CHAPTER 3

PROCESSING OF RAW INGREDIENTS FROM

Amorphophallus paeoniiifolius CORM
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OVERVIEW OF THE CHAPTER

This chapter discusses steps required for production of flour and starch. Blanching of slices, dehydration and maceration proceeded to flour production. Flour granule structure, proximate, nutritional composition analysis and starch extraction, physiochemical characterization and resistant starch production is discussed in this chapter.

Quality characterization of shelf-stable flours and production of resistant starch produced thereof from the A. paeoniifolius would lead to the development of new acceptable food products and enhance its commercial potential.
3.1 INTRODUCTION

Root and tuber crops are the most important food crops after cereals. Tropical root crops occupy an important place in human nutrition, especially among the low-income groups of population in the developing world. Food insecure households look to these crops as a major calorie contributor. Researchers have focused on investigation of structure and physicochemical properties of several underutilized tropical tuber and root starches[74–76] . Neglected plants like aroids are gaining interest as food source as they are also rich in tuber and root starches. Optimal processing techniques and product development potential of these tuberous crop has not been utilized due to the lack of knowledge. In attempt to increase peoples’ preference towards these underutilized food sources, there is a need to transform these commodities into value added products such as flours or starches [77]. In fortification with other nutrients, transforming the microstructure and transformation into starch could improve the functionality of food product as well as increase the storage efficiency of A. paeoniifolius.

Flours and starch isolated from roots and tuberous crops generally have superior viscosity, higher paste clarity and less starchy flavors compared to that of cereal flours [78-79]. Along with these physiological qualities as well as presence of higher amount of dietary fibre in these tuberous crop therefore attributes to offer several other health benefits like preventing obesity, constipation, cardiovascular disease, diabetes and colon cancer. Tuber flours could be rich in fibre content but also be devoid of gluten which may turn to be boon for celiac disease (CD) patients [80].

Starch is second most versatile and useful biopolymer on earth, next to the organic compound cellulose[81]. It is used in numerous food, beverage industry as a multifunctional ingredient that contributes to important characteristics like thickener, stabilizer, extender, binder, glazing and texture-modifier aid [82-83] and also find applications in paper, textile, plywood, adhesive, pharmaceutical, fuel and cosmetics industries[78],[84].

There is need to look for native starches can find application in food industry (syneresis, turbidity, freeze/thaw stability), not requiring chemical or genetic modifications is of great importance [85].
*A. paeoniifolius* contain good source of protein as well as starch and is an underutilized crop. Its nutritional quality has potential to be a valuable food source for human consumption. Traditionally it has been used for the treatment of piles, abdominal disorders, tumours, enlargement of spleen, asthma and rheumatism [7].

Physiochemical characterization of *A. paeoniifolius* flour, starch and its food application study is not been reported. We have studied the nutritional properties of *A. paeoniifolius* flour as well as analysed physicochemical properties of starch which can aid its future applications and end use suitability.

### 3.2 MATERIAL AND METHODS

#### 3.2.1 Material

Mature *Amorphophallus paeoniifolius* corm used in this study was collected from local market of Ghaziabad. After washing with clean water the corms were stored at 5°C till use.

#### 3.2.2 Flour preparation

The corm was peeled, rinsed with distilled water, cut into 1-2 cm cubes and sliced into thick chips (~5mm). These chips were then soaked in boiling water for ~5 min and oven dried at 30-40°C for 24 hours until the moisture content reached 8–10%. Subsequently, the dried chips were milled into flour and sieved through a 100-mesh screen. The dried flour was packed in zip lock bags and stored in air tight containers left at room temperature.

