

A Novel Enzymatic Process to Produce Oxalate Depleted Starch From Taro

Kunal Kumar and Prasanna D. Belur*

A novel process comprising treatment of Taro (*Colocasia esculenta* (L.) Schott) tuber flour with oxalate oxidase enzyme is developed to deplete the oxalate content. Oxalate oxidase enzyme produced by an endophyte, *Ochrobactrum intermedium* CL6 is employed to treat taro tuber flour. The treatment followed by extraction of starch results in a 97% reduction in total oxalate content. Further, several physicochemical properties such as paste clarity, swelling power, solubility, amylose content, granule size of starch produced out of enzyme treatment are studied and compared with properties of taro starch produced without enzyme treatment. The study reveals that enzyme treatment does not bring appreciable changes in the studied parameters. The taro starch produced by enzyme treatment shows very low paste clarity (9.38%), high swelling power (15.32 g/g), very low solubility (21.66%), and low amylose content (7.52%) at 100 °C compared to potato and sweet-potato starches. X-ray diffraction data reveal that taro starch possesses an A-crystalline form, unlike the B-crystalline form found in potato and sweet potato starch. To the best of the authors knowledge, for the first time, the use of oxalate oxidase to produce oxalate depleted taro starch is reported. One of the interesting food industry applications of oxalate-depleted taro starch, among many other uses could be for baby food formulation because of its small granule size.

order to meet food industry demands. Therefore, finding new starch sources, developing process technology to extract starch, knowledge on physicochemical and functional properties of starches from these crops would be beneficial to the food industry immensely.


Taro (*Colocasia esculenta*) is a starch source that has not been explored by food industry due to several reasons including the presence of oxalates, which is an anti-nutritional factor. Oxalate crystals impart acrid taste cause irritation to mouth and throat.^[2] Ingestion of foods containing oxalates causes caustic effects, irritation to the intestinal tract and absorptive poisoning. Oxalates are also known to interfere with the bioavailability of calcium.^[3] Oxalates interfere with absorption of minerals like calcium, magnesium, zinc, and copper by forming complexes with them during the course of digestion and assimilation in the intestine.^[4] The consumption of even the moderate amounts of oxalate has been reported to play a key role in calcium oxalate induced kidney stone disease.^[5] Therefore, removal of oxalate from taro starch is crucial before introducing it to the food industry.

1. Introduction

Starches from various biological origins, such as corn, potato, wheat, and rice, have received widespread attention with respect to functional and physicochemical properties.^[1] The physicochemical properties of starches command their functionality in various applications. Starches with desirable functional properties play important role in improving the quality of different food products and are therefore in great demand in the food industry. Hence, interest in new value-added starch products has encouraged researchers to investigate the physicochemical and functional properties of starches produced from different genotypes and botanical sources in

Several attempts have been made to reduce oxalate content in taro. Although it has been reported that traditional methods of drying reduce oxalate content, it does not eliminate completely.^[3] Soaking and blanching of taro tuber flour could reduce oxalate content by 85%.^[6] Substantial reduction in oxalate content (around 80%) has been reported by cooking for 40 min at 90 °C.^[7] However, none of these methods is suitable for the production of oxalate-free starch, as they impair the quality of starch seriously.^[8] Therefore, research on designing an alternative method that would reduce oxalate content in taro starch, while maintaining the starch quality is warranted. Such a method would encourage the food industry to explore the possibility of utilizing this cheap and abundant source of starch. Sit et al.^[9] have reported the production of starch from taro by employing a combination of physical and enzymatic methods (cellulase and xylanase). However, reports on the use of oxalate oxidase enzyme in enhancing the quality of taro starch are not available. This research was therefore aimed at developing an enzymatic treatment to reduce oxalate content in the extracted starch and evaluating physicochemical properties of the starch thus produced.

Dr. K. Kumar, P. D. Belur
Department of Chemical Engineering
National Institute of Technology Karnataka
Surathkal, Srinivasanagar, Mangalore-575 025, India
E-mail: prsnbhat@gmail.com

 The ORCID identification number(s) for the author(s) of this article can be found under <https://doi.org/10.1002/star.201700352>.

DOI: 10.1002/star.201700352

2. Experimental Section

2.1. Production of Oxalate Oxidase Enzyme

The enzyme, oxalate oxidase (OxO) was produced by *Ochrobactrum intermedium* CL6, an endophytic bacterium isolated earlier from rhizomes of taro (*Colocasia esculanta*) in our laboratory. The isolate was identified based on 16S rDNA sequence analysis and its GenBank accession number is KM658164. The organism was cultured in a defined medium and enzyme was purified from fermentation broth by a two-step process as explained before.^[10]

2.2. Collection and Processing of Taro Tubers

Taro tubers were purchased from the local market of Mangalore, India. The tubers were thoroughly washed with tap water, peeled, and sliced into pieces (Approximately 1 cm thickness). The slices were dried in a hot air oven at 60 °C overnight. The dried slices were ground to powder in a mortar and pestle and passed through 500 µm screen to get fine flour. A known weight of taro tuber flour was taken (0.125 g), mixed with OxO (Oxalate Oxidase) enzyme solution (6 mL), and incubated at 55 °C in an incubator shaker. The OxO solution was prepared by dissolving the known quantity of lyophilized enzyme in 6 mL of 50 mM sodium succinate buffer of pH 3.8.

