CHARACTERIZATION OF STARCH FROM DIFFERENT TARO (*COLOCASIA ESCULENTA*) CULTIVARS OF ASSAM AND EFFECT OF ULTRASOUND-ENZYMATIC TREATMENT ON YIELD AND PROPERTIES OF STARCH

A thesis submitted in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

By

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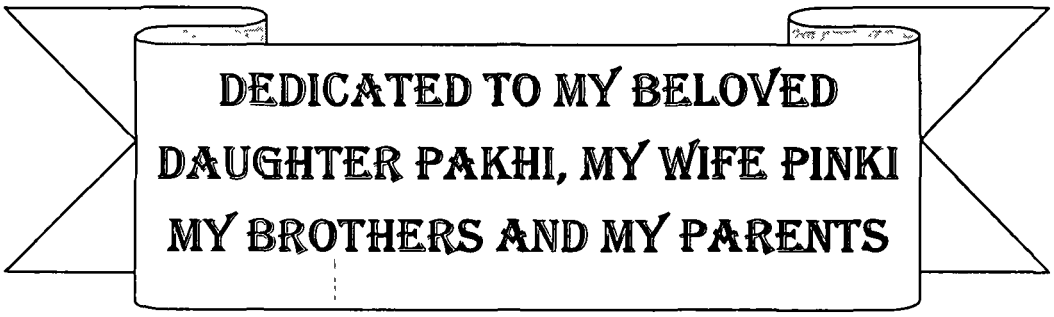
NOVEMBER 2013


DEDICATED TO MY BELOVED
DAUGHTER PAKHI, MY WIFE PINKI
MY BROTHERS AND MY PARENTS

Characterization of starch from different taro (*Colocasia esculenta*) cultivars of Assam and effect of ultrasound-enzymatic treatment on yield and properties of starch

ABSTRACT

The thesis includes extraction and characterization of starches from various taro cultivars available in Assam, India, and their application in food models. The present investigation deals with characterization of various physicochemical, functional, textural and colour properties of taro starches. In order to establish the application of taro starch it was incorporated in preparation of ketchup. In addition, the extraction of starch from taro tubers was studied using enzymes, ultrasound and their combination with an intention to increase the yield of starch.

The thesis is divided into seven chapters which are discussed below:

Chapter 1 presents the general introduction about taro, its production and utilization. It also deals with compositions, physicochemical and functional properties of starch, structure of amylose and amylopectin molecules, and how the composition and other properties are related to each other. The processes of extraction of starch from various agricultural materials are described. Application of enzymes and ultrasound for isolation of starch is included in this chapter. Finally the scope and objectives of the present investigation are also included.

Chapter 2 deals with the characterization of starches isolated from various taro cultivars of Assam, India. Various physicochemical and functional properties like proximate composition, amylose content, granule size and shape using SEM, XRD pattern and FTIR spectra of the starches, swelling, solubility, freeze-thaw stability, clarity, pasting properties, gelatinization properties, etc. of the isolated starches were investigated. Texture and colour properties of the starches were measured using Texture analyzer and Hunter colorimeter. The properties of the taro starches were compared with rice, maize and potato starches in this chapter.

Chapter 3 investigates the effect of incorporation of taro starch as thickener on quality parameters of tomato ketchup during storage. In this chapter the process of preparation of tomato ketchup using different concentrations of taro starch is described. The properties of tomato ketchup investigated were serum loss, texture

and colour. The ketchup prepared using taro starch was compared with the ketchup prepared using maize starch and also with control (ketchup prepared without using any starch). Sensory evaluation of the different ketchup samples were carried out.

Chapter 4 includes the optimization of starch extraction process using various cell wall degrading enzymes. Different parameters like concentration of enzymes, incubation time and temperature of incubation were optimized to maximize the yield of starch. After optimization, the properties of the starch isolated by using the optimized enzymatic process were compared with the starch isolated by conventional method.

Chapter 5 reports the effect of various ultrasound processing parameters on yield of taro starch. Various parameters of ultrasound like time, cycle and amplitude were tested. The effect of ultrasound on functional properties of taro starch like swelling, solubility, clarity, freeze-thaw stability, texture and colour were investigated in this chapter.

Chapter 6 describes the starch extraction methods from taro tubers using combination of enzymes and ultrasound. The effect of enzyme concentration and time of ultrasound treatment on yield of starch was investigated. The changes in properties of the starches isolated by the various experimental combinations were studied in this chapter.

Chapter 7 presents the conclusions of the work carried out, salient findings and future scopes of the present investigation. It is concluded that the taro starches studied were found to be closer to the premium cereal starches i.e. rice and maize with respect to its structure and various properties. It could be used as a substitute for cereal starches in many food and non-food applications. Extraction of starch using enzymes, ultrasound alone and in combination were able to increase the yield of starch significantly from taro tubers compared to conventional method and resulted in improved functional properties.

Declaration by the candidate

The thesis entitled **"Characterization of starch from different taro (*Colocasia esculenta*) cultivars of Assam and effect of ultrasound-enzymatic treatment on yield and properties of starch"** is being submitted to School of Engineering, Tezpur University in partial fulfilment for the award of the degree of Doctor of Philosophy in the Department of Food Engineering and Technology is a record of bonafide research work accomplished by me under the supervision of **Prof. S. C. Deka**.

All helps from various sources have been duly acknowledged.

No part of the thesis has been submitted elsewhere for award of any other degree.

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CERTIFICATE OF THE SUPERVISOR

This is to certify that the thesis entitled "**Characterization of starch from different taro (*Colocasia esculenta*) cultivars of Assam and effect of ultrasound-enzymatic treatment on yield and properties of starch**" submitted to the School of Engineering, Tezpur University in partial fulfilment for the award of the degree of Doctor of Philosophy in the Department of Food Engineering and Technology is a record of research work carried out by **Mr. Nandan Sit** under my supervision and guidance.

All helps received by him and from various sources have been duly acknowledged.

No part of this thesis has been submitted elsewhere for award of any other degree.

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ACKNOWLEDGEMENTS

I feel extremely privileged to express my deep sense of reverence and indebtedness to my supervisor and Chairman of the Doctoral Committee, Dr. S. C. Deka, Professor, Department of Food Engineering and Technology, Tezpur University, Assam for his sincere exhortation, meticulous guidance and sustained interest, constant encouragement and constructive criticism, and painstaking efforts throughout the course of the investigation and preparation of the thesis.

I express my deepest gratitude and veneration to the esteemed members of my Doctoral Committee, Dr. C. L. Mahanta, Professor, Department of Food Engineering and Technology & Dean R&D, Tezpur University, and Dr. S. Ray, Associate Professor, Department of Molecular Biology and Biotechnology, Tezpur University for their valuable suggestions and encouragement at various stages of the investigation and thesis writing.

Sincere regards are due to Prof. M. K. Chaudhury, Vice Chancellor, Tezpur University, Prof. D. K. Bhattacharya, Dean, School of Engineering, Tezpur University and Dr. M. K. Hazarika, Head, Department of Food Engineering and Technology, Tezpur University for providing necessary facilities to carry out the research work.

With a sense of gratitude and great pleasure, I acknowledge the whole hearted cooperation extended by Dr. S. K. Dolui, Professor, Department of Chemical Sciences, Tezpur University.

Thanks are due to the members of Departmental Research Committee, all my friends and colleagues for getting all sorts of help from them during my research work.

Thanks are also due to staffs of Department of Food Engineering and Technology, for their assistance and cooperation from time to time throughout the period of this investigation.

A very special thank to Mr. Sudip Misra for constantly helping me throughout my work.

Words would never be able to fathom the depth of feelings for my beloved daughter Pakhi, my wife Pinki and revered parents and brothers, for their constant showering me with blessings and love.

My abstruse regard goes to The Ministry of Food Processing Industries for the financial help received during the course of my doctoral programme.

Lastly, I thank the God, almighty for his blessings during all the years of my life and studies.