#### 3.2.3 Starch Extraction

*Amorphophallus paeoniifolius* starch was extracted by the method of (Vanna, T., Khajee Boondee., Thanachan Mahawanich 2004) [86]. Flour samples were further grounded in a blender with 0.2% sodium hydroxide solution in the ratio of (1:2). The slurry was filtered through double fold cheese cloth and allowed to settle starch pellets at bottom after 2 hours sediment was suspended in distilled water and centrifuged at 6000 rpm 20 °C for 30 min. The pellet was re suspended in two volumes of water and centrifugation process repeated. The washing steps were repeated until the supernatant was clear and the starch was free of colour. The colour free pellet was then dried in a hot air oven at 45 ±5°C for 16 hours until the
moisture content was less than 10%. The dried starch samples were ground, sieved through 100 mesh sieves and stored in air tight containers.

3.2.4 Proximate analysis and nutritional composition of Flour

Total Ash and Acid insoluble ash, was done according to method 923.03 (AOAC, 1990). Moisture content of flour was determined according to method 964.22 (AOAC, 1990). All analysis was done in triplicate. Total Fat, Protein, Carbohydrate, sugar, dietary fibre, Vitamin C, sodium, Potassium, Phosphorous, calcium, Iron, cholesterol, Vitamin A, Vitamin D₃ and gluten content of *A. paeoniifolius* flour were tested as per the protocol mentioned in Table 3.2.

3.2.5 Characterization of flour and starch

3.2.5.1 Microscopy

Scanning electron micrographs (SEM) of *A. paeoniifolius* flour and starches were obtained using a scanning electron microscope (Model ZEISS EVO 50, UK) from Amity Noida. Isolated starches were sprinkled on double-sided adhesive tape fixed on an aluminium stub. The sample was coated with gold and an accelerating potential of 3 kV was used during electron microscopy in this study.

3.2.5.2 X-ray diffraction

X ray diffraction of starch and flour sample was carried out using X-ray diffractometer, XRD-6000 Shimadzu Analytical, Japan with target voltage 40 kV current 30 mA scanning range (2theta) 10-60 degree for starch and 20-80 degree for flour with continuous scan and scan speed 2.0 degree/min receiving slit width 15mm operating conditions.

3.2.6 Physicochemical properties of starch

3.2.6.1 pH of starch

Starch (5 g) was suspended in 20 ml of distilled water in a beaker and stirred for 5 min. Starch content was allowed to settle and the pH of the water phase was measured using a calibrated pH meter [87].

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3.2.6.2 Water binding capacity

Water binding capacity (WBC) was determined in triplicate by modified method of Yamazaki 1953, Medcalf and Gilles 1965) [88-89]. 5 gram of starch and 75 ml of distilled water was added to pre-weighed centrifuge tubes at room temperature and then kept at 25 ºC for 30 min with every 5 min shaking period. Tubes were centrifuged at 5000 rpm for 30 min, the supernatant was decanted and the tubes were allowed to drain for 10 min at a 45º angle. The tubes were then weighed and the gain in weight was used to calculate the water binding capacity.

3.2.6.3 Swelling power

The swelling power and solubility were measured according to (Lan et al. 2008)[90]. 1% (w/v) starch suspensions was prepared and incubated in a shaking water bath for 30 min at temperature ranges from 50 to 90ºC and left for cooling to room temperature. Suspensions were centrifuged for 15 min at 5000 rpm. The supernatant was decanted and the sediment weight taken. Swelling power is defined as the ratio in weight of the wet sediment to the initial weight of dry starch.

3.2.6.4 Paste clarity

Starch suspensions (1%, w/w) in screw cap tube were placed in boiling water for 30 min with mixing. After cooling to room temperature samples were refrigerated to 4ºC for 7 days and every 24 h transmittance (%T) was determined at 650 nm against water as blank using spectrophotometer.

