and carbohydrate content

Protein content of the polysaccharide preparations was analysed using the Kjeldahl method with the nitrogen content being multiplied by a factor of 6.25 to give crude protein (AOAC 1990). The total carbohydrate content was determined by the phenol-sulfuric acid method using D-glucose as standard (Dubois et al. 1956) and was then expressed in mg.mg⁻¹. Total uronic acid (UA) content of water-soluble polysaccharides was estimated according to the method of Blumenkrantz and Asboe-Hansen (1973), and expressed in g galacturonic acid eq.kg⁻¹.

Table 1 Proximate composition (g/ 100 g) of the different varieties of taro flour

Parameters	Varieties					
	RIE	RIN	CE	CN	KW	WSC
Moisture	8.2±0.21 ^a	9.0±0.43 ^{bc}	9.6±0.11 ^c	8.5±0.42 ^{ab}	8.5±0.13 ^{ab}	8.8±0.18 ^b
Proteins	3.1±0.10 ^{ab}	3.4±0.12 ^c	4.6±0.14 ^d	4.9±0.24 ^c	2.9±0.09 ^a	3.3±0.12 ^{bc}
CHO	94.8	93.9	90.5	91.0	95.5	90.8
Fats	0.50±0.105	1.17±0.324	0.53±0.154	0.30±0.072	0.33±0.064	0.37±0.065
Ash	1.6±0.35 ^a	1.5±0.41 ^a	4.4±0.27 ^b	3.8±0.87 ^b	1.3±0.63 ^a	5.5±1.11 ^c

Each observation is a mean ± SD of three replicate experiments (*n*=3). CHO carbohydrate evaluated by difference. Means in the same row followed by different superscript are significantly different (*p*<0.05). CE Country Ekona variety; RIE Ibo Ekona variety; CN Country Ngaoundere variety; RIN Ibo Ngaoundere variety; KW Kwanfre variety; WSC white sosso chad variety

Monosaccharide composition and amino acids profile

Monosaccharide composition was determined according to Nguema-Ona et al. (2006). Briefly, 3–10 mg of dried polysaccharides was hydrolysed using trifluoroacetic acid. The hydrolysed monosaccharides were primarily converted into methyl glycosides, and then into trimethylsilyl (TMS) derivatives. TMS derivatives were analyzed by gas chromatography using a GC 3800 Varian instrument. Myo-inositol was added to the mixture as the internal standard. Raw data were integrated and analyzed using Varian GC Star Workstation software. The level of monosaccharide was expressed as mg.g^{-1} carbohydrates.

The taro mucilage sample containing 40 mg of nitrogen, determined by the Kjeldhal method, was weighed out into digestion tubes containing 6 N HCl in 1% phenol. The tubes were then flushed with nitrogen, sealed and incubated for hydrolysis in a Pico tag station (Waters, Milford, MA, USA) set at 150 °C for 60 min. Hydrolysed samples were centrifuged, filtered into vials and submitted to analysis on an auto derivatiser-analyser 420a (Applied Biosystems, Applera Corp, Foster City, CA, USA). The pre-column derivatisation used phenylisothiocyanate (PITC) and the separation was operated on a reverse phase column PTC RP-18 (2,1*220 mm) N° Series 238638, Lot N°02M9-40531 Brownlee (Applied Biosystems, Applera Corp, Foster City, CA, USA). Amino acid analysis was performed on a HPLC applied Biosystems model 172 A instrument (Applera Corp, Foster City, CA, USA). The amino acid derivatives were detected at 254 nm. The level of amino acid was expressed as mg.g^{-1} proteins.

Microscopy and viscosity analysis

Microtome sections (1.5–2 μm) of fresh taro corms showing mucilage duct and vesicles were prepared for light microscopy as earlier described (Njintang et al. 2006).

Mucilage was also observed from a fresh slice (2 cm thick) using stereomicroscopy.

Viscosity analysis was done on mucilage dispersed in distilled water at 2.5%. After mixing, viscosity of the samples was measured using a Rheostress rheometer equipped with a cone and plate geometry (Rheologica instruments AB). The cone angle and the diameter were 4° and 20 mm, respectively. The gap was set at 0.19 mm and sample volume was 0.5 mL. Twenty shear stresses of 80 s with intervals of 1 s were applied, increasing continuously from 0.02980 to 3,000 Pa. All the experiments were done in triplicates. Apparent viscosity was calculated from the linear fit of the shear strain versus shear stress curves during the upper phase for Newtonian samples and a power law fit was applied (Aboubakar et al. 2008).

Statistical analysis The means $\pm$ standard deviations are average values of triplicate determinations. One-way analysis of variance was applied to evaluate the variation within variety using Statgraphics Plus statistical software version 5.0 (Statistical Graphics Corp).