(Nandan Sit)

Tezpur
November, 2013

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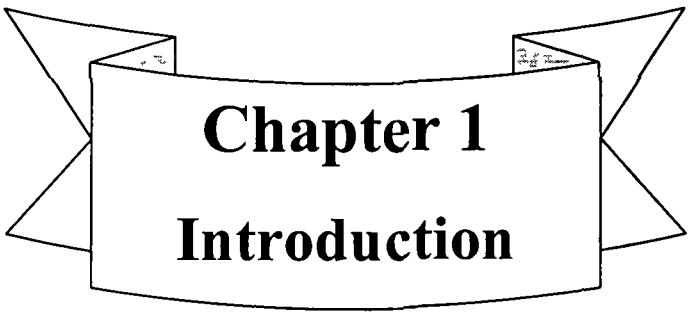
List of Abbreviations

%T	Per cent transmission
a*	Redness
AFM	Atomic force microscope
ANOVA	Analysis of variance
AO	Ammonium oxalate
b*	Yellowness
BV	Breakdown viscosity
CCRD	Central composite rotatable design
CWDE	Cell wall degrading enzymes
db	Dry basis
DoBS	Dodecylbenzene sulfonate
DSC	Differential scanning calorimeter
FAO	Food and Agriculture Organization
FT-IR	Fourier transform infrared
FV	Final viscosity
HV	Hold viscosity
L*	Lightness or whiteness
LPL	Lysophospholipids
LSD	Least significant difference
MW	Molecular weight
OA	Oxalic acid
p	Probability
PRESS	predicted residual sum of squares
PT	Pasting temperature
PV	Peak viscosity
rpm	Revolutions per minute
RVA	Rapid visco analyzer
SDS	Sodium dodecyl sulphate

SEM	Scanning electron microscope
SLS	sodium lauryl sulfate
SSL	sodium stearoyl lactylate
SV	Setback viscosity
T_c	Conclusion temperature of gelatinization
TEM	Transmission electron microscope
T_o	Onset temperature of gelatinization
T_p	Peak temperature of gelatinization
TSS	Total soluble solids
w/v	Weight by volume
w/w	Weight by weight
wb	Wet basis
XRD	X-ray diffraction
ΔH	Enthalpy of gelatinization



Chapter 1

Introduction

1.1 Taro origin and production

After cereals, root and tuber crops are the most important food crops. They provide a major part of the world's food supply. Approximately 55 percent of roots and tuber production is consumed as food and the rest are used as planting material, animal feed or in the production of industrial products such as starch, distilled spirits, alcohol and a range of other minor products.[1]

India has a rich genetic diversity of tropical root and tuber crops. The major root and tuber crops grown in India are cassava, potato, sweet potato, aroids and yams. Apart from these several minor tuber crops are also grown. The two major regions of global biodiversity in India are North Eastern Himalayas and Western Ghats which are particularly rich in wild relatives of tropical root and tuber crops.[2]

Aroids are perennial herbs belonging to the family *Araceae*. They are tuber bearing plants which include taro (*Colocasia*), giant taro (*Alocasia*), tannia (*Xanthosoma*), elephant foot yam (*Amorphophallus*), and swamp taro (*Cyrtosperma*).[2] Among all the aroids, taro is the most widely cultivated crop and occupies 1.32 million hectares throughout the world and the production is around 9.97 million tonnes of tubers.[3] The production, area harvested and average yield of taro in major taro producing regions of the world is shown in Table 1.1.

Table 1.1 Production, area harvested and yield of taro in major regions of the world

Sl. No.	Region/ Continent	Production, tonnes	Area Harvested, ha	Yield, kg ha^{-1}
1	Africa	7360196	1130762	6509
2	Asia	2195042	134564	16312
3	Oceania	385260	48418	7957
4	North America	1590	160	9938
5	Caribbean	19210	1921	10000
6	South America	5900	1160	5086

Source: FAOSTAT[3]

Taro is an important tuber crop grown in several tropical countries. It is mostly cultivated in Asia, Africa and Pacific, Caribbean Islands and South America. It is the most important crop in the Pacific Islands and is a staple in many countries like Fiji, Papua New Guinea, Western Samoa, Vanuatu etc in the South pacific

region. In India, taro is cultivated in almost all the states, right from the foot hills of Himalayas to the coastal areas in the South. The major area under taro cultivation lies in Eastern and Northern States of India. Taro is believed to have originated in South East Asia including India and Bangladesh.[4] North East India is thought to be one of the centres of origin of taro.[5-8] From there, it probably spread to Egypt, Arabia and the Pacific many centuries ago.

Cultivated taro (Fig. 1.1a) is classified as *Colocasia esculenta*, but the species is considered to be polymorphic.[9] There are at least two botanical varieties: i) *Colocasia esculenta* (L.) Schott var. *esculenta* with a large cylindrical central corm and few cormels and agronomically referred to as the "dasheen" type of taro (Fig. 1.1b), and ii) *Colocasia esculenta* (L.) var. *antiquorum* (Schott) Hubbard and Rehder which has a small globular central corm with many relatively large cormels arising from the central corm (Fig. 1.1c). Agronomically this variety is referred to as the "eddoe" type of taro.[9, 10]

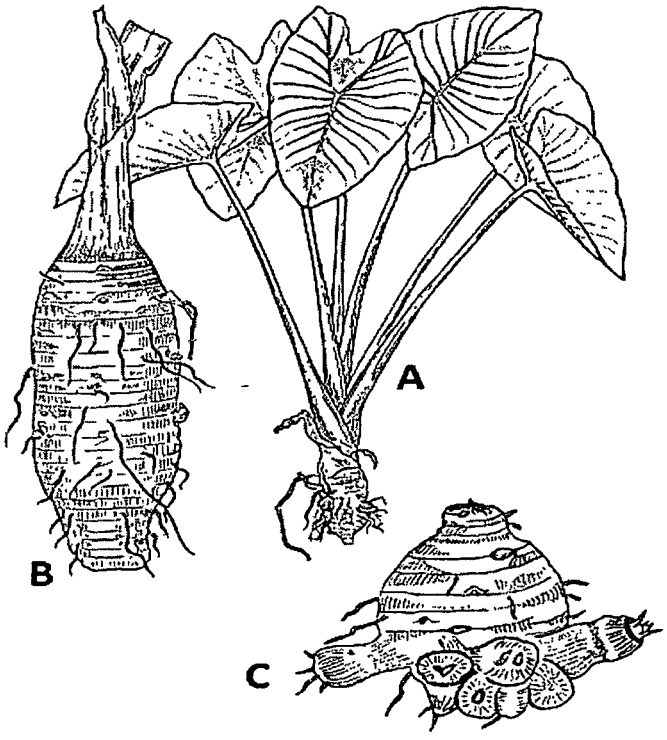


Fig. 1.1 A) Taro plant, B) corm of "dasheen" type taro, and C) corm and cormels of "eddoe" type taro

Taro is also called the "potato of the tropics," or "elephant ears". It is a perennial herb with huge "elephant ear" like leaves (Fig 1.1a). It grows to a height of 1-2 m and produces heart shaped leaves 0.5-2 m long and 0.3-0.6 m across on 1 m long petioles that all emanate from an upright tuberous rootstock, called a corm. The petioles are thick, succulent, and often purplish. The leaf petiole attaches near the centre of the leaf. The leaf is peltate; the root system is fibrous and lies mainly in the top one metre of soil. The corm is the nutrient storage organ of the plant. Corms, cormels and daughter corms are very similar in their internal structure. The tuber consists of an outer thick brownish layer called the periderm. Inside this there is the starch-filled ground parenchyma. Vascular bundles and laticifers are present throughout the ground parenchyma. Idioblasts i.e. the cells which contain raphides or bundles of calcium oxalate crystals are also present in the tubers and other tissues of the plant. The acridity in taro is due to the presence of the raphides. The density and woodiness are more with the older corms.