3.2.6.5 Syneresis: stability to freezing and refrigeration

Aqueous starch suspensions containing 6% solids were rapidly heated up to 95 ºC for 15 min under constant agitation; cooled to 50 ºC and these suspensions were then held at this temperature for 15 min. The aliquot of 50 ml was subjected to three temperature conditions: cold storage at -20ºC, 4 ºC and ambient temperature for 5 days. Every 24 h the samples were centrifuged at 8000 rpm for 15 min. The percentage of water separated after each freeze–thaw cycle was measured. Syneresis was expressed as the percentage of water released after centrifugation.
3.2.7. Resistant starch

The RS content was analysed according to the method of (Goñi et al. 1996)[91]. Starch samples (equivalent to 100 mg of dry matter) mixed with KCl–HCl buffer (10 ml, pH 1.5) and homogenized. Pepsin solution (0.2 ml) (1 g pepsin/10 ml KCl–HCl) was added, mixed thoroughly and shaken continuously for 60 min at 4°C and cooled to room temperature. 9 ml Tris Maleate buffer (0.1 M, pH 6.9) and 1 ml of α -amylase solution (40 mg α-amylase/ml Tris Maleate buffer) was added and incubated at 37 °C for 16 h with constant shaking. After 16 hours samples were centrifuged and the supernatants were discarded, re-suspended in 10 ml distilled water and the centrifugation repeated. 3 ml KOH (4 M) was added mixed well and kept at room temperature with constant shaking for 30 min. Then, 5.5 ml of HCl (2M), 3 ml of sodium acetate buffer (0.4 M, pH 4.75) and amylloglucosidase were added, mixed thoroughly and incubated at 60 °C for 45 min with constant shaking. Samples were centrifuged and the supernatants collected. Supernatant were checked for glucose. Total glucose was analyzed using a GOD-POD reagent. The resistant starch was calculated as glucose (mg) * 0.9.

3.3 RESULT AND DISCUSSION

3.3.1 Proximate, Nutritional analysis of flour and health claims

Proximate composition of dried flour has moisture content 4.72%, total ash and acid insoluble ash 4.0 and 2.0% respectively. Results are shown in Table 3.1.

Moisture content of the flour was within acceptable range (5%–8%), thus implying that the flour can be stored for a long period. Acid-insoluble ash value of the flour is 2.0% shows that small amount of the inorganic component is insoluble in acid and adulteration of raw ingredients by silica and rice husk substances is very less. Ash values determine the inorganic composition and other impurities present along with the flour. These values are within the limits of Ayurvedic Pharmacopeia of India. The proximate composition testing was carried out in Organic India, Lucknow.
Table 3.1: Proximate composition of flour

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Result</th>
<th>API Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>4.72%</td>
<td>NMT 10%</td>
</tr>
<tr>
<td>Total Ash</td>
<td>4.0%</td>
<td>NMT 8.0%</td>
</tr>
<tr>
<td>Acid Insoluble Ash</td>
<td>2.0%</td>
<td>NMT 2.0%</td>
</tr>
</tbody>
</table>

Nutritional profile of flour was checked as per standard guidelines in NABL certified laboratory, Sigma Test & Research Centre, Delhi. Results are shown in Table 3.2.