2.3. Extraction of Starch From Taro Flour

Starch was extracted from taro flour by the method of Aboubaker et al.^[11] with modifications. After incubation with OxO, the suspension was centrifuged at 5000 rpm for 10 min. The supernatant was discarded and sediment was washed twice. Washing was carried out by the addition of distilled water, followed by centrifugation at 5000 rpm for 10 min. The supernatant was discarded and the sediment was dried at 60 °C for 12 h. Around 20 g of dried sediments were collected as mentioned above and steeped in (500 mL) distilled water at 35 °C overnight to obtain a slurry. The slurry obtained was homogenised for 15 min using a commercial blender. The suspension obtained was filtered using a filter medium having a pore size of 150 µm. The filter cake was discarded and the filtrate was allowed to stand for 24 h to facilitate starch sedimentation. The supernatant was decanted carefully and the settled starch was then suspended in warm distilled water. The suspension with starch was centrifuged at 5000 rpm for 10 min. The liquid supernatant was discarded and the process was repeated until reaching a transparent supernatant. Then purified settled starches were dried for 48 h at 60 °C and stored in airtight container. Starch was also extracted from untreated (without the addition of enzyme) taro tuber flour in a similar fashion as mentioned above.

2.4. Determination of Oxalate Content

The oxalic acid is found to exist in both soluble and insoluble forms in the plant materials.^[12] The soluble oxalic content and total oxalate content were determined as described below and the

insoluble oxalate content was calculated by subtracting soluble oxalate content from total oxalate content.

To calculate soluble oxalate content, 0.125 g of a sample of taro tuber flour was weighed and dispersed in 6 mL of distilled water. The slurry was incubated in a water bath at 80 °C for 15 min. The extract was allowed to cool and then the volume of the mixture was made to 12.5 mL with distilled water and left undisturbed for 30 min prior to oxalate estimation.^[12] For determining total oxalate content, 0.125 g of a sample of taro tuber flour was weighed and suspended in 6 mL of 2 M HCl. The slurry was incubated in a water bath at 80 °C for 15 min. The extract was allowed to cool and then the volume of the mixture was made to 12.5 mL with 2M HCl and left undisturbed for 30 min prior to oxalate estimation.

1 The extracts were centrifuged at 3000 rpm for 10 min and 10 mL of the supernatant was filtered through a 0.45 µm cellulose acetate membrane. 20 µL filtrate was analysed using an HPLC system (Shimadzu, Japan), and UV/VIS detector set at 254 nm. Data capture and processing were carried out using the Lab Solution software. The chromatographic separation was carried out using an RP C18 (4.6 mm I.D × 250 mm) analytical column attached to a guard column, using an isocratic elution at 0.5 mL min⁻¹ with 0.3% acetic acid as a mobile phase. The analytical column was held at 30 °C and the column was equilibrated at a flow rate of 0.5 mL min⁻¹ prior to use and in between sample sets. The oxalate peak was identified by comparison of the retention time to a range of oxalate standards.

2.5. Experimental Design

To study the effect of incubation period on the removal of oxalates, taro flour was mixed with 2 Units of OxO activity and incubated for a varying period (15–120 min) (Table 1A). Similarly, the effect of enzyme loading on the reduction in oxalate content was assessed by mixing taro flour with increasing activity of the enzyme (1–10 Units) and incubated for 120 min (Table 1B). After optimizing the enzyme load, trials were conducted to ascertain the effect of peroxidase enzyme (Sigma-Aldrich Switzerland, Prod. Code 77332) on oxalate reduction kinetics. Two different activities of peroxidase enzyme (5 and 10 units) were used for the study. In each characterization study, Corn starch (S.D.Fine Chemicals Ltd. India) was taken as a control for comparison of results.

2.5.1. Determination of Paste Clarity

Paste clarity of the starch suspension was determined by measuring the light transmittance of the starch paste using the method of Huang et al.^[13] Starch aqueous suspension (2% w/v) was heated in a boiling water bath with stirring for 1 h and then cooled to room temperature. The light transmittance of the starch solution was determined at 640 nm with a UV-Vis spectrophotometer (Lab India UV 3000, India).

2.5.2. Swelling Power and Solubility

The swelling power and solubility were measured by stirring an aqueous suspension of starch (2% w/v) in a water bath kept at

Table 1. Experimental design for oxalate depletion in Taro flour.

