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Isolation, Determination and Characterization of Taro (*Colocasia esculenta*) Starch

Feroz A. Jaffery[a], Abid Hasnain*[a], Khalid Jamil[b] and Tahir Abbas[b]
[a]Department of Food Science and Technology, University of Karachi, Karachi, Pakistan
[b]Food and Marine Resources Research Centre, PCSIR Laboratories Complex, Karachi, Pakistan

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Abstract. The starch content of taro (*Colocasia esculenta*) was determined using DNS colourimetric, acid-hydrolysis and enzymatic methods. Starch content varied from 80.3 to 81.3% as determined by DNS colourimetry. For the extraction of taro starch, different techniques were used. It was found that there was a noticeable improvement in the yield of starch using the freeze-thaw method. The various physicochemical properties of the extracted starch were also compared with the starch obtained by ammonia and alkali extractions. It was found that the hydration capacity, swelling volume, moisture absorption, freeze-thaw stability, as well as swelling power, were generally higher while solubility was lower of the freeze-thaw extracted starch than that extracted by ammonia and alkali. The DNS colourimetric method is recommended as a simple method for the determination of taro starch.

Keywords: taro starch, freeze-thaw extraction, starch extraction, DNS colourimetry, *Colocasia esculenta*, starch characterization

Introduction

Edible aroids are starchy tuber crops of the humid tropical and subtropical regions of the world. They are herbaceous plants (Family: Araceae), consisting of five genera of which *Colocasia esculenta* is the most important food crop. This species is commonly known as taro or old cocoyam, (vernacular: arvi, dasheen). It is an important low-cost starchy food source (Hong and Nip, 1990). Taro has been reported to have 70-80% starch on a dry weight basis (Jane *et al.,* 1992; Tu *et al.,* 1979), comprised of small granules, having dia between 1.4 and 5 µm (Sugimoto *et al.,* 1986). Taro starch, in view of its small granule size, has been considered to be easily digestible, hence it is widely used in baby foods and the diets of people allergic to cereals and children sensitive to milk (Wang, 1983). In addition to the food use, taro has found some industrial applications as well. The small size of taro starch granules makes them ideal in cosmetic formulations, such as face powder, and in dusting preparations which use aerosol dispensing systems (Griffin and Wang, 1983). Taro starch has been considered to be a suitable filler in biodegradable plastics and as a fat substitute (Daniel and Whistler, 1990).

The starch content of edible aroids has been determined using a variety of analytical techniques. Average starch value of 679 g/kg, on a dry weight basis, was determined from cormels (*Colocasia esculenta*) grown in Bangladesh, by hydrolysis of starch (Chowdhury and Hussain, 1979). Using the glucoamylase hydrolysis and copper reduction method, the starch content of a single taro cultivar was reported to be 540 g/kg on a dry weight basis (Hussain *et al.,* 1984). In another study, the starch content was calculated to be 70.6%, on a dry weight basis (Agbor-Egbe and Rickard, 1990). Starch values in this study were determined using acid hydrolysis and the ferricyanide reduction method. Since in the selection of edible aroid cultivars in a germplasm collection, the starch content is considered to be a very important characteristic, there is a need for the development of an accurate but simple and inexpensive method of starch analysis.

In spite of the versatile uses of the taro starch, large-scale extraction and utilization of taro starch is not practiced anywhere. This has been probably due to the difficulty in extracting the taro starch from fresh tubers, which also contain a lot of mucilaginous materials. Moorthy (1991) made an attempt to extract taro starch by using dilute solution of ammonia. In another study, the starch was extracted by using NaOH solution (Jane *et al.,* 1992). The quality, as well as yield of the starch, using these methods, was found to be reduced due to the chemical treatments involved. An easy and convenient method is, therefore, also needed to be developed for the extraction of taro starch to produce good quality starch with better yield. The purpose of the present study was to explore a new technique for the isolation and determination of taro starch and to compare the so developed method with the reported methods by evaluating the physicochemical properties of the extracted starch.

*Author for correspondence

Materials and Methods

Materials. Taro (*Colocasia esculenta*) was purchased from the local market of Karachi, Pakistan. The chemicals used were of Analytical grade (E. Merck and Sigma).