Table 3.2: Nutritional analysis of *A. paeonifolius* flour

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Test Parameters</th>
<th>Unit</th>
<th>Result</th>
<th>Test Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total Fat</td>
<td>g/100g</td>
<td>0.31</td>
<td>AOAC 920.85</td>
</tr>
<tr>
<td>2</td>
<td>Protein</td>
<td>g/100g</td>
<td>9.72</td>
<td>IS: 7219-1973</td>
</tr>
<tr>
<td>3</td>
<td>Carbohydrate</td>
<td>g/100g</td>
<td>73.48</td>
<td>AOAC 986.25</td>
</tr>
<tr>
<td>4</td>
<td>Total Sugar</td>
<td>g/100g</td>
<td>5.10</td>
<td>AOAC 923.09</td>
</tr>
<tr>
<td>5</td>
<td>Total Dietary Fibre</td>
<td>g/100g</td>
<td>12.15</td>
<td>AOAC 985.29</td>
</tr>
<tr>
<td>6</td>
<td>Vitamin C</td>
<td>mg/100g</td>
<td>N.D(D.L -1.0)</td>
<td>AOAC 967.21</td>
</tr>
<tr>
<td>7</td>
<td>Sodium</td>
<td>mg/100g</td>
<td>67.54</td>
<td>AOAC 966.16</td>
</tr>
<tr>
<td>8</td>
<td>Potassium</td>
<td>g/100g</td>
<td>1.47</td>
<td>AOAC 965.30</td>
</tr>
<tr>
<td>9</td>
<td>Phosphorus</td>
<td>mg/100g</td>
<td>180.31</td>
<td>AOAC 965.17</td>
</tr>
<tr>
<td>10</td>
<td>Calcium (as Cu)</td>
<td>mg/kg</td>
<td>393.05</td>
<td>AOAC 985.35</td>
</tr>
<tr>
<td>11</td>
<td>Iron (as Fe)</td>
<td>mg/kg</td>
<td>42.44</td>
<td>AOAC 985.35</td>
</tr>
<tr>
<td>12</td>
<td>Cholesterol</td>
<td>g/100g</td>
<td>N.D(D.L -0.3)</td>
<td>STRC/STP/F050</td>
</tr>
<tr>
<td>13</td>
<td>Vitamin A (By HPLC)</td>
<td>µg/RE/100g</td>
<td>N.D(MDL -7.5)</td>
<td>STRC/STP/F048</td>
</tr>
<tr>
<td>14</td>
<td>Vitamin D3 (By HPLC)</td>
<td>IU/100g</td>
<td>N.D(MDL -25)</td>
<td>STRC/STP/F049</td>
</tr>
<tr>
<td>15</td>
<td>Gluten content</td>
<td>mg/kg</td>
<td>45</td>
<td>ELISA</td>
</tr>
</tbody>
</table>
As per Codex guidelines and Food safety and Standards Authority of India rules any product can be labelled fat free if it is containing not more than 0.5 g of fat per 100 g/ml of food. Natural claim can be used when packaging is done without chemicals and preservatives and Cholesterol free is used where the product contains not more than 5 mg cholesterol / 100g /ml of food. A. paeoniifolius flour has 0.31 g /100 g of fat; this result shows that flour is fat free, cholesterol not detected and flour is processed without any preservative so it is natural, cholesterol free. Fat free diet has several benefits like it may reduce the risk of some cancers.

As per Food Safety and Standards Authority of India guidelines, food that is high in fibre, may only be made where the product contains at least 6 g of fibre per 100 g or 100 ml or 20% of RDA per serving. In our case we reported 12.15 g/100 g dietary fibre this result shows that A. paeoniifolius flour can be labelled as high in dietary fibre or rich in dietary fibre.

The official limits described in the Codex Draft are 20 ppm for foodstuffs that are considered naturally gluten-free and 200 ppm for foodstuffs rendered gluten-free and also as per Codex Standard 118 and recent EC Regulations define “Gluten Free” (GF) foods for Particular Nutritional use (PARNUTS) as containing less than 20 Parts per Million (PPM; mg/kg). PARNUTS foods above 20 PPM & below 100 PPM must be labelled “Very Low Gluten” special dietary use by persons intolerant to gluten foods, should be labelled as reduced-gluten, suitable for most coeliacs”. A. paeoniifolius flour has 45 ppm gluten that indicates that this can be labelled as rendered gluten free and suitable for most coeliacs.

Apart from being fat free, high in fibre, A. paeoniifolius flour are having reduced gluten which offers significant opportunities for exploring this flour use in different food systems. The number of gluten intolerant people has doubled in the past decade and the number of new gluten-free products increased in the United States by five-fold, i.e. 135 in 2003 to 832 in 2008 [92] The global market for GF products was valued at $ 3770 million in 2012 and is projected to reach $6,206.2 million at a CAGR of 10.2% by 2018. Gluten free diet is not only a medically accepted treatment for people suffering from celiac disease but also for people who are health conscience. The additional benefits of weight management, nutrition & sound digestive health is further creating a shift even among gluten tolerant consumers, driving up the demand for gluten free products.
3.3.2 Granule Morphology

SEM studies revealed that flour and starch granules are round, elliptical and polygonal in shape. SEM images of flour with small agglomerates of large and small starch granules attached with remnants of the protein matrix are shown in Figure 3.1. SEM image of starch is shown in Figure 3.2. The diameter of starch granules ranged from 5–20 µm which is considered to be small when compared to other starch types e.g. potato (10–65 µm)[93].