1.2 Nutritive value and uses of taro

1.2.1 Nutritive value

The most important parts of the taro plant are the corms and the cormels, as well as the leaves. Taro corm is rich in carbohydrate, and is low in fat and protein. The protein content of taro corm is however slightly higher than that of other tubers like yam, cassava or sweet potato, which makes it nutritionally superior. The protein content of taro tubers is around 7% on a dry weight basis.[11] The protein is rich in some essential amino acids, but is low in isoleucine, tryptophan and methionine.[12] The chemical composition of the taro corms[13] is given in Table 2 and that of the carbohydrate fraction[13] is given in Table 3. Starch is the major carbohydrate present in taro corms. It consists of 17-28% amylose, and the rest is amylopectin.[14] The starch is 98.8% digestible which might be attributed to its small granule size, and are approximately a tenth that of potato starch granules, which make it ideal for people with digestive problems.[15] Taro is good for people allergic to milk or cereals and can be consumed by children who are sensitive to milk.[16, 17] The corm is also rich in potassium and fibre. Taro corms are a good source of calcium and iron as well. It is a moderately good source of water soluble vitamins, such as thiamine, riboflavin and ascorbic acid, compared to other tropical roots and tubers.[18, 19]

Table 1.2 Composition of taro corm

Sl. No.	Component	Content, % wet basis
1	Moisture	63-85
2	Carbohydrate (mostly starch)	13-29
3	Protein	1.4-3.0
4	Fat	0.16-0.36
5	Crude Fibre	0.60-1.18
6	Ash	0.60-1.30
7	Vitamin C	7-9 mg/100 g
8	Thiamine	0.18 mg/100 g
9	Riboflavin	0.04 mg/100 g
10	Niacin	0.9 mg/100 g

Source: Onwueme[13]

Table 1.3 Fractions of taro corm carbohydrate

Sl. No.	Carbohydrate fractions	%
1	Starch	77.9
2	Pentosans	2.6
3	Crude Fibre	1.4
4	Dextrin	0.5
5	Reducing sugars	0.5
6	Sucrose	0.1

Source: Onwueme[13]

Taro leaves contain higher levels of protein compared to the corms and are also rich source of carotene, potassium, calcium, phosphorous, iron, riboflavin, thiamine, niacin, vitamin A, vitamin C and dietary fibre which are important constituents of human diet.[12, 20-22] It contains about 23% protein on a dry weight basis. The moisture contents of fresh taro leaf lamina and petiole are 80% and 94%, respectively.[11]

1.2.2 Uses of taro

Taro is a staple food in many countries in the Pacific Island such as Fiji, Papua New Guinea, Western Samoa, Vanuatu etc. It is also important crop in many parts of Africa.[10, 22] Taro corms are used as staple in place of rice or potato.[23] The corms are generally cooked by baking, boiling or baking in the traditional ovens. The mature root is boiled as a starchy vegetable. In Hawaiian Islands; it was the most important food. It was the used to make poi. The young leaves are cooked like leafy vegetables and used for human consumption as a very nutritious vegetable. These young leaves are boiled or covered with coconut cream, wrapped in banana or breadfruit leaves and cooked on hot stones.[24] These large leaves are cooked like mustard or turnip greens and the resulting product is called 'Callaloo' in the Caribbean. Taro plant is one of the few major crops where both the leaf and underground parts are important in the human diet.[17] Taro flour is made by drying the taro corms and is available in some places. It is used for making of soups and other preparations as a thickener. Taro peels and wastes are used as feed.

1.3 Chemical composition of starch

Starch is a natural, renewable and biodegradable polysaccharide found in nature. It is the main storage carbohydrate in plants. It is one of the most abundant natural biopolymer next only to cellulose. Starch is an important constituent of human nutrition. It is the major carbohydrate consumed by humans and is the basic source of energy. Starch is present throughout the plant world. Cereal grains, legume seeds, and tubers are the most important sources of starch. The physicochemical properties of food products made from cereals, tubers, roots, legumes, and fruits are due to the starch present in the raw materials. Starch is present in granular form in the chloroplasts of green leaves and in the amyloplasts of storage organs and other parts of the plant.

Starch is one of the most important raw materials for industrial use. It is an important ingredient in the food industry and is used for variety of purposes such as thickening, gelling, stabilizing and as a replacement or extender for more costly ingredients. Starches are favoured as they are readily available, comparatively low cost and have unique properties. It is also an important raw material for paper, pharmaceutical, textile and cosmetic industry.[25-28]

Corn, potato, wheat, tapioca and rice are the major sources of industrial starch. There are substantial differences in the properties of the starches obtained from different sources. These differences depend not only on differences in the ratio of amylose to amylopectin and the structural characteristics of these molecules, but also on the differences in the content of the non-starch components such as lipids, proteins and phosphate groups present in the starch granules.

Starches are primarily composed of two kinds of polysaccharides, amylose and amylopectin. Amylose is linear, whereas amylopectin is a branched polysaccharide. The ratio of amylose to amylopectin varies with source of the starch and ranges from 17 to70% amylose which corresponds to 30 to 83% amylopectin. Amylopectin is the major component in starches of normal varieties of different crops, with a common ratio of approximately 1:3. There are mutant varieties of crops such as waxy barley, waxy maize, waxy rice, waxy potato, and waxy sorghum starch of which contain 100% amylopectin. Also, there are the high-amylose starches such as amylomaize-5 (53% amylose), amylomaize-7 (70% amylose) and wrinkled pea (66% amylose).[29]

Amylose is a linear polymer of α – D –glucopyranose residues linked together by α (1 → 4) glycosidic linkages (Fig. 1.2). The degree of polymerization varies from 100 to 10,000. Each macromolecule contains one reducing end and one non-reducing end. Two to eight branch points may be present per molecule of amylose in starches from some sources and the chain length of these branch chains generally varies from 4 to 100 glucose residues.[30] In some plant species, amylose has some phosphate groups attached to the C-6 position of glucose residues.[31]

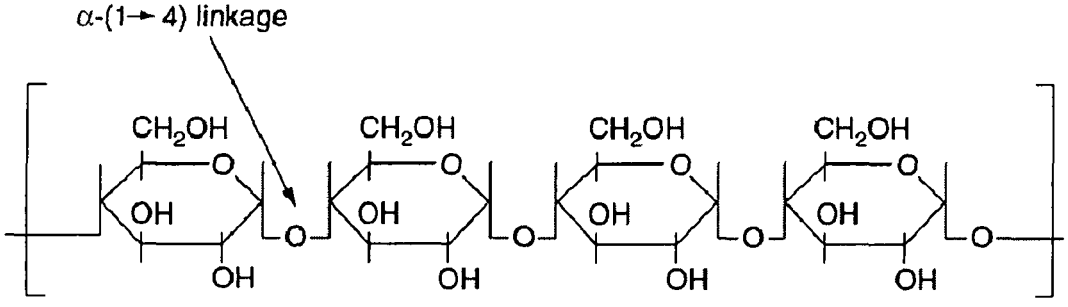


Fig. 1.2 Chemical structure of amylose showing α (1 → 4) glycosidic linkage

Numerous hydroxyl groups are present throughout the amylose molecules (Fig. 1.2) which gives hydrophilic properties. Due to the linear nature of the amylose

molecules, their mobility, and the presence of many hydroxyl groups, they have a tendency of orienting themselves parallely and come close enough which allows hydrogen bonding between two nearby chains, due to which, the affinity of the molecule for water is diminished and the solution appears to be opaque.[32]

The amylose content values ranging from 13.6-23.8% for cassava, 20-25% for sweetpotato, and 3-43% cocoyam starches have been reported depending on variety.[33, 34] Peroni et al.[35] found higher levels of amylose in yam (32.6%), canna (31.7%) and ginger (26.5%) starches than in cassava (19.8%), arrowroot (20.8%) and sweetpotato (22.6%) starches. The amylose contents obtained by Jiranuntakul et al.[36] for normal rice, maize and potato were 21.72%, 25.19% and 28.97% respectively, whereas for waxy rice, maize and potato starches were 1.64%, 2.06% and 3.92% respectively. Shujun et al.[37] found amylose contents ranging from 20.74-25.94% for four different varieties of Chinese yam (*Dioscorea opposita*). Amylose content of five varieties of taro determined by iodine potentiometric titration and gel permeation chromatography ranged from 18 to 22% and 19 to 24% respectively. Dasheen and Bun-long taro starches gave the highest amylose contents while Hawaii White and Hawaii Red had the lowest.[38]

Moorthy et al.[39] reported variation in total and soluble amylose content ranging from 14 to 19% and 4 to 11% respectively for 10 cultivars of taro from India. Falade and Okafur[40] observed wide variation in the amylose contents of the starches from three *Colocasia* and two *Xanthosoma* cultivars investigated. It was observed that amylose content varied from 11.55 to 33.77% among the five cultivars. The amylose contents as determined by differential scanning calorimetry (DSC) method varied from 14.70 to 26.05% and 16.65 to 30.85% respectively for flours and native starches of six varieties of taro from Ngaoundere, Cameroon.[41] Nwokocha et al.[42] reported that the amylose content of taro starch was higher than that of cassava starch.