Results and discussions

Yield of the mucilage and extraction The yield and composition of mucilage showed considerable differences between the five varieties tested (Table 2). The WSC showed the lowest yield while the RIN variety exhibited the highest yield. Jiang and Ramsden (1999) reported a similar yield ranges varying from 30 to 190 g.kg^{-1} . The proportion of carbohydrates and proteins in the mucilage also varied significantly between the varieties. In fact, a negative correlation ($r=-0.87$; $p<0.05$) was observed between the crude protein levels and the yield, while a positive correlation was observed between the carbohydrate levels and the yield ($r=0.77$). This observation differs from that

Table 2 Yields, protein and total carbohydrate and uronic acid compositions of the taro mucilage

Flours	Yield of mucilage (g.kg^{-1})	Total proteins (mg.mg^{-1})	Total carbohydrates (mg.mg^{-1})	Uronic acids μg (eq. galacturonic acid) mg^{-1}
RIE	50.1 $\pm$ 5.52 ^{cd}	0.50 $\pm$ 0.041 ^{ab}	0.46 $\pm$ 0.101	15.8 $\pm$ 0.42 ^a
WSC	30.2 $\pm$ 4.62 ^d	0.48 $\pm$ 0.102 ^{ab}	0.50 $\pm$ 0.082	9.4 $\pm$ 0.13 ^c
CN	70.1 $\pm$ 3.69 ^c	0.43 $\pm$ 0.049 ^{bc}	0.55 $\pm$ 0.104	13.1 $\pm$ 0.22 ^b
KW	70.3 $\pm$ 6.43 ^c	0.40 $\pm$ 0.065 ^{bc}	0.56 $\pm$ 0.196	16.3 $\pm$ 0.43 ^a
RIN	189.9 $\pm$ 6.10 ^a	0.30 $\pm$ 0.072 ^c	0.69 $\pm$ 0.303	15.6 $\pm$ 0.34 ^a
CE	99.8 $\pm$ 4.54 ^b	0.47 $\pm$ 0.076 ^{ab}	0.56 $\pm$ 0.105	13.3 $\pm$ 0.19 ^b

Each observation is a mean $\pm$ SD of three replicate experiments ($n=3$). Means in the same row followed by different superscript are significantly different at $p<0.05$. CE Country Ekona variety; RIE Ibo Ekona variety; CN Country Ngaoundere variety; RIN Ibo Ngaoundere variety; KW Kwanfere variety; WSC white sosso chad variety

Table 3 Sugar composition expressed as mg. g⁻¹ of carbohydrates present in the mucilage

Sugars	Taro varieties					
	CE	CN	KW	RIE	RIN	WSC
Arabinose	5.9±0.11	7.5±0.22	7.1±0.02	5.2±0.31	4.4±0.12	8.3±0.34
Rhamnose	1.5±0.01	1.8±0.03	1.4±0.09	1.4±0.22	3.5±0.33	3.6±0.65
Fucose	3.3±0.23	3.0±0.32	3.5±0.23	1.0±0.30	1.7±0.08	1.0±0.01
Xylose	1.5±0.02	1.1±0.21	1.4±0.02	0.9±0.02	0.8±0.07	13±1.32
Galacturonic acid	4.2±1.24	4.6±0.22	5.7±1.22	5.6±1.07	12.3±0.31	7.4±1.11
Gluronic acid	9.7±1.09	11.7±1.44	13.3±1.31	7.0±1.38	9.6±0.63	7.5±1.07
Mannose	15.6±0.42	16.2±0.82	12.9±0.41	10.4±1.22	8.1±1.10	6.4±0.38
Galactose	48.1±0.33	46.2±1.34	45.2±0.22	62.0±1.52	56.3±1.41	59.7±0.76
Glucose	3.0±0.72	2.7±0.01	3.4±0.54	3.8±0.47	4.0±0.01	4.1±0.22
N acetyl galactosamine	1.0±0.04	0.72±0.012	1.0±0.10	1.0±0.03	0.32±0.023	0.12±0.011
N acetyl glucosamine	0.70±0.031	0.52±0.032	0.72±0.109	0.32±0.012	0.43±0.014	0.33±0.009
N acetyl mannosamine	4.4±0.73	2.9±0.22	3.3±0.93	0.91±0.119	1.6±0.13	0.90±0.120
2-O-Methyl Xylose	0.32±0.009	0.31±0.011	0.21±0.120	0.91±0.012	0.23±0.021	0.91±0.126