Generally botanical sources have round, oval, although some may be elliptical, lenticular, polyhedral, polygonal and irregular sausage-shaped starch granules.[94]. The difference in the size and shape of starch granules is attributed to the plant biological origin [95]. Physicochemical properties, like swelling power, percent light transmittance, water-binding capacity and amylose content and other functional properties were highly different due to difference in plant sources and significantly correlated through the average granule size of the starches separated from those sources[96].

Figure 3.1: Scanning Electron Microscopy (SEM) of A. paeoniifolius flour
The smaller granule sizes have been found to get better the digestibility because of greater surface area and are more rapidly digestion by enzymes [97-98]. Increasing knowledge of the granular structure and functional properties of the starches has enabled researchers to process them in a novel way to modify starches to meet special demand of food and other industries [95].

**Figure 3.2**: Scanning Electron Microscopy (SEM) of *A. paeonifolius* Starch

3.3.3 XRD Analysis

The X-ray diffraction patterns obtained for both starch and flour are shown in Figure 3.3 and Figure 3.4 respectively. Starches tend to present pertinent crystalline arrangements depending on their botanical origin [99]. The positioning of the peaks and their intensity of diffraction peaks depend on the starch granule source. Three main patterns, A-, B- and C-types, have been identified to describe native starch granules. Generally the A-type starch granules include the cereal starches, the B-type the starches from tubers and fruits and C-type from cassava starches [100]. For cereal or A pattern, these peaks appear predominantly as one doublet at 18° (2θ) and a singlet at 23°(2θ). Tubers or B pattern are recognized by the intensity of the corresponding band to one doublet at 5°(2q) and 6°(2θ), two singlets at 15°(2θ) and 17°(2θ) and one doublet at 22°(2θ) and 24°(2θ).
The crystalline peaks were analyzed for starch sample in the interval from 10° to 60°, identifying the most intense peaks and calculating the distances between the planes of the crystals \(d\) (Å) from the diffraction angles \(\theta\) according to Bragg’s law. Starch shows the diffraction of the crystalline structure appears, with three major peaks at 17.28°, 18.08°, 15.3421°, corresponding to inter planar spacing \(d = 5.12\) Å, 4.90 Å, and 5.77 Å respectively.
As shown in Figure 3.4, *A. paeoniifolius* flour sample had clear A-type diffraction patterns with main reflections at 2θ= 22.9°, 26.36°, 27.93°, 38.05°. The crystallinity characteristics of *Amorphophallus* flour was different to its starch, however, the interplaner spacing of *A. paeoniifolius* flour was lower than starch. Flour granules contained proteins and fat which influence the granule structure and crystallinity of grains.

### 3.3.4 Paste clarity

Paste clarity is another important property of flour and starch this property governs its applicability in food processing. Improved paste clarity is a useful property in the manufacture of some foods like jellies, sausages and fruit pastes, which require transparency [101]. Transparent starch paste is required to thicken fruit pies where as opaque paste, which is more suitable for salad dressing[86] As depicted in Figure 3.5, maximum paste clarity (94.95%) light transmittance is reported at 2-day of storage. Several factors that may also influence paste clarity such as presence of amylose, lipids and protein contents of the sample. The botanical source, particle size of granules, total solids concentration, degree of granule dispersion and the capability of granules to form aggregates also affects paste clarity [102].
3.3.5 Water absorption capacity, pH and yield

The water absorption capacity of *A. paeoniifolius* starch is 70.35%, pH of 7.2 with 10.5 % yield as shown in Table 3.3. Water absorption capacity (WAC) is significant for certain product characteristics, such as the moistness of the product, starch retrogradation and the subsequent product staling. Starches with high WAC have more hydrophilic constituents such as polysaccharides. Hydration or rehydration is the first and perhaps most critical step in imparting desirable functional properties to proteins in a food system. Interactions of water in starch are very significant characteristic because of their effects on the flavour and texture of foods. pH 7.2 is suitable for acting of amylase enzyme on the starch since optimum temperature of amylase is pH 7.0.