Amylopectin is a large, highly branched polymer of glucose. The structure is composed of α – D -glucopyranose residues linked primarily by α (1 → 4) glycosidic linkages. Branching in amylopectin molecules occurs through non-random α (1 → 6)-linkages (Fig. 1.3). Branching in amylopectin molecule takes place every 24 to 30 glucose residues.[43] It is one of the largest natural molecules and its molecular weight varies from 10^6 to 10^9 g × mol^{-1}, depending on the source.

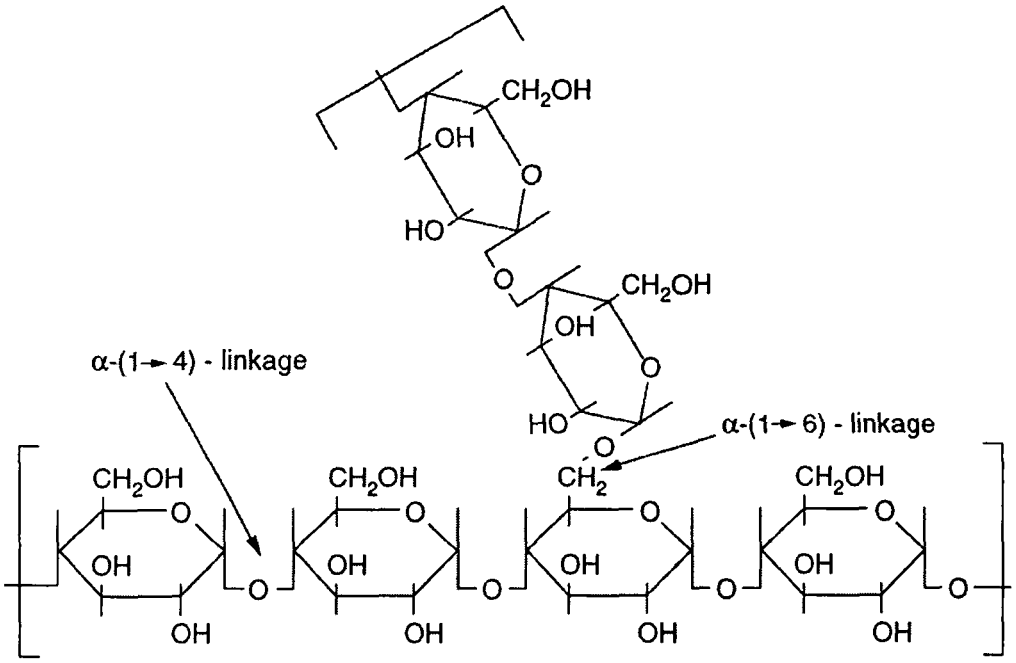


Fig. 1.3 Chemical structure of amylopectin molecule showing α (1 → 4) and α (1 → 6) glycosidic linkages

Due to the large size of amylopectin molecule and its highly branched nature its mobility in solution is reduced which eliminates any possible interchain hydrogen bonding.

Covalently linked phosphate monoesters are present in amylopectin. They are linked to the C3 or C6 position of the glucose residue, and occur particularly in starch of tuber crops, especially potato starch. The gelatinization enthalpy is found to be related to the degree of phosphorylation of amylopectin and decreases as the degree of phosphorylation at the C6 position increases.[44] The change of gelatinization and pasting properties of starch is due to the charged nature of phosphate monoesters which increases the electrostatic repulsion between molecules.

The functional properties of starch are greatly affected by the ratio of amylose-to-amylopectin, branch chain length distribution of amylopectin,[45] and the presence of phosphate monoester, phospholipids and lipid in starch granules.[46, 47]
The amylose content of starch is found to affect the functionality of starch. Swelling and solubility of taro and wheat starches were found to decrease with an increase in

amylose content of starch.[48, 49] Lower peak and hot paste viscosities of sweet potato starches were observed with higher levels of amylose.[50] Increase in onset and peak gelatinization temperatures was associated with lower amylose content of starch.[51] Waduge et al.[52] investigated the effect of annealing on high amylose barley starches. It was observed that the responses towards annealing were affected by amylose to amylopectin ratio and packing of the starch chains within the amorphous and crystalline regions of the native granules.

Aboubakar et al.[41] observed that amylose content is negatively correlated to the hydrolyzability of starch. Starches with high level of amylose hydrolyzed more slowly compared to starches with low level of amylose. Amylose content was found to be positively correlated to final viscosity, setback viscosity and pH of starch, and negatively correlated to the water absorption capacity.[40]

Amylopectin branch chain-length is found to be related to the crystalline structure of starch.[45, 53-56] Gelatinization, retrogradation,[38, 57-63] and pasting properties of starch[38, 64, 65] are also affected by the branch chain length of amylopectin.

Starches where the proportion of long branch chains of amylopectin is high, displays higher gelatinization temperature and higher enthalpy changes.[45, 66, 67] Chain length distribution also affects the pasting properties of starches.[45, 65, 67] Decrease in the gelatinisation temperature of starches was observed due to the presence of phosphate monoester groups on the amylopectin molecule.[68] It was observed that amylopectin contributes to the swelling of starch granules, whereas amylose and lipids contents inhibit it. Tattiyakul et al.[69] investigated the effect of amylopectin structure on the functional properties of taro starch. It was observed that the solubility of taro starch was inversely proportional to the average amylopectin chain length, average external and internal chain lengths. It was further observed that starch with shorter average amylopectin chain length, average external and internal chain length had a higher peak and breakdown viscosities.

Extracted starches from various sources may contain varying amount of other components like fibre, proteins, lipids, and minerals, depending upon a number of factors such as maturity of the crops, time of harvest, environmental and storage conditions, method of starch isolation etc. Starch usually contains 0.5 to 2% (w/w) non-carbohydrates, including 0.05% to 0.5% (w/w) proteins, 0.1 to 1.7% (w/w) lipids, and 0.1 to 0.3% (w/w) ashes.[32] Even though these non- starch components are present in very low amount, they are important in determining the physicochemical

properties of starch. Proteins present in starch can lead to development of unwanted flavours or colours in starch and starch products via Maillard reactions. They may also affect rates of hydration and interfere with the interactions between starch granules and hydrolytic enzymes. Cereal starches have comparatively higher protein content than tuber starches. Lipids are another important minor constituent and can form an amylose-lipid complex that enhances the resistance of starch to enzyme hydrolysis.[32] Starch-lipid complex improve the textural properties of various foods. The interaction of starch and lipid is particularly important in cereal starches where lipids are present in noticeable amounts. Lower quantities of lipids are present in tuber starches and therefore the effect of lipid is not so pronounced. Depending on the plant source lipid may be present on the surface or inside of the starch granule. The main components of surface lipids are triglycerides, free fatty acids, glycolipids, and phospholipids. Monoacyl lipids (containing a single fatty acid residue which has a chain length of 16 or 18 carbon atoms) are the main components of internal lipids of some maize and wheat starches.[70] Ash in starch mainly contains phosphorus, calcium (CaO), potassium (K_2O), sodium (Na_2O), and silicon (SiO_2).[71] Tuber starches, particularly starch from potato has higher phosphorus than cereal starches. Phosphorus in potato starch is attached as esterified phosphate at carbon 6 and 3 of various D -glucose units; C6 bound phosphate constitutes about two thirds of the total starch bound phosphate.[44] The fibre content of starch also varies and depends on various factors such as source of starch, sieve used for extraction of starch, age of the tubers etc.