Isolation of taro starch. Three different techniques were used for the extraction of taro starch as given below.

Extraction by the freeze-thaw method. This is a simple and convenient method, recently developed by the present authors. In this method, peeled taro was ground in a grinder, blended in distilled water in the ratio of 1:2, and mixed well in a mechanical mixer for 1 h. The slurry was filtered through an 80 mesh sieve, followed by passing through 260 mesh sieve. The residue on the sieve was removed and the filtrate was kept in a freezer at -10 °C for 5 days. The frozen extract was thawed at room temperature for 4 h. The separated water was decanted. Residue was carefully dried at 45 °C and then pulverized.

Extraction with ammonia. The extraction was carried out following the procedure of Moorthy (1991), using 0.03 M ammonia solution.

Extraction with NaOH. The method of Jane *et al*. (1992) was used. In this method, 0.05% NaOH solution was used for the extraction of taro starch.

Purification of starch. Defatting of starch was carried out by Soxhelt extraction with 75% aqueous *n*-propanol for 7 h. The solvent was removed by vacuum evaporation and the starch was air-dried to a moisture content of 10%. Deproteinization of the starch was done by the method of Jane *et al*. (1992), using NaCl solution and toluene.

Starch analysis. Three different methods were used for the determination of starch present in the dehydrated taro powder.

DNS colourimetric method. DNS (3,5-dinitrosalicylic acid) colourimetric method was used for the determination of starch (Geirwyn, 1995). About 0.1 g of the powdered sample was hydrolyzed in 10 ml of 1.5 M H_2SO_4 for 20 min in a boiling waterbath. 12 ml 10% NaOH was added and the contents were filtered in 100 ml volumetric flask (hydrolysate). Solutions of 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 mg/ml of glucose were prepared in 100 ml volumetric flask. To six labelled test tubes was separately transferred 1 ml of each concentration of the standard glucose solution (0.25 - 1.5 mg/l). A tube containing 1 ml distilled water served as the blank. To each of these test tubes was then added 1.0 ml of DNS reagent (2,6-dinitro-salicylic acid) and 2.0 ml of distilled water. All the tubes were heated in a boiling waterbath for 5 min and then cooled. The volume of each test tube was adjusted to 20 ml with distilled water. Absorbance of each standard glucose solution was measured at 540 nm to prepare the standard curve. The taro starch sample for analysis was prepared in a similar manner by replacing the glucose solution and the absorbance taken at 540 nm. The concentration of taro starch was determined from the standard glucose calibration curve and then the percentage of starch was calculated.

Enzymatic method. The enzymatic method used was in accordance with Jane *et al.* (1992). Taro flour (50 mg, dsb) was suspended in 90% dimethyl sulfoxide (3 ml) and boiled in a water bath at 96 °C for 1 h. After cooling, methanol was added to precipitate the solids. The mixture was centrifuged, and the supernatant was discarded. A phosphate buffer (pH 6.9, 0.1 M, 3 ml) and porcine pancreatic α-amylase (1,330 units) was added to the solid residues. The mixture was incubated in a shaker waterbath at 35 °C for 4 h. At the end of the incubation, glucoamylase (25 units) in an acetate buffer solution (pH 4.3, 0.1 M, 0.55 ml) was added to the digestion mixture, and the pH of the mixture was adjusted to 4.5. The digestion mixture was then incubated in the shaker waterbath at 55 °C for 4 h. Glucose produced in the digest was analyzed by measuring the absorbance at 520 nm, using a mixture of hexokinase and glucose-6-phosphate dehydrogenase.

Acid-hydrolysis method. The starch content of the taro powder was determined by the AOAC (1984) direct acid hydrolysis procedure (Method 22.045), as modified by Rickard and Behn (1987).

Moisture absorption. Moisture absorption by starch was determined using the method of Nyqvist (1983), with few modifications. The starch powder was dried at 60 °C for 6 h until the moisture level was < 1%. The hygrostate was prepared using a saturated Na_2SO_4 solution in the wells of a glass dessicator. A starch sample of 2 g was placed in a watchglass and kept in the hygrostate. The percentage moisture absorbed was determined from the weight gained after 240 h (10 days).