A		
Incubation time (Min)	Total oxalate (mg/100 g DW)	% Reduction
0	790 ± 5.77 ^a	0
15	736.4 ± 5.99 ^b	6.76 ± 1.13 ^a
45	631.8 ± 4.50 ^c	20 ± 1.15 ^b
75	623.2 ± 8.22 ^d	21.06 ± 1.55 ^b
120	602.44 ± 2.14 ^e	23.73 ± 0.63 ^c
B		
Enzyme load (U)	Total oxalate (mg/100 g DW)	% Reduction
0	790 ± 5.77 ^a	0
1	736.4 ± 5.99 ^b	6.86 ± 1.07 ^a
2	620.66 ± 13.48 ^c	21.37 ± 1.62 ^b
4	388.48 ± 16.34 ^d	50.83 ± 1.92 ^c
6	277.83 ± 2.68 ^e	64.83 ± 0.44 ^d
8	247.6 ± 6.86 ^f	68.66 ± 0.66 ^e
10	242.4 ± 12.03 ^f	69.33 ± 1.33 ^e

Experiments in table A were conducted with 2 Units of Oxalate Oxidase, and incubated for varying period at 55 °C. Experiments in table B were conducted with varying enzyme load, and incubated for 120 min at 55 °C.

65 °C, 80 °C, 100 °C for 1 h, respectively. The suspension was cooled to room temperature and centrifuged at 5000 rpm for 10 min. The liquid supernatant was decanted out carefully and evaporated overnight at 100 °C. The swollen starch residue was weighed. Swelling power and solubility were calculated using the method described by Gomand et al.^[14]

Swelling power and solubility were calculated as follows:

$$\text{Swelling power (g/g)} = \frac{(\text{SW} \times 100)}{\text{Starch}_{\text{dwb}}} \times (100\% - \% \text{SOL}) \quad (1)$$

$$\text{Solubility (SOL\%)} = \frac{\text{Weight of dried supernatant}}{100/\text{Starch}_{\text{dwb}}} \quad (2)$$

where, SW is weight of wet sediment, and starch_{dwb} is the dry water basis starch weight.

2.5.3. Determination of Amylose Content

The amylose content of extracted starches was determined by colorimetric method as described by Sukhija et al.^[15]

2.5.4. Microscopic Examination of Starch Granules

Starch granule morphology was studied by using Scanning electron microscope (JEOL JSM 6380, USA). For Scanning electron microscopy, samples were scattered on the double-sided adhesive tape mounted on a metal stub and it was then coated with gold to make the sample conductive and images were examined at an accelerating potential of 20 kV.

2.5.5. X-Ray Diffraction (XRD) of Starch

X-ray diffraction of starch was recorded by Rigakuminiflex 600 X-ray powder diffraction (Rigaku, Tokyo, Japan) with Nickel filtered Cu K α radiation ($\lambda = 1.54056 \text{ \AA}$) at a voltage of 40 kV and current 15 mA. The scattered radiation was detected in the angular range of 3 – 40° (2 θ), with a scanning speed of 2° (2 θ)/min and a step size of 0.02° (2 θ). The degree of crystallinity was calculated approximately by the software origin 8.5 based on the method reported by Nara and Komiya.^[16]

2.6. Statistical Analysis

All the measurements were taken in triplicate and the mean and SD were presented. Statistical comparisons between means were made by one-way analysis of variance (ANOVA) at a significance level of 5%, using the OPSTAT statistical software package of HAU, Hisar, India. The statistical significance is specified by appropriate letters, wherever applicable.

3. Results and Discussion

Oxalate oxidase (oxalate: oxygen oxidoreductase, EC 1.2.3.4) catalyzes the oxidative cleavage of oxalate to carbon dioxide with the reduction of molecular oxygen to hydrogen peroxide.^[10] Hydrogen peroxide is relatively unstable and hence subsequently decomposes into water and molecular oxygen leaving behind no undesirable residues. In the current study, initially the taro flour was produced from taro tubers and then taro flour was treated with OxO. The incubation time and OxO load were optimized by the one-factor-at-a-time method to achieve maximum oxalate reduction in taro flour. Thereafter starch was extracted from enzyme-treated taro flour. Various physico-chemical properties of starch thus produced was studied.

3.1. Effect of Incubation Time and Oxalate Oxidase Enzyme Load

The effect of incubation period on the oxalate content of taro flour is presented in Table 1A. The result shows that with the increase in incubation time, the oxalate content decreased progressively. After 2 h of incubation with 2 Units of OxO, a reduction of 23% oxalate content was noticed. However, about 20% oxalate reduction was achieved within 45 min and rest in remaining 75 min. The results indicate that there is a scope for enhancing oxalate reduction by increasing enzyme load. Moreover, the slowing down of oxalate conversion during the later stage of incubation suggests that OxO is being inhibited by one or the other component of either taro flour or the products of enzymatic conversion. However, the addition of peroxidase enzyme to the reaction mixture could not alter oxalate reduction kinetics, ruling out the possibility of feedback inhibition (Data not shown). Further, an addition of taro tuber water extract (freshly prepared from wet tuber) to the OxO solution quickly diminished the enzyme activity indicating the presence of inhibitors in the taro flour (Data not shown). The reduction in