The fat and protein content of different rice varieties varied from 0.06-0.08% and 0.08-0.39% respectively.[72] It was also observed that the protein and fat contents of the rice starches influenced the turbidity, pasting properties, and gel textural and retrogradation properties. Nwokocha et al.[42] observed that the ash and nitrogen content of cassava starch was found to be higher than that of taro starch. Aboubakar et al.[41] obtained lipid content of 0.2-0.6% and ash content of 2% for taro starches isolated from six varieties of taro grown in Cameroon.

In addition to amylose, amylopectin and minor constituents of starch, starch absorbs water when it is in equilibrium with the environment. The moisture content of extracted starch after drying varies from 6-16% and depends on the drying process and the storage conditions of the dried starch. Microbial spoilage of the starch may take place if the moisture content is high and can lead to deterioration in quality

which will make the starch unfit for further use. The maximum moisture content prescribed for safe storage by most of the starch producing countries is 13%.[73, 74] Considerable variation in moisture content among tuber starches has been reported by several workers.[34, 75-80]

1.4 Morphological and physicochemical characteristics of starch granules

1.4.1 Starch morphology

Starches are stored in the chloroplasts of the leaves or amyloplasts of other tissues of the plant in the form of granules. These starch granules are insoluble in cold water. Biosynthesis of starch granules takes place mainly in the amyloplasts and where it starts in the hilum and grows by apposition.[81]

The granules are relatively dense particles of compact molecules, with semi-crystalline properties. They have a density of about 1.5 g/cm^3.[82] Starch granules are insoluble in cold water due to this stable semi crystalline structure.

Wide variation in shape and size of starch granules has been observed from starches obtained from different plant sources. The size of starch granules from different sources may vary from < 1 μm to 100 μm in diameter.[29, 83]

The size of starch granules is usually expressed as a range or average of the length of the longest axis. Potato starch granules are the largest among all the starches. Most cereal starch granules are comparatively smaller in size than granules of tuber, root and legume starches. It was usually observed that shapes of cereal starch granules such as maize, oat, and rice are generally polygonal or round. Starch granules from roots and tubers are voluminous and oval in shape and have an eccentric hilum. Filamentous granules (budlike protrusions) are observed for high amylose maize starch. Starch granules from legume seeds are bean-like and have a central elongated or starred hilum. For very small starch granules the hilum may not be always distinguishable.[32]

The industrial application of a particular starch may be influenced by the shape and size of the starch granules as the functional properties of starches are affected by the granule shape and size. Granule size and shape affects many properties of starch such as gelatinisation, viscosity properties, susceptibility of the starch to enzyme, swelling power and solubility, and crystallinity.[84] Granule shape and size are also important because it determines the mesh size for extraction and purification sieves for starch extraction industry.[85]

Higher solubility and water absorption capacity was observed for smaller granules.[34] For potato starch lower peak viscosity temperature was observed with smaller granule size whereas an increase in granule size increased peak, breakdown and setback viscosity temperatures.[86, 87]

Starch granule morphology can be studied using various methods. The external surface characteristics can be determined using optical microscopy or light scattering. Optical microscopy can also be used to determine the shape and size of the particle. Other complementary methods like scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) have been frequently used to study the detailed structure of the starch granules.[32]

Moorthy et al.[39] determined the size of the starch granules from ten cultivars of taro using ocular micrometer. They observed wide variation in the average granule sizes among the cultivars. The average granule size varied from 2.96 to 5.19 μm for the ten cultivars investigated. Falade and okafor[40] found wide variation in granule size of five cocoyam varieties from Nigeria. The size of the starch granules, as measured by light microscopy, ranged from 6.47 to 13.63 μm in length and 5.36 to 8.45 μm in width. The granules were found to be similar in shape and were found to be round. It was observed that size of starch granules varied with the size of tubers.[69] Starch from larger corm of taro had a smaller granule size. Aboubakar et al.[41] investigated the morphology and size of starch granules from six varieties of taro from Cameroon and found that the mean diameter varied from 3 to 20 μm which were much larger than that of taro starches reported by other authors. The starches were found to be polygonal and irregular in shape. Granules of rice starches from Nigeria observed using SEM had polygonal and irregular shape and ranged from 3 to 10μm.[88] Granule sizes ranged from 14.3-53.6 μm, 3.6-14.3 μm, and 7.1-25.0 μm for potato, tapioca and corn starches, respectively as observed by Mishra and Rai[89] using light and scanning electron microscopy. Potato starch granules were oval/flattened and ellipsoid in shape, while those of corn were polyhedral and those of tapioca were spherical and truncated. *Dioscorea nipponica* starch had smaller granule size (9.5 μm) compared to tapioca (14.7 μm) and potato (30.5μm) starch.[90] *D nipponica* starches were mostly oval shaped granules with some sausage shaped while tapioca starch granules were mostly spherical. Bello-Pérez et al.[91] reported that banana starch granules are mostly lenticular in shape with an average size of 39 μm.

Starch granule size may also be influenced by season. Starches from cocoyam (*Xanthosoma sagittifolium*) and taro (*Colocasia esculenta*) tubers planted in summer exhibited larger granule sizes than those of starches from tubers planted in spring and winter.[48, 92] The exact mechanism affecting the size of starch granules is not very clear,[48] however, the differences in average granule size in different seasons could be attributed to significant differences in soil temperature.[93]

1.4.2 Starch crystallinity

Starch is a semi-crystalline material. The arrangements of amylose and amylopectin molecules in the granules are such that it forms complex structures which consist of amorphous as well as crystalline regions. Amylopectin short chains are arranged into double helices, some of which then form crystalline lamellae (or crystallites).[94, 95] The remaining double helices and the crystallites form the ordered part i.e. semi-crystalline part of the starch granule; the rest part is the disordered or amorphous part. The amorphous part of the starch granule is generally considered to be consisting of amylose and long chains of the amylopectin molecules.[94] Alternate layers of semi-crystalline and amorphous region are present in the starch granules.[94, 96] Birefringent nature of the starch granules is due to the presence of crystallites which can be observed under polarized light using light microscope fitted with cross polarizers.

Presence of crystalline structure in starch granules and their characteristics is studied by X-ray powder diffraction diffactometry.[97]

Three distinct X-ray diffraction patterns for starches from various sources have been observed, A-type, B-type, and C-type patterns.[94, 98] The A-type patterns are characteristic of starches from cereals such as maize, wheat, oat, rice and waxy maize. The B-type patterns are associated with starches from tuber, fruit, and stem, such as canna, potato, sago, banana starches and the mutant maize starches such as amylomaize-5 and amylomaize-7.[94, 98] C-type patterns are found in roots, beans, and peas. C-type pattern is intermediate between A- and B-types.[98, 99]

Starches with A-type show stronger diffraction peaks at 15°, 17°, 18° and 22° 2θ angles while B-type has four main reflection intensities at 5.5° and 17° 2θ angles. And there were also a few small peaks at around 2θ value of 20°, 22° and 24°. The B-type X-ray pattern of starch is usually characterized by the position and relative peak intensity in the range of 2θ = 5 - 6°, while the absence of the peak of 2θ = 5 - 6° is

characteristic of A-type starch. The C-type X-ray pattern reflects at 5.5°, 17°, 18°, 20° and 23.5° 2θ, which is thought to be a combination of the A- and B-type patterns.[100] Cassava starch possesses A, C, or a mixed pattern with three major peaks at 2θ = 15.3°, 17.1° and 23.5°. Sweetpotato starch shows variable X-ray patterns between C and A. Cocoyam starch also exhibits A-type pattern.[33, 97, 101] Variations in crystallinity of starches from the same crops were associated with variety, sample preparation, growth conditions and maturity of the plant or the part of the plant from where the starch was extracted at the time of harvest.[102-105]

The degree of crystallinity can be defined as the percentage of the crystalline portion of the material with respect to the total amount of the material. It is an important characteristic as it may influence the physical, chemical, and functional properties of the material.[106]

The crystallinity of starch granules can be determined from the X-ray diffraction pattern by calculating the percentage ratio of diffraction peak area to the total diffraction area.[107] Generally the crystallinity of B-type starch is lower than that of A-type starch. The crystallinity of starches varies from 15 to 45%[107, 108] and depends on source of starch and methods used for calculating the crystallinity. Amount of moisture present in the starch granules greatly affects the crystallinity of starch.[109] For A type starches crystallinity is not much affected by the Amylose content of the starch. However, for B type starches lower crystallinity is observed with higher amylose content (e.g., amylomaize). This may be due to the typical molecular structure of starches with high amylose, such as longer chain length and unique chain length distribution.[110]

Chávez-Murillo et al.[111] studied the XRD patterns and cystallinity of several Mexican rice cultivars. They observed that all the rice cultivars presented A-type crystallinity with strong peaks at 2θ = 15°, 16.8°, 17.8° and 22.75°, and the per cent crystallinity varied from 32.7 to 36.3%. Higher crystallinity was shown by starches from rice cultivars having lower amylose content. Qin et al.[112] investigated the XRD spectra of transgenic rice and their wild varieties during kernel development. They observed that all the rice varieties showed A-type crystalline pattern with peaks 15°, 17°, 18° and 23° 2θ and decrease in relative crystallinity with increase in amylose content with kernel development.