Hydration capacity. Hydration capacity, or water absorption, was determined by the method of Komblum and Stoopak (1973). 400 mg starch samples were placed in each of the 15 ml plastic centrifuge tubes. Distilled water was added from a burette, the tubes were covered with parafilm and their contents mixed on a vortex mixer (Vortex_Gennie Scientific Industry, USA) for 2 min. The mixture in each tube was left to stand for an additional 3 min and then immediately centrifuged at 2000 xg for 10 min in a Gallenkamp bench centrifuge. The supernatant was decanted and the sediment was weighed. The weight of water adsorbed and retained was determined as the gain in weight of the dry sample.

Swelling power and solubility. Swelling power was determined by using the method of Lauzon *et al.* (1995). Starch suspension (1%, w/v) was heated to 55, 65, 75, 85 and 95 °C and

was kept at each temperature for 30 min, followed by cooling down to the room temperature rapidly in an ice waterbath. The cooled sample was centrifuged at 5,000 xg for 20 min. The swelling power was measured from the precipitate. The solubility was determined by using 0.4% (w/w) starch dispersion according to the method of Leach *et al*. (1959). The amount of soluble material in the supernatant was estimated from its volume and concentration.

Swelling volume. The swelling volume was determined by heating 15 ml of an aqueous (w/v) starch suspension in screw top 40 ml universal sample bottles. Samples were heated in a 95 °C waterbath, with gentle shaking until gelatinization occurred, and then left in the waterbath for a further period of 1 h. After cooling, the samples were transferred into 15 ml conical centrifuge tubes and centrifuged at 2200 rpm (approx 1000 xg) for 20 min. The swelling volume was obtained directly by reading the volume of the sediment in the tube. The swelling volume is the volume of sediment per 100 ml of the starch sample.

Freeze-thaw stability. A modified method based on Narkrugsa (1996) and Schoch (1968) was used to determine the freeze-thaw stability. Starch sample of 15 g was mixed in 300 ml distilled water in a beaker at 95 °C, stirred with a mechanical mixer for 20 min. The mixture was poured into a plastic cup and frozen at -10 °C, for 7 days. The frozen mixture was then thawed in a water bath at 30 °C, for 4 h. From this, 100 ml of the mixture was centrifuged at 8000 rpm for 30 min. The amount of water separated from the mixture, after centrifugation, was measured. Results used for calculation were the means of triplicate measurements.

Viscography. Starch suspension (6%) was subjected to viscography, using a Brabender Viscoamylograph. Starch suspension was gradually heated from 25 to 92.5 °C at a rate of 1.5 °C/min, held at 92.5 °C for 10 min, and then cooled at the same rate.

Results and Discussion

The variations in the taro starch content, using different analytical methods studied, are shown in Table 1. Significant differences were observed in the quantity of starch determined. The overall mean starch values of all taro samples for DNS colourimetric method (80.3 - 81.3%) were found to be higher as compared with the values obtained using the enzymatic method (73.0 - 74.3%) and the acid-hydrolysis method (76.3 - 77.1%). These observations indicate that the DNS colourimetric method is suitable for the determination of taro starch accurately in a simple procedure.

Taro contains large quantities of mucilagenous materials and it is difficult to extract starch from this tuber. The settling of starch is too slow because of these mucilagenous materials, leading to a reduction in the yield and also rendering extract susceptible to microbial degradation during the settling process over 1-2 days. A noticeable increase in the yield, on extraction with ammonia and sodium hydroxide solution, followed by the conventional settling process, can be used for the large-scale taro starch production. It was observed, that although ammonia and sodium hydroxide solution improved the settling of taro starch and prevented the microbial decay, yet the starch obtained had a brownish colour, which could not be removed even by repeated washing. Ammonia and sodium hydroxide solution also disrupted the starch granules to some extent and affected their functional properties. Extraction using the freeze-thaw technique yielded good quality taro starch, with high yield (upto 19.8%), as compared with starch extracted using the ammonia and alkali methods (Table 2).