oxalate conversion rate during the last phase of incubation suggests the presence of either small quantity of inhibitors or slower inhibition kinetics. To enhance the oxalate conversion further, increased OxO load was tried in the next set of experiments. With the increase in OxO load from 1 to 10 Units of activity, progressive reduction in oxalate content was observed (Table 1B). About 65% oxalate conversion was observed with the enzyme load of 6 Units, and a further increase in enzyme load to 10 units, increased the oxalate conversion by another 4%. After treating the taro flour with 6 Units of OxO for 120 min, the starch was extracted and the oxalate content was estimated (Table 2). Total oxalate content was reduced by 97% in the extracted starch. This drastic reduction in total oxalates could be due to the enzyme action as well as subsequent starch extraction procedure. Enzymatic processing led to the reduction of both soluble and insoluble oxalates. The major reason for the reduction in total oxalates could be due to the oxalate treatment and subsequent starch extraction procedure. Perhaps, calcium oxalate micro-crystals which are known to be bound to the fibres of taro,^[12] remained entangled with the discarded filtrate.

Published literature suggest that several methods are available for reducing oxalate content in food materials. Boiling, blanching with hot water, steeping with sodium bicarbonate and subsequent water leaching are some of the popular methods to reduce oxalate content in food materials. Cooking reduces the oxalate content of common foodstuffs like Silverbeet, Spinach, Rhubarb, Beetroot, and Broccoli by leaching losses into the cooking water in the range of 15 to 83% for different cooking time.^[12] In case of taro, soaking and blanching were not found to be a suitable method to reduce total oxalate content above 18%.^[6] Treating taro flour with sodium bicarbonate could reduce the oxalate content to 61%.^[2] However, a substantial reduction of around 80% was achieved by cooking taro slices for 40 min at 90 °C.^[7] Reduction of 80% oxalate content of taro, by cooking, looks attractive, but the methods are unacceptable as cooking will affect the quality of the extracted starch if the objective is to extract oxalate free starch from taro flour.^[17] Native starches cannot withstand the stringent processing conditions of high temperature, which lead to abundant granule disruption and undesirable product properties. When heated in the presence of water, starch undergoes an irreversible order-disorder transition termed as gelatinisation and leads to decomposition of glucose rings,^[8] formation of lumping and affects the physical properties like expansion ratio, water absorption, water solubility,^[18] and affect the mouth feel property of the starch.^[19]

Oxalate content reduced from 790 to 24.2 mg in 100 g starch (dry weight basis), due to this novel strategy. Total oxalate level obtained in the study (24.2 mg in 100 g starch) is less than 71 mg/100 g, which is the permissible oxalate levels in food.^[2,3] Under this scenario, oxidative cleavage of oxalate in taro flour by

OxO is worthwhile, which would prevent detrimental physical modifications of starch and maintain the level of oxalate much below the permissible level.

3.2. Physico-Chemical Properties of Starch

Various physico-chemical properties of starch such as paste clarity, swelling power, solubility, amylose content and crystalline properties were studied. Properties of starch extracted from enzyme-treated taro flour (ET) and untreated taro flour (NT) were determined and compared with laboratory reagent grade corn starch supplied by S.D.Fine Chemicals Ltd., India (Control).

3.2.1. Paste Clarity

The Paste clarity (% transmittance) was 9.38 and 10.44% for ET and NT respectively (Figure 1c) indicating a marginal increase in opaqueness due to enzymatic treatment. Compared to control, taro starch, in general, showed very low transmittance indicating higher opaqueness. Paste clarity obtained here for taro starch (ET & NT) found to be in agreement with the findings of Deepika et al.^[20]

3.2.2. Swelling Power and Solubility

Swelling power of NT increased almost linearly with the increase in temperature from 65 to 100 °C (Figure 1a). In case of ET, swelling power remain unaltered from 65 to 80 °C, increased linearly though, from 80 °C onwards. Control showed low swelling power of 8.17 at 100 °C as against 20.66 and 15.32 for NT and ET respectively. The swelling power of NT and ET increased with temperature, indicating a high hydrating ability under different temperature and excess water, which was otherwise not seen in control. These results are concurring with the results reported by Tattiyakul et al.^[21] while studying raw taro starch of Thailand.

The solubility of NT was quite low at a temperature of 65 °C and was comparable with ET only at 100 °C (Figure 1b). Control, however, showed a linear increase in solubility with temperature reaching 83% at 100 °C, which was about four times higher than ET and NT. The increase in solubility of control starch could be a consequence of depolymerization and structural weakening of the starch granules.

In general, swelling power and solubility of starches and fibre residues increases gradually with the increase in temperature in a particular range. Perhaps, this temperature range signifies the onset of gelatinization.

Table 2. Oxalate content of Taro flour and starch, obtained through Oxalate oxidase treatment of Taro flour. Oxalate content is expressed in mg in 100 g of starch on dry weight basis.