1.4.3 Spectral features

Vibrational spectroscopy has been used since long time to study the various properties of starch such as the crystallinity and changes due to gelatinization because it reflects the change in vibrational frequency and energy of chemical groups associated with the starch molecules.[113, 114]

Vibrational spectroscopic techniques such as the Fourier transform infrared (FT-IR) and Raman (FT-Raman) methods have been frequently used to investigate changes due to processing and to measure quality of food.[115-118] Hrebicik et al.[119] investigated the effect of irradiation on oat and rice starches using FT-IR and observed an increase in the intensity of 1734 cm^{-1} band in due to irradiation.

Li et al.[120] investigated the effect of gelatinization of different starch types on the crystallinity using FT-IR. FT-Raman spectroscopy was used by Schuster et al.[121] to study the kinetics of starch hydrolyzed by α-amylase and amyloglucosidase.

FT-IR studies on various tuber starches revealed very minor differences, although the crystallinity and granule sizes of the starches were different.

Aboubakar et al.[41] investigated the FT-IR spectra of six different varieties of taro starch and found that the spectra obtained for the six starches were similar based on the form, but differed in the intensity of the major peaks. The spectra showed high absorption at the wave numbers 574, 1020 – 1026, 1056, 1151, 1365, 1631, 2922 and 3400 cm^{-1} confirming the carbohydrate nature of the samples.

Fan et al.[114] studied the structural changes in rice starch due to rapid cooking by conventional method and microwave cooking using FT-IR and Raman spectra. They observed that the native starch, rapid cooked starch and microwave cooked starch had the same peak position and absorption peak numbers. The results indicated that rapid heating and microwave heating neither changed the type of chemical groups in starch molecules nor produced a new chemical group.

1.4.4 Colour of starch (dry powder)

Colour is an important criterion for the measurement of starch quality. Any pigment in the starch negatively affects its acceptability and will be carried over to its products.[122] A high value of lightness and a low value of chroma are desired for starches. Deng et al.[123] studied the colour characteristics of sweet potato starches isolated by various methods in the laboratory and industry. They observed that the

starches isolated in the laboratory were whiter and were less yellow and red compared to the commercial starches isolated by similar methods. Pelissari et al.[124] found that the whiteness of banana starch was 90.9 (L* value) and can be used in products requiring uniform colour. Aboubakar et al.[41] measured the colour of six taro starches from Cameroon and found that the L*, a* and b* values varied from 83.2 to 94.9, 1.5 to 5.7 and 3 to 13.8, respectively.

1.5 Functional properties of starch

Industrial application of starch is dependent on the numerous functional properties such as thickening, gelling, coating, pasting, encapsulation, stability to freeze-thaw cycles etc., which decide the specific use of a particular kind of starch. There is considerable variation in the functional properties of starches obtained from different sources.

Starch pastes and gels are used to control the consistency and texture of sauces, soups, dressings, and spreads.

The functional properties of starch are affected by the structural characteristics of starch molecules such as amylose content and branch chain length distribution of amylopectin.[45] The functional properties are also affected by the presence of minor constituents of the starch granules like phosphate monoester, phospholipids and lipid contents.[46, 47] Starches with higher proportion of long branch chains of amylopectin molecules display higher gelatinisation temperature and enthalpy changes.[45, 66, 67] Amylopectin branch chain length distribution also affects the pasting properties of starch.[45, 65, 67] Gelatinization temperature of starches is decreased due to the presence of phosphate monoester groups on the amylopectin molecule.[68] It was observed that amylopectin contributes to granule swelling, whereas amylose and lipids contents inhibit it. Granule shape and size also affect the functional properties of starches such as gelatinisation and pasting properties, susceptibility to enzyme, swelling power and solubility are also affected by.[84]

1.5.1 Swelling and solubility

The changes that occur in a starch granule in an aqueous system depend on temperature and amount of water available. When starch molecules are heated in excess of water, the crystalline structure is disrupted and the water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of amylose and

amylopectin, which causes an increase in granule swelling and solubility.[125] Swelling and solubility of starches provide information about the magnitude of interaction between starch chains within the amorphous and crystalline regions of the starch granules. Swelling power and solubility are influenced by ratio of amylose to amylopectin, and structural characteristics of amylose and amylopectin such as molecular weight distribution, degree and length of branching, and conformation.[97, 126] Lower amylose content and higher proportion of long chains of amylopectin tends to increase the swelling power of starch granules.[49, 111, 112] Swelling and solubility are influenced by the presence of minor constituents like phosphate and lipid contents of starches. Amylose in the presence of lipids forms insoluble complexes limiting swelling and solubility of the starch.[127] Increased swelling and solubility of starches was reported with higher contents of phosphate monoesters as repulsions between phosphate groups on the adjacent amylopectin molecules increased.[125] Swelling and solubility are also found to be dependent on granule size and shape. It was observed that starches with large granules display higher swelling but lower solubility compared to starches with smaller granule size.[128]

Swelling is a very important property for certain food applications where the quality of products is based on the capacity of starch granule to retain water and swell. Starch swelling is directly related to the increase of solubility, paste clarity and viscosity which are very much important in determining the use of starch in the food industry. The swelling power of native starch increases with the temperature increasing as a result of crystalline structure relaxation so that both amylose and amylopectin can form easily hydrogen bonds with water molecules.[129]

Thus, swelling and solubility of starches are temperature dependent and increase with increase in temperature due to weakening of internal associative forces which maintain the granular structure.[35]

1.5.2 Clarity

Clarity of the starch pastes is one of the most important attributes of the starch, and determines their application in the food, textile, paper and the adhesive industry.[33] It is particularly important in the food industry as it directly influences brightness and opacity of products, an important factor to determine the clarity of starch paste being the physical arrangement of molecules which contribute to swelling ability of granules.[129] Starches used to thicken fruit pies are preferably

transparent while those in salad dressings are opaque. Starches with clear pastes are also preferred for combining with other colouring agents.[130]

Light transmittance offers information regarding the starch paste behaviour when light goes through it, thus the higher the transmittance value the paste is more transparent or clear. Differences in clarity and its stability of cereal starches in comparison to tuber starch can be attributed to the physical arrangement of amylose and amylopectin molecules within granules and presence of other minor constituents, which obstructs their ability to swell and disperse in water. In addition, the starch paste clarity during storage can be influenced by the interaction of several factors such as granule swelling, granule remnants, leached amylose and amylopectin, amylose and amylopectin chain length, intra- or inter-bonding, lipid and cross-linking.[131]

The decrease in the transmittance value indicates re-association of initial broken bonds in orderly structure (retrogradation) and it could be observed by separation of liquid phase and sediment formation.[129]