Each observation is a mean ± SD of four replicate experiments ($n=4$). *CE* Country Ekona variety; *RIE* Ibo Ekona variety; *CN* Country Ngaoundere variety; *RIN* Ibo Ngaoundere variety; *KW* Kwanfre variety; *WSC* white sosso chad variety

reported by Jiang and Ramsden (1999) who found that the proportions of protein and carbohydrate levels in mucilages are independent of the yield. In addition we found, in agreement with the report of Jiang and Ramsden (1999), a relatively high proportion of proteins (30–51%) in the taro mucilage. This result could be due to the saline buffer extraction step which we used to isolate our mucilage-rich fraction. Proportion of carbohydrate in mucilage varied from

46% to 69%, suggesting that carbohydrates are the main components. The differences observed within taro varieties in the present work compared with those in other studies may reflect differences in protein and carbohydrate structures present (length and number of carbohydrate chain, etc...). Plants used in this study were grown in different agro-ecological conditions, which suggest that different growth behaviors could have affected the mucilage yields and the

Fig. 1 Proportion of amino acids in the mucilages of the five varieties of taro flour. *ASX* represents the sum of asparagine (ASN) and aspartic acid (ASP) residues; *GLX* represents the sum of glutamine (GLN) and glutamic acid (GLU) residues

Table 4 Comparative composition of the amino acid profiles expressed as mg. g⁻¹ proteins in flour or mucilage of two varieties of taro

Amino acids	Varieties			
	RIN		CE	
	Mucilage	Flour	Mucilage	Flour
Asx	144.3	152.3	159.7	160.7
Glx	102.9	123.3	117.3	120.3
Ser	77.0	57.6	83.7	60.7
Gly	101.7	60.8	109.6	61.8
His	183	25.2	20.7	22.2
Arg	60.3	67.2	43.1	63.2
Thr	62.9	41.0	42.3	44.5
Ala	81.2	60.8	62.6	59.3
Pro	56.9	47.3	50.0	47.8
Tyr	20.2	10.5	26.4	14.6
Val	39.9	62.7	44.1	64.0
Met	7.8	1.0	11.8	1.2
Cys	11.4		13.8	
Ile	61.9	41.9	60.4	40.4
Leu	86.0	103.7	86.2	107.2
Phe	38.4	55.7	38.2	59.3
Lys	28.9	55.5	30.1	54.3

Values are average of triplicate measurements. Coefficients of variation were systematically lower than 0.05%. *Asx* represents the sum of asparagine and aspartic acid residues; *Glx* represents the sum of glutamine and glutamic acid residues. *CE* Country Ekona variety; *RIN* Ibo Ngaoundere variety

proportions of carbohydrates and proteins. In particular, response to environmental stress has been commonly associated with the variation of mucilage yield (Jiang and Ramsden 1999; Charles et al. 2008).

Monosaccharide composition The total monosaccharide composition of the mucilage is shown in Table 3. In general the percentage of glucose level appears generally lower when compared to earlier reports: 3.8–8.9% (w/w) for taro mucilage and 24.5–51.5% (w/w) for cassava mucilage (Jiang and Ramsden 1999; Charles et al. 2008). A little starch contaminated mucilage was obtained, as suggested by the low level of glucose found after treatment with α -amylase and amyloglucosidase. Galactose, mannose and arabinose were the main monosaccharides in the hydrolysate, suggesting that it is rich in galactomannan, glucomannan, or galactoglucomannan polysaccharides (Moreira and Filho 2008). Since the extraction buffer used in this study was primarily used to extract soluble cell wall proteins called arabinogalactan proteins (AGPs), it is reasonable to consider that AGPs are probably present in this fraction (Jiang and Ramsden 1999). The ratio of galactose to arabinose varied from 6:1 to 13:1 while that of mannose to galactose varied from 2:1 to 9:1. The ratio of galactose to arabinose was very high in our samples as compared to the 3:1 ratio reported by Jiang and Ramsden (Jiang and Ramsden 1999) for some varieties which are within the range reported for arabic gum. Jiang and Ramsden (1999) also reported high levels of mannose (11.2–16% w/w); and they argued that it may be associated with the arabinogalactan proteins (AGPs) or may represent separate mannans or galactomannans. We did not succeed

Fig. 2 Mucilage duct surrounded by secretory cells

in purifying AGPs using the Yariv reagent. Since the nature of molecular interaction between AGPs and this reagent is not well defined, it is not possible to say that our extract does not contain AGPs. We assume that the AGPs isolated were not sensitive to Yariv induced immunoprecipitation. Protein extracts presenting carbohydrate epitopes associated to AGPs were shown to be insensitive to this immunoprecipitation (Nguema-Ona et al. 2006). Uronic acids were found in relatively low amounts and consisted of glucuronic and galacturonic acids. It is worthwhile mentioning that the uronic acid levels measured in this study agree with the data in literature despite the different methods of analyses used. Jiang and Ramsden (1999) reported uronic acid level ranges from 1.4% to 3.8%. The presence of these negatively charged monosaccharides probably justify the presence of a core protein which is positively charged, and linked by electrostatic interactions. In this respect, the amino acid composition of this core protein is useful in the comprehension of the interaction between carbohydrate and protein moieties in the mucilage. However, most of the information on taro mucilage recognizes its glycoprotein nature and the presence of galactose, arabinose and mannose as the major components of the analyzed polysaccharides on the basis of their relative abundance, but the quantitative composition of amino acids in its protein has not been given as it has been done in this work.