Sample	Total oxalate	Soluble oxalate	Insoluble oxalate
Taro flour	790 ± 5.77	639 ± 5.8	150 ± 11.56
Taro starch	24.2 ± 0.2	22.42 ± 0.02	1.78 ± 0.21

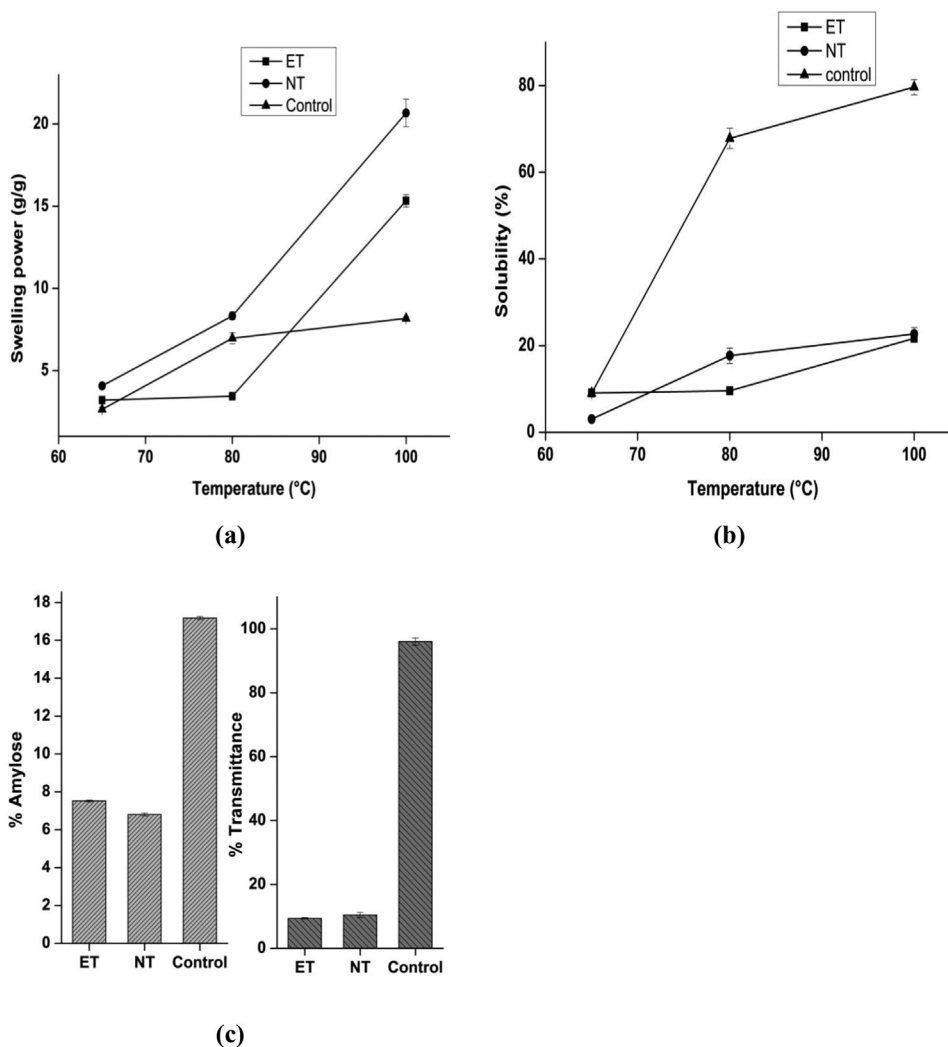


Figure 1. Physico-chemical properties of Taro starch produced without enzyme treatment (NT), with enzyme treatment (ET) and control (Corn starch). a) Swelling power, b) Solubility, c) Amylose % and Paste clarity.

4. Amylose Content

Both NT and ET had similar but low amylose content of around 7%, which was three times less than that of the control (Figure 1c). The low amylose content of ET and NT corroborates the results of swelling power of the starch varieties. Comparison of the swelling power and amylose content of taro tuber starch and control suggests that swelling power is negatively correlated to amylose content. Amylose acts as both a diluent and an inhibitor of swelling.^[22] It has been suggested that amylose plays a role in restricting initial swelling, but swelling proceeds rapidly after leaching of amylose molecules. The extent of leaching of amylose mainly depends on the lipid content of the starch and the ability of the starch to form amylose-lipid complexes. The amylose-lipid complexes are insoluble in water and require higher temperatures to dissociate.^[23] Therefore, in the present study increase in swelling power with increasing temperature could be phenomena of low amylose content and progressive dissociation of insoluble amylose-lipid complex if any with the

rise in temperature. Such low amylose starch has excellent solution stability, including freeze-thaw stability, and prevents retrogradation. The starch is useful in a wide variety of food, pharmaceutical, and industrial applications, either with or without chemical modification. Low amylose starch increases the shelf life of products in bread making industry, improve the texture of noodles and common Chinese spaghetti (Cuisine made out of long, thin, solid, cylindrical pasta).^[24] In addition to this, low amylose starch finds application in textile, paper and adhesive industry.^[25]