As evident from Table 3, the hydration capacity of the freeze-thaw extracted starch (TS-1) was noted to be generally higher than that extracted with ammonia (TS-2) and alkali (TS-3). The absorbed water was undoubtedly taken up by the granules on their surfaces (Medcalf and Gilles, 1965). The lower values obtained with TS-2 and TS-3 indicate that the ammonia and sodium hydroxide treatment had caused breakage of some starch granules, and thus the capacity to absorb water by the granules had decreased. Due to relatively more cleavage of starch granules, TS-3 absorbed much less water among the starches extracted by the three methods. Similarly, the enhanced moisture absorption of TS-1 showed that it was much more hygroscopic than TS-2 and TS-3 starches. It shows that greater surface area was available to absorb the moisture in the case of TS-1, which indicates the superiority of TS-1 over TS-2 and TS-3 starches.

When starch gels were subjected to freeze-thaw cycling, the water used in the preparation of the gel was noted to get

Table 1. Starch content (%, dry weight basis) of taro flour* as determined by different methods

DNS colourimetric method	Enzymatic method	Acid-hydrolysis method
80.8 ± 0.5	73.8 ± 0.5	76.7 ± 0.4

*mean ± sd (n = 3); DNS = 3,5-dinitrosalicylic acid

Table 2. Yield (%, fresh weight basis) of taro starch* extracted by using different methods

Freeze-thaw extraction	Extracted with NH_3	Extracted with NaOH
19.3 ± 0.5	16.3 ± 0.5	15.0 ± 0.5

*mean ± sd (n = 3)

Table 3. Moisture absorption, hydration capacity, swelling volume and freeze-thaw stability of taro starches* extracted by using different methods

Starch	Moisture absorption (%)	Hydration capacity (g/g)	Freeze-thaw stability (%)	Swelling volume (ml/g)
TS-1	14.25 ± 0.35	2.85 ± 0.05	62.8 ± 0.42	34.6 ± 0.50
TS-2	9.50 ± 0.28	2.50 ± 0.04	50.5 ± 0.35	27.8 ± 0.45
TS-3	7.85 ± 0.24	2.18 ± 0.04	43.2 ± 0.32	19.8 ± 0.42

*mean ± sd (n = 3); TS-1: freeze-thaw method; TS-2: ammonia method; TS-3: NaOH method

separated due to the tendency of starch molecules to reassociate, thus forming insoluble aggregates. The stability of starch to freeze-thaw cycling makes it suitable for use in the frozen food products (Jaroslaw *et al.,* 2004). The starches that are most stable to freeze-thaw cycling are also the most stable during refrigerated storage (White *et al.,* 1989). The freeze-thaw stability of a starch gel is evaluated by the amount (%) of water released (syneresis) when the starch chains retrograde (reassociate) during the freeze-thaw cycle. The present results show that TS-1 had higher freeze-thaw stability than the counterpart TS-2 and TS-3 starches. This is probably due to the greater degree of reassociation between starch chains in the TS-1 starch.

The swelling volume of starches is also affected by various chemicals (Moorthy, 1991). The swelling volume of starches extracted with ammonia and alkali were, therefore, compared with that of the freeze-thaw extracted starch. The swelling volume of taro starch was noted to increase on using ammonia solution and much more in the case of alkali extraction. Higher swelling indicated a lowering of associative forces between the starch granules, and hence the taro starch appeared to have undergone some reduction in associative forces when extraction was done using ammonia and sodium hydroxide solution.

Solubility of starch is observed to be a function of temperature (60 to 90 °C). At temperature below their gelatinization temperature, starches are less soluble. Solubility of starch greatly increased at higher temperaturs (80 and 90 °C). Solubility characterization of starch reportedly depends upon the degree of substitution and the degree of polymerization. The present results show that the TS-1 starch was the least soluble than the TS-2 and TS-3 (Fig. 1). It is probably due to weakening of starch granules during the chemical treatment, which caused the ease in solubilization of TS-2 and TS-3 starches. The swelling power of starch was found to be a function of temperature and it followed a pattern similar to that of solubility characteristics. Prior to gelatinization there is only a slight increase in the swelling capacity of starches. Once the gelatinization process sets in, however, swelling increases rapidly with increasing temperature. The swelling power rises significantly at the gelatinization temperature. When the crystal region in the starch granules begins to melt, it enhances the swelling power. As the temperature of an aqueous suspension of starch granules is raised above the gelatinization range, hydrogen bonds between polymers continue to be disrupted and water migrates into the interior of the molecule (Shi and BeMiller, 2002). The present results showed that the swelling power of TS-1 was higher than the TS-2 and TS-3 starches (Fig. 2). It was probably due to weakening of the hydrogen bonds by the ammonia and sodium hydroxide treatments.