Amino acid composition Of the 17 amino acids analyzed (Fig. 1), aspartic acid and asparagine are predominant in all the mucilage samples and constituted from 14.4% to 17.2%

Fig. 4 Viscosimetric behavior of some taro mucilage dispersion in water (2.5 g/100 mL) *CE* Country Ekona variety; *RIE* Ibo Ekona variety; *CN* Country Ngaoundere variety; *KW* Kwanfre variety

of total amino acids; followed by glutamic acid and glutamine (10.3–13.6%), glycine (10.17–11.60%), leucine (8.46–8.60%) and serine (7.7–8.4%). Those found at lower levels were methionine (0.78–1.18%), cysteine (0.78–1.4%), tyrosine (1.99–2.64%) and histidine (1.83–2.87%). These proportions of amino acids highly reflected the amino acid content of the flour samples as whole. In fact, our recent findings revealed that aspartic acid and asparagine are the most abundant (Table 4) in the flour samples, followed by glutamic acid and glutamine (12.0–12.3%) (Mbofung et al. 2006). This observation gives some clarification to be claimed by some authors Jiang and Ramsden (1999) who closely associated the tuber

Fig. 3 Infra red spectrum of taro mucilage **a**, **b**, **c** and **d** are respectively Country Ngaoundere, Red Ibo Ekona, Red Ibo Ngaoundere and Kwanfre varieties

proteins with those of the mucilage. The sum of the positively charged amino acids in the core protein of the mucilage does not vary significantly (7.4–10.75%) with the varieties while that of the uronic acids widely varies. This suggests that greatest difference in the mucilage is based on its monosaccharide profile while the protein composition is relatively stable.

Microscopy, infrared spectroscopy and viscosimetric behaviour of taro mucilage When fresh taro is cut, mucilage comes out from its ducts. Light microscopy of a fresh taro section shows mucilage ducts surrounded by secretory cells (Fig. 2). The shape of the duct in the transverse section seems to be circular, in concordance with findings of Harris et al. (1992).

A typical infrared analysis of mucilage extracted straight in the mucilage duct of each taro variety (represented in Fig. 3), showed stretches at near 1608, 1412, 1371, 1305, 1231, 1143, 1072, 1037, 975, 922, 897 and 842 cm^{-1} . A very weak band appeared at 1,735 cm^{-1} , and according to Tipson and Parker (1980), this may correspond to the frequency of un-ionised carboxylic acid (COOH) groups. It has been shown that carboxylic ions show a strong C-O stretching band at 1,635–1,600 cm^{-1} that distinguished carboxylate anions from esters, and a medium strength band at 1,420–1,300 cm^{-1} . The C-O group (carboxylic acid) also exhibits small stretching around 1,320–1,210 cm^{-1} . Our findings confirmed those of many other authors (Lin and Huang 1993; El-Mahdy and El-Sebaiy 1984; Jiang and Ramsden 1999) who had reported the presence of uronic acid in the structure of mucilage. In addition, the moderate stretching bands around 1,420 cm^{-1} and 1,400 cm^{-1} have been shown to correspond to the primary amide group (C-N), which together with the high vibration at 1,608 cm^{-1} , could justify the presence of proteins. Lin and Huang (1993) made similar observations on taro mucilage, but their wave-lengths were at 1,550 cm^{-1} and 1,650 cm^{-1} . Also, El-Mahdy and El-Sebaiy (1984) observed the usual bands at 1,700–1,550 cm^{-1} and 1,150–1,000 cm^{-1} which they attributed to amide deformation. The band at 1,370 cm^{-1} could be that of a lactone C-O stretching. Bands between 1,200 and 1,000 cm^{-1} , similar to those observed by Lin and Huang (1993), may result from strong stretching of C-OH alcohol groups mostly from the carbohydrate structures. It can be seen from the spectra that the C-O as well as the C = O are the major groups in mucilage, and thus confirm the carbohydrate nature of mucilage. The observation of medium NH stretching suggests the presence of some glycoproteins like AGPs. The viscosity analysis of some taro mucilage solutions is shown in Fig. 4. Only a slight difference can be observed between the curves among the different varieties which behave to a very good approximation ($r=0.95\text{--}0.99$) as power law liquids over this range of shear rates.

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