4.0.3. Crystalline Properties

The crystalline information obtained by the X-ray diffraction spectra of the ET, NT, and Control is presented in **Figure 2**. There were three strong diffraction peaks (2θ) at 15° , 17.8° , 23.2° , and few additional sharp peaks at 14.95° , 26° , 32° for NT. This is a typical A-type pattern of starch granular arrangement, with

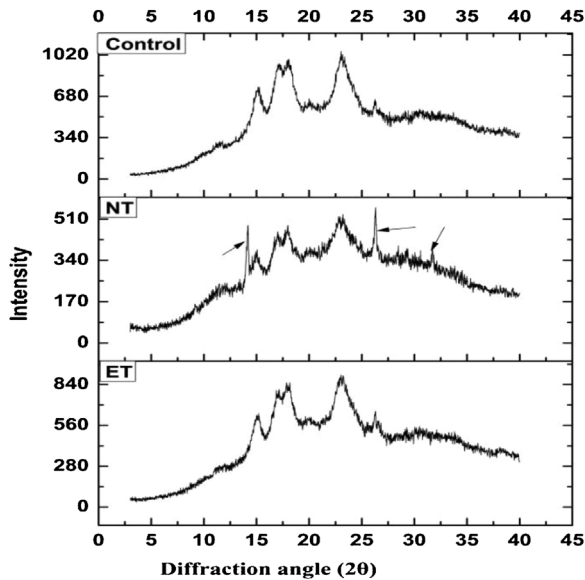


Figure 2. XRD patterns of Taro starch produced without enzyme treatment (NT), with enzyme treatment (ET) and control (Corn starch). Peaks with arrow in case of NT represents characteristics peaks of calcium oxalate.

strong reflections at 2θ , 15° , and 23° and an unresolved doublet at 2θ , 17° , 18° . Unlike these starches, tuber starches like potato show the B-form of the crystalline structure of the starch granules.^[23] A-type crystals tend to be more resistant to enzyme digestion than the B-crystal form.^[26] Starch with an A-type crystal structure is not degraded rapidly by human digestive enzymes in the upper gut and known to be associated with health benefits due to a slower release of glucose into the bloodstream resulting in reduced postprandial glycemic and insulin responses.^[26,27] The corresponding crystallinity levels calculated from the ratio of diffraction peak area and total diffraction area of starches were about 9.63, 10, and 15% for ET, NT, and control, respectively. Additional sharp peaks in NT (indicated by arrows in Figure 2) are characteristics peaks of calcium oxalate monohydrate crystals.^[28] The absence of characteristics peaks of calcium oxalate in case of ET gives a qualitative insight of the whole

strategy of insoluble calcium oxalate reduction in the present study.

The reduction of insoluble oxalates in ET due to the enzymatic treatment of taro flour followed by extraction is quite unusual. The enzymes are expected to attack only soluble substrates. However, it is rarely found that an enzyme can bound to its crystalline substrate. There are few reports of crystalline cellulose being hydrolyzed by cellulase enzyme.^[29] We suspect a similar mechanism could be responsible for the reduction in insoluble oxalates to a small extent. The major reason for the reduction in insoluble oxalates could be due to the starch extraction procedure. The calcium oxalate crystals might be bound to the fibres of taro^[12] which remained entangled with the discarded filtrate. The XRD spectra prove that insoluble oxalate content was significantly low in ET also corroborates the HPLC analysis (Table 2).

4.0.4. Scanning Electron Micrograph (SEM)

The morphological characteristics of ET, NT, and control starches using scanning electron microscopy are represented in Figure 3. The SEM of both ET and NT starch showed the smooth surface and irregular shapes of granules without any damage or fissures similar to the starch granules of Control. The SEM images of ET and NT samples did not show much difference in granule morphology, suggesting enzymatic treatment with OxO did not alter the physical structure of starch. The mean diameter of both the ET and NT starch granules were approximately 1–2 μm , whereas that of control ranging from 5 to 15 μm . The granule size from other sources of taro tubers as reported in literature varied from 1 to 10 μm in diameter.^[3,30,31] The granule size is also variable among starches from different cultivars and ranges from 1 to 120 μm .^[19] Variation in starch granule morphology may be due to the biological origin and physiology of the plant and the biochemistry of the amyloplast. This may be also due to variations in amylose and amylopectin content and its structure, which in turn play an important role in the control of the starch granule size and shape.^[32] Because of its small granular size, taro starch could be easily digestible and therefore, it is widely used in baby foods and the diets of people allergic to cereals and children