Viscosity of taro starch solution (5, 6 and 7%) was determined by Brabender Viscoamylograph. Important characteristics were recorded during the heating and cooling cycle, including pasting temperature, viscosity at 95 °C, viscosity after holding at 95 °C for 30 min, and viscosity after cooling to 50 °C (Table 4). The results show that considerable viscosity stability was observed throughout the heating-cooling cycle. This is an evidence of restricted swelling and solubilization, and of resistance to mechanical disintegration. When the pasted starch was cooled, setback (retrogradation) was observed. Viscosity can be considered as a measure of the strength of starch granules that are intact, while the starch which has undergone chemical and microbiological damage loses viscosity (Moorthy, 1991). The present data indicate that TS-1 starch began to swell at a slightly higher temperature than the TS-2 and TS-3 starches. This observation may be related

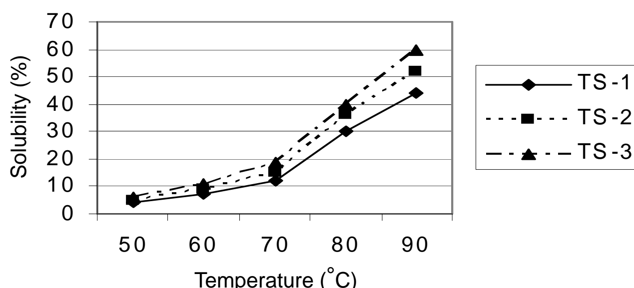

Fig. 1. Solubilities of taro starches extracted by different methods (TS-1: freeze-thaw; TS-2: ammonia; TS-3: alkali).

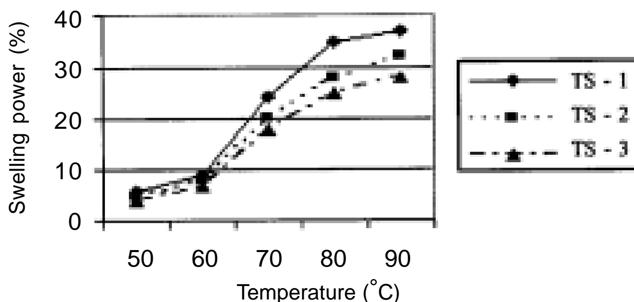

Fig. 2. Swelling power of taro starches extracted by different methods (TS-1: freeze-thaw; TS-2: ammonia; TS-3: alkali).

Table 4. Viscosity of taro starches extracted using different methods

Starch* type	Starch concentration (%)	V_P (BU)	V_{97} (BU)	V_H (BU)	V_C (BU)	Pasting temperature (°C)
TS-1	5	380	350	290	360	84-97
TS-1	6	620	590	540	600	84-97
TS-1	7	950	800	660	910	82-97
TS-2	5	330	320	270	310	80-83
TS-2	6	580	560	470	580	80-83
TS-2	7	860	700	610	880	80-84
TS-3	5	310	290	250	300	71-75
TS-3	6	550	540	440	540	71-75
TS-3	7	730	660	580	720	70-75

V_P = peak viscosity; V_{97} = viscosity at 97 °C; V_H = viscosity after holding at 97 °C for 30 min; V_C = viscosity after cooling to room temperature; *extraction method used (TS-1: freeze-thaw; TS-2: ammonia; TS-3: NaOH)

to the suggested more compact granule structure of the TS-1 starch. Similarly, the TS-1 starch had high pasting temperature in contrast with TS-2 and TS-3 starches. The relatively lower viscosity breakdown can be exploited in food uses where a short non-cohesive texture is required. Chemical treatment disrupts the structure of starch granules, rendering them more susceptible to hydration and swelling and eventually complete disintegration takes place at a lower temperature (White *et al.,* 1989).

Conclusion

The results of the present study have indicated that DNS colourimetric method is an inexpensive and simple method for the determination of taro starch. The quality, as well as yield, of taro starch can be improved by using the freeze-thaw extraction method. Better understanding of the functional and physicochemical properties of taro starch may, therefore, lead to its new applications in the food industries.

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