<table>
<thead>
<tr>
<th>Starch Sample</th>
<th>Colour</th>
<th>Water binding capacity (%)</th>
<th>pH</th>
<th>Starch Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. paeoniifolius</em> Starch</td>
<td>Pale white</td>
<td>70.35 %</td>
<td>7.2</td>
<td>10.5%</td>
</tr>
</tbody>
</table>
3.3.6 Freeze-thaw stability

The syneresis tendency of the starch was expressed as liquid liberated from their pastes after freeze-thaw cycles. Starch presented poor stability to freezing as reflected by high amount of water drained during the experiment. At room temperatures lowest level of water liberated in the fourth and fifth cycles as depicted in Figure 3.6. Starch gels are metastable and non-equilibrium systems so undergo structural changes during storage[103].

![Figure 3.6: Effect of syneresis (%) of A. paeoniiolius starch after storage at different temperature](image)

This Syneresis phenomenon is undesired for the use of starch in both food and non-food industries. As shown in Fig. 3.6 at room temperature the pastes are quite stable, meaning that they can form elastic gels with water binding capacity. In contrast, the refrigerated and frozen starch gels are more unstable, the released amount of water increasing with the decrease in storage temperature and the increase in the period of storage.
3.3.7 Swelling power

Swelling power is an indicator of water absorption index of the granule. During heating it contributes to important characteristics of starchy food products, such as pasting and rheological behaviour with excess water. In this study the swelling power of starch samples were measured at 10 °C intervals from 50-90 °C (see Figure 3.7). Swelling power slowly increased from 50 °C to 60°C and rapidly increased upto 90 °C.

Swelling power determines the hydration ability of starch granule and it is measured by weighing the swelled starch and retained water. Such property is very important for certain starch application like those from food industry where the quality of starch-based products is strongly related to the capacity of starch granule to retain water and swell.

![Figure 3.7: Effect of temperature on swelling power of A. paeoniifolius starch](image)

It is well known that starch cannot be dissolved in cool water, this characteristics attributed to the starch crystal structure. However, when starch was heated in excess water, the crystalline structure was disrupted and water molecules became linked by hydrogen bonding to exposed hydrogen group of amylose and amylopectin results in dissociation of the amylose and amylopectin in suspension, and the solubility of starch was increased [93].
3.3.8 Resistant starch

The RS content is an significant parameter mainly considered from a nutritional point of view as the starch in this form is less easily digested and this may impart health benefits [104]. Table 3.4 shows the Total, digestible and resistant starch content of A. paeoniifolius.

Table 3.4: Total starch, digestible starch and RS contents (mg/100g) of A. paeoniifolius

<table>
<thead>
<tr>
<th>Total starch</th>
<th>Resistant starch</th>
<th>Digestible starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>142 mg</td>
<td>115 mg</td>
<td>27 mg</td>
</tr>
</tbody>
</table>

3.4 CONCLUSION

The information regarding nutritional aspects of flour and physiochemical properties of starch are important for developing new products using its flour and starch. Nutritional analysis of flour revealed some unique characteristics like fat and cholesterol free, high dietary fibre containing flour. These properties propose the application of its flour and starch to suitable products. One particular area where A. paeoniifolius flours could be used is development of gluten-free products, which have shown a tremendous marketing potential in recent years. In the future, the possibility of these samples to partially substitute wheat flour to produce composite flours with new properties that are suitable for certain food application needs to be studied.

Amount of resistant starch and the slow digestibility make the use of this starch as a valuable alternative carbohydrate source, this property could offer health benefits like, aiding in the prevention of certain diseases such as obesity and hypertension.

This study shows that A. paeoniifolius flour and starches may be seen as having very broad applications within the food and other industrial applications and could become attractive alternatives for food developers, depending on their characteristics and functional properties.