Paste clarity, just like other functional properties of starch, varies with its source.[125, 130, 132] Potato starches have higher paste clarity (96%T) than corn (31%T), wheat (28%T) and rice (24%). Craig et al.[130] compared clarity of potato, tapioca, wheat, and corn starch pastes among others. They reported higher paste clarity for potato (96%T), than for tapioca (73%T), wheat (62%T) and corn (41%T) starches. Tetchi et al.[132] also found that potato starch pastes were more transparent (79%T) than cassava (47%T), sweetpotato (17%T) and cocoyam (16%T) starch pastes. These differences in paste clarity have been attributed to differences in chemical composition such as phosphate and amylose. Potato starch pastes have higher paste clarity than cereal starches due to high content of phosphate monoesters as opposed to higher contents of phospholipids in cereal starches. Phospholipids present in starches form complexes with amylose and long chain fractions of amylopectin resulting in limited swelling and hence lower light transmittance. On the other hand, phosphate monoesters covalently bond to amylopectin fraction and due to repulsions between phosphate groups on adjacent amylopectin groups swelling is enhanced and hence light transmittance.[125, 130] Amylose reorganisation forms aggregates that reduce light transmittance of starch pastes.[132] High amylose starches reassociate more readily amylopectin starches thereby resulting in more opacity.[133]

1.5.3 Pasting properties

Rheological properties of a material are because of the intermolecular interactions occurring within its molecular structure. During gelatinization of starch, granules swell to several times its initial size and, consequently, the constituents inside the granules are leached out, particularly amylose, leading to a three dimensional network.[46, 134] These changes are responsible for the rheological characteristics of starch suspensions during heating and shear rate. The rheological behaviour of starch is influenced by several factors such as amylose content, lipid contents and by branch chain-length distribution of amylopectin. granule size and shape, and also on granule-granule interaction.[125, 128, 135-137] Amylopectin contributes to swelling of starch granules and pasting, whereas amylose and lipids tend to inhibit the swelling of granules.[46] Viscosity of starch suspension is strongly related to molecular chain length and, on the spatial arrangement of the molecule determined by intermolecular bonds as well as hydrogen or Van der Waals bonds.[138] Studies on rheological properties of starches showed non-Newtonian behaviour of starch suspensions i.e. a non-linear relation between the shear rate and shear stress. The dependence of shear rate on shear stress indicates the pseudoplastic character of the starch suspensions.[129]

Providing viscosity to the food products is one of the major functions of starch and pasting properties of starch suspensions i.e. changes in the viscosity of starch suspension during heating and cooling cycles is useful in deciding the application of a particular starch in food industry. Viscosity of a starch suspension is dependent on temperature. Rheological or properties of starch can be studied using a Brabender Visco Amylograph and Rapid Visco Analyzer (RVA). The RVA provides more versatile information on starch characteristics compared to the Brabender Visco Amylograph. The sample size required for RVA is small and has other advantages like shorter testing time, and the testing conditions can be modified according to the requirement. When starch granules are heated in excess water, they absorb large amounts of water and swells to multiple times of their original size. There is increase in viscosity due to shearing when these swollen granules have to squeeze past each other. Pasting temperature is the temperature when this rise in viscosity starts. The pasting temperature provides information about the minimum temperature required to cook a given starch sample. A rapid rise in the viscosity of the starch suspension is observed when sufficient numbers of starch granules have swollen. An equilibrium

point is reached between swelling and polymer leaching, after which no further increase in viscosity takes place. This viscosity is known as the peak viscosity. Peak viscosity and temperature indicate the water binding capacity of the starch. As the temperature increases further and the starch suspension is hold at this high temperature for a certain period of time, granules continue to rupture and subsequent polymer alignment occurs, which decreases the apparent viscosity of the paste. This process is defined as breakdown. The viscosity at this stage is known as the hold viscosity and gives an indication of paste stability. As the system is subsequently cooled, re-association between starch molecules, especially amylose, occurs to various degrees. In sufficient concentration this usually causes the formation of a gel, and the viscosity will be increased to a final viscosity. This phase of the pasting curve is commonly referred to as the setback region, and involves retrogradation of the starch molecules. The final viscosity gives an indication of the stability of the cooled, cooked paste under low shear.[32]

Oladebeye et al.[139] found that starch paste of sweetpotato had higher viscosity values than that of red cocoyam starch paste. Yuan et al.[90] compared viscosity of starches from yam (*Dioscorea nipponica* Makino), cassava and potato using a Brabender Viscoamylograph. They observed lower peak viscosity, higher setback and lower breakdown viscosities for yam starch compared to potato and cassava starch. Like paste clarity of the starches, differences in chemical composition account for the variations in paste viscosity of different starches. High phosphate monoester content is known to increase paste viscosity while an increase in phospholipids results in lower paste viscosity.[125] Viscosity variations also exist between different varieties of crops. Sefa-Dedeh and Sackey[140] reported varying paste characterisics between three varieties of cocoyam (*Colocasia esculenta*, red and white *Xanthosoma sagittifolium*). *Colocasia esculenta* starch exhibited lower hot paste viscosity but higher thermal stability when compared to *Xanthosoma* starches. *Colocasia esculenta* starches from Hawaii Red and Hawaii White varieties gave the highest peak viscosities, whereas Bun-long starch had the lowest.[38]

Pasting properties of starch are influenced by the composition of the starches such as amylose and other minor components present in the starch. The amylopectin chain-length and amylose molecular size produce synergistic effects on the viscosity of starch pastes.[64]

1.5.4 Gelatinization

Gelatinization is one of the most important properties of starch when it is heated in excess of water. Native starch granules are insoluble in cold water but swell in warm water. Gelatinization of starch is a phase transition phenomenon. Starch gelatinization is the collapse (disruption) of molecular orderliness. It is associated with transformation of granule crystalline phase in amorphous one and causes concomitant and irreversible changes of different functional properties such as granular swelling, solubility, loss of optical birefringence, viscosity development etc. The point of initial gelatinization and its range are governed by many factors such as concentration of starch, method of observation, granule shape and size, and heterogeneity within the granule population.[64] Gelatinization occurs initially in the amorphous regions of the granule, as opposed to the crystalline regions, as hydrogen bonding is weakened in these areas.[125] Thus, the differences which can appear in transition temperatures for different kinds of starches may be attributed to the differences in the degree of crystallinity. A high degree of crystallinity resulted in high transition temperatures, which provides structural stability and makes the granule more resistant towards gelatinization.[141] Both gelatinization and swelling are properties partially controlled by the molecular structure of amylopectin (unit chain length, extent of branching, molecular weight, and polydispersity), amylose to amylopectin ratio and granule architecture (crystalline to amorphous ratio).[46] Gelatinization is of great importance in food processing as gelatinization of starch occurs during the processing of foods containing starch. Product quality is greatly influenced by starch gelatinization. Various analytical techniques have been used to study the phenomenon and to understand its mechanism of starch gelatinization. Differential scanning calorimetry (DSC) is known as an extremely valuable method to investigate the gelatinization process of starch.[142] It provides a quantitative measurement of the enthalpy, ΔH, which means the energy required for the gelatinization process to take place. It also, determines the temperature range where gelatinization occurs.

The thermal properties of the starches are influenced by the source of the starch. Peroni et al.[35] compared properties of cassava, arrowroot, sweetpotato, yam, canna, and ginger starches. Cassava, arrowroot and sweetpotato starches exhibited lower onset gelatinization temperatures (61.5, 62.6 and 62.8°C, respectively) and enthalpy changes (10.4, 11.3 and 12.9 J g^{-1}, respectively), whereas yam and ginger

starches gave the highest onset temperatures (70.7 and 82.4°C, respectively) and enthalpy changes (14.3 and 15.9 J g^{-1}, respectively). Pérez et al.[143] reported lower onset (56°C), peak (60°C) and conclusion temperatures (73°C) for Peruvian carrot than cocoyam (74, 78 and 87°C, respectively) and potato (66, 69 and 80°C, respectively) starches. However, taro starch exhibited lower gelatinization enthalpy (3.98 J g^{-1}) than Peruvian carrot (4.19 J g^{-1}) and potato (4.64 J g^{-1}) starches. Yuan et al.[90] reported higher gelatinization temperatures for *Dioscorea nipponica* Makino starch (67.4, 76.0 and 81°C for onset, peak and conclusion temperatures, respectively) than those of tapioca (64.9, 69.1 and 75.9°C) and potato starches (59.2, 64.1 and 73.0°C). Tapioca starch had lower enthalpy of gelatinization (14.8 J g^{-1}) than *D. nipponica* Makino starch (18.6 J g^{-1}) while potato starch had the highest enthalpy of gelatinization (23.4 J g^{-1}). Van Hung and Morita[144] compared starch from canna with cassava, potato and sweetpotato starches grown in Vietnam. They reported higher transition enthalpy for edible canna (14.5 J g^{-1}) starch than potato (14.1 J g^{-1}), cassava (12.4 J g^{-1}) and sweetpotato (12.3 J g^{-1}) starches. Canna starch had a gelatinization temperature range (67.4-76.1°C) similar to that of cassava (66.9-77.0°C) and potato (64.9-76.4°C) starches but higher than that of sweetpotato starch (57.4 -74.5°C). Jiranuntakul et al.[36] reported onset, peak and conclusion temperatures for gelatinization of normal rice starch as 69.9, 74.1 and 80.26°C respectively, whereas for corn and potato starches onset, peak and conclusion temperatures were 66.5, 70.4 and 75.36, and 62.7, 66.2 and70.80 °C respectively.