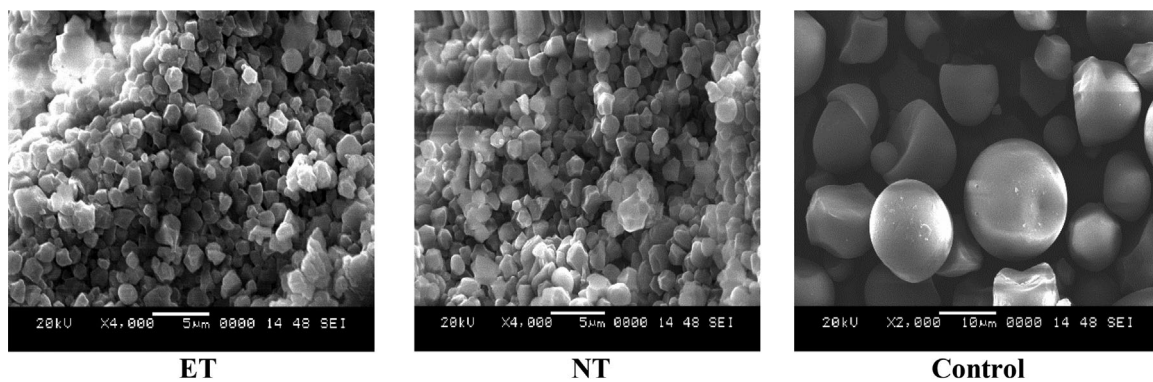


Figure 3. SEM micrographs of Taro starch produced without enzyme treatment (NT), with enzyme treatment (ET) and control (Corn starch). ET (4000 \times), NT (4000 \times), Control (2000 \times).

Table 3. Comparison of physico-chemical properties of starches from different sources.

Starch Source	Amylose content (%)	Swelling ^{d)} power (g/g)	Solubility ^{d)} (%)	Paste clarity (% Transmittance)	Granule pattern	Granule size (μm)
Present study	7.52 ± 0.049	3.19 ± 0.153	9.02 ± 0.268	9.38 ± 0.198	A	1–2
Taro starch	13.5–27.66 ^{a)}	0.89–1.40 ^{a)}	7.42 ^{b)}	10.6 ^{b)}	–	5–10 ^{a)}
Potato starch	20.1–31.0 ^{c)}	19.39 ^{b)}	6.3 ^{b)}	96 ^{c)}	B ^{c)}	1–110 ^{c)}
Sweet potato	–	15.48 ^{b)}	6.47 ^{b)}	8.0 ^{b)}	B ^{c)}	–

^{a)} Deepika et al.^[20], ^{b)} Pramodrao and Riar^[33], ^{c)} Singh et al.^[23], ^{d)} Swelling power and solubility % values estimated at 65 °C for the present study and at 60 °C for all the other starch samples given.

sensitive to milk. Small granules of taro starch can be a good filling agent for biodegradable polyethylene film, entrapping agent for flavoring compounds like vanillin and is a potential fat substitute. It is also appropriate for cosmetic formulations like face powder and in dusting preparations that utilize aerosol-dispensing systems.^[11,30]

All the studied parameters were compared with the physicochemical properties of potato and sweet-potato starch (Table 3). Thus the starch extracted after enzyme treatment showed very low transmittance indicating higher opaqueness, high swelling power, very low solubility, and low amylose content at a temperature range of 80–100 °C, compared to potato and sweet-potato starch. Moreover, the starch possesses A-crystalline form, unlike B-crystalline form found in potato and sweet-potato starch.

This research aimed at processing taro flour to get oxalate free starch. The outcome of the work would support taro to gain market value and enhance its commercial potential with the permissible level of anti-nutritional oxalate residue left in the extracted starch and interesting physicochemical and functional properties. This research work would also address another burning issue of storage of taro tubers. Taro has a high post-harvest loss due to its high moisture content with an estimated 30% loss during storage of these tubers.^[11] Transformation of perishable taro tubers into non-perishable starch of high purity as reported here would reduce post-harvest loss and would resolve starvation problems in non-developed countries. Physicochemical and functional properties of taro starch studied here and elsewhere would unravel the prospects offered by this root crop and help their utilization.

5. Conclusion

Flour produced from the taro tuber (*Colocasia esculanta*) was treated with OxO enzyme. Subsequently, the starch was extracted. This novel process resulted in 97% reduction in total oxalate content and was less than the permissible oxalate levels in food. Comparison of several physicochemical properties such as paste clarity, swelling power, solubility, amylose content, granule pattern and size of starch produced out of enzyme treatment (ET) and without enzyme treatment (NT) suggests minor changes. Low amylose content, high swelling power and A-type crystal structure of this starch are known to impart higher shelf life, higher solution stability, higher freeze-thaw stability, and lower glycaemic index. Considering these desirable properties, oxalate depleted taro starch produced by this novel

process could be an important source of starch for many food-processing industries. Oxalate depleted taro starch could be ideal for baby food formulation because of its small granule size. The low amylose content of oxalate-depleted taro starch would brighten its use in bread making industry.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

Colocasia esculenta, oxalate, oxalate oxidase, starch

Received: December 19, 2017

Revised: February 6, 2018

Published online: March 12, 2018

- [1] M. H. Madsen, D. H. Christensen, *Starch/Stärke* **1996**, *48*, 245.
- [2] A. C. Kumoro, R. D. A. Putri, C. S. Budiayati, D. S. Retnowati, Ratnawati, *Procedia Chem.* **2014**, *9*, 102.
- [3] S. Sefa-Dedeh, E. K. Agwir-Sackey, *Food Chem.* **2004**, *85*, 479.
- [4] J. L. Kelsay, *Am. J. Gastroenterol.* **1987**, *82*, 983.
- [5] R. P. Holmes, M. Kennedy, *Kidney Int.* **2000**, *57*, 1662.
- [6] E. O. James, I. A. Peter, N. I. Charles, N. Joel, *Niger. Food J.* **2013**, *31*, 113.
- [7] C. I. Iwuoha, F. A. Kalu, **1995**, *54*, 61.
- [8] X. Liu, Y. Wang, L. Yu, Z. Tong, L. Chen, H. Liu, X. Li, *Starch/Stärke* **2013**, *65*, 48.
- [9] N. Sit, S. C. Deka, S. Misra, *J. Food Sci. Technol.* **2015**, *52*, 4324.
- [10] K. Kumar, P. D. Belur, *Prep. Biochem. Biotechnol.* **2016**, *46*, 734.
- [11] Aboubakar, Y. N. Njintang, J. Scher, C. M. F. Mbofung, *J. Food Eng.* **2007**, *86*, 294.
- [12] G. P. Savage, L. Vanhanen, S. M. Mason, A. B. Ross, *J. Food Compos. Anal.* **2000**, *13*, 201.
- [13] H. Huang, Q. Jiang, Y. Chen, X. Li, X. Mao, X. Chen, L. Huang, W. Gao, *Food Hydrocolloids* **2016**, *55*, 244.
- [14] S. V. Gomand, L. Lamberts, R. G. F. Visser, J. A. Delcour, *Food Hydrocolloids* **2010**, *24*, 424.
- [15] S. Sukhija, S. Singh, C. S. Riar, *Food Hydrocolloids* **2016**, *55*, 56.
- [16] S. Nara, T. Komiya, *Starch/Stärke* **1983**, *35*, 407.
- [17] Aboubakar, N. Y. Njintang, J. Scher, C. M. F. Mbofung, *J. Food Eng.* **2009**, *91*, 373.
- [18] Dunford, N.T., (Ed.) John Wiley & Sons, Oxford **2012**.
- [19] BeMiller, J.N., Whistler, R.L., (Eds.), *Starch: Chemistry and Technology*, 3rd edn., Academic Press, Amsterdam **2009**.

- [20] V. Deepika, K. Jayaram Kumar, P. Anima, *Carbohydr. Polym.* **2013**, *96*, 253.
- [21] J. Tattiyakul, P. Pradipasena, S. Asavasaksakul, *Starch/Stärke* **2007**, *59*, 342.
- [22] R. F. Tester, W. R. Morrison, *Cereal Chem.* **1990**, *67*, 558.
- [23] N. Singh, J. Singh, L. Kaur, N. Singh Sodhi, B. Singh Gill, *Food Chem.* **2003**, *81*, 219.
- [24] Jeffcoat, R., Hanchett, D., Tayal, A., Merk, A., US20030108649 A1. Retrieved from <http://www.google.com/patents/US20030108649>, (2003, June 12).
- [25] Tuberosa, R., Graner, A., Frison, E., (Eds.), *Genomics of plant genetic resources*, Springer Science & Business Media, Berlin **2013**.
- [26] L. Copeland, J. Blazek, H. Salman, M. C. Tang, *Food Hydrocolloids* **2009**, *23*, 1527.
- [27] S. Simsek, S. N. El, *Food Chem.* **2015**, *168*, 257.
- [28] A. Okada, S. Nomura, Y. Saeki, Y. Higashibata, S. Hamamoto, M. Hirose, Y. Itoh, T. Yasui, K. Tozawa, K. Kohri, *J. Bone Mineral Res.* **2008**, *23*, 1629.
- [29] D. Gao, S. P. S. Chundawat, A. Sethi, V. Balan, S. Gnanakaran, B. E. Dale, *Proc. Natl. Acad. Sci.* **2013**, *110*, 10922.
- [30] E. Agama-Acevedo, F. J. Garcia-Suarez, F. Gutierrez-Meraz, M. M. Sanchez-Rivera, E. San Martin, L. A. Bello-Perez, *Starch/Stärke* **2011**, *63*, 139.
- [31] F.-K. Zeng, H. Liu, G. Liu, *Starch – Stärke* **2014**, *66*, 142.
- [32] L. Kaur, J. Singh, O. J. McCarthy, H. Singh, *J. Food Eng.* **2007**, *82*, 383.
- [33] K. S. Pramodrao, C. S. Riar, *Food Hydrocolloids* **2014**, *35*, 613.