1.5.5 Freeze-thaw stability

A variety of new frozen food products are continuously launched in the market due to increase in demand for ready to eat food products.[145] During freezing water present in the food turns into ice, and results in damage to the food matrix. When this frozen food is thawed for consumption, the water easily separates out from the product and alters the texture of the product making it softer, and causing deterioration of overall quality.[146] Starch has been used in a variety of "ready-to-eat" frozen food products. During freezing of such products, starch pastes or gels are frozen and phase separation occurs due to formation of ice crystals. Upon thawing, syneresis occurs with starch pastes and gels, a phenomenon in which water gets separated from the complex network of the food.[113] Syneresis in freeze-thawed gel is due to the increase of molecular reassociation between starch chains, in particular

retrogradation of amylose,[147] expelling water from gel structure.[148] Thus the amount of syneresis is a useful indicator for the tendency of starch to retrograde.[113] Stability to freezing and thawing cycles is defined as the ability of the starch to resist the physical changes occurring during the freezing and thawing phases, which acts as an indicator of the extent of retrogradation of starch pastes[149] and is also important in deciding the potential use of starches in frozen products.[150] The freeze-thaw stability of a starch paste or gel can be easily evaluated by measuring the amount of water gravimetrically that separates from starch pastes or gels.[149, 151] Repeated freeze–thaw cycles that involve subjecting samples to repeated freezing and intermittent thawing to room temperature over a period of 2 – 4 h are known to drastically accelerate retrogradation and syneresis.[73]

1.5.6 Texture

Textural properties of starch gels are very important criteria, used to evaluate the performance of starch in a food system. Significant differences in the gel properties of starches from selected corn lines were observed by Ji et al.[152] by using a texture analyzer. Seetharaman et al.[153] reported significant differences in the textural properties starches of 13 selected Argentinean corn landraces during storage. Sandhu and Singh[154] reported a positive correlation between amylose content of corn starches and hardness and gumminess of the starch gels.

1.6 Uses of starch

Starch is a natural, versatile, cheap, readily available, renewable, and biodegradable polymer produced by most plants as a source of stored energy. Starch is present throughout the plant. It is found in the leaves, stems, roots, bulbs, nuts, stalks and seeds of plant. It is the major component of staple crops such as rice, wheat, corn, cassava, and potato. It has wide range of uses in the food, textiles, cosmetics, pharmaceuticals, paper, plastics, and adhesive industries.[25-28] In the food industry, starch is used as a thickening, binding, filling and gelling agent, and as stabilizer for snack foods, meat products, fruit juices, etc.[155] It is either used as "native starch" i.e. as extracted from the plant, or undergoes one or several modifications in order to improve specific properties to suit specific application and is called "modified starch".[156] Worldwide the biggest user of starch is the sweetener industry. About 70% of starch produced throughout the world is converted to syrup

for use in food industry.[157] Apart from the sweetener industry; starch is used in various other food and non-food industries for various purposes. The various application of starch in food and non-food industries is given in Table 4.[158]

Table 1.4 Industrial uses of starch

	Application
Food Industries	
Canning	Filling viscosity aid, suspension aid for particulates, opacity agent, body or texture agent for soups, sauces, puddings and gravies, aseptically canned products, beverages such as coffee, teas or chocolate
Cereals and snacks	Hot extruded snacks, chips, pretzels, extruded and fried foods, ready-to-eat cereals
Bakery	Pies, tarts, fillings, glazes, custards and icings, cakes, donuts, Danish, icing sugar
Frozen foods	Fruit fillings, meat pies, Oriental foods, soups, sauces, entrees, cream-based products
Confectionery	Dusting powder, licorice, jelly gums, hard gums, panned candies, confectioners' sugar
Flavours and beverage clouds	Encapsulation of flavours, fats, oils, vitamins, spices, clouding agents; spray dried flavours for dry beverage mixes, bartender mixes, beverage emulsions,liquid and powdered non-dairy creamers
Dressings, soups and sauces	Mayonnaise-type spreads, pourable salad dressings (high shear), spoonable dressings, instant dry salad dressing mixes, low-fat dressing, canned gravies and sauces, frozen gravies and sauces, soups and chowders
Non-Food Industries	
Textiles Industry	Warp sizing, fabric finishing, printing
Pharmaceutical and cosmetic industry	Tablet binder/dispersing agent , pill coating , dusting agent, facial creams, soap filler/ extender, dusting powder
Explosives industry	Wide range binding agent, match-head binder
Mining industry	Ore flotation , ore sedimentation, oil well drilling muds
Construction industry	Concrete block binder , asbestos, clay/limestone binder, fire-resistant wall board, plywood/chipboard adhesive, gypsum board binder, paint filler
Others	Biodegradable plastic film, dry cell batteries, printed circuit boards , leather finishing etc.

Source: FAO[158]

It is evident that application of starch in both food and non-food industries requires specific properties of starch and starch obtained from different sources has different physicochemical and functional properties. Therefore, information about the physicochemical and functional properties of starches in "native" form, as well as the "modified" starches obtained from different sources becomes very much important in deciding the application of a particular starch for specific purpose.[156]

1.7 Isolation of starch from plant materials

Starch can be extracted using various processes, depending on the plant source and end use of the starch, as plant materials differ in their tissue structure and composition. For cereals wet milling process is widely used for starch extraction and the major steps include steeping, milling, and separation. The starch extraction process from roots and tubers involves grating of the raw material, in order to break vegetal cells and release the starch. This step is followed by passing the fiber through sieves of different mesh sizes and subsequent slurry concentration by decantation or centrifugation.[25] The high water content and other morphological similarities of tuber crops require a familiar technological process of starch extraction from these crops.[159] Investigations have been carried out by various authors on the effect of extraction conditions on the yield and properties of starch isolated from different plant materials. Traditionally, starch from maize is extracted by wet milling process. The wet milling process steeps the whole kernels counter currently in a 0.1-0.2% sulfurous acid solution at 50-55°C for 24-38 h to soften the kernels, inhibit growth of microorganisms, remove solubles and break down the disulfide bonds in the protein matrix to facilitate the release of starch. Subsequently, wet milling is done and the germ and fibers are separated by skimming and screening respectively.[160] Finally, the filtrate containing the starch is then centrifuged or allowed to sediment to separate starch from protein.

Eckhoff et al.[161] reported that higher yield of starch can be achieved, steep time and sulphurous concentration can be reduced to 6 h and 0.1% respectively, if maize grits are used instead of whole kernels. Mistry et al.[162] used alkali instead of sulphurous acid for extraction of starch from maize. The effect of alkali concentration, steep temperature and steep time were evaluated on starch yield, protein and sodium contents. Higher yield of starch with low protein and sodium

content was obtained when alkali concentration and steep time was low and steep temperature was high.

Starch obtained from maize by using hydrocyclones gave higher levels of residual protein content when compared to tabling method i.e., using starch tables where the heavy starch fraction settles on the table and lighter protein fraction remains suspended in the water and flows off the end of the table.[163] Ji et al.[164] compared sedimentation with centrifugation for separation of starch from protein during maize starch extraction. Sedimentation resulted in starch with lesser protein and greater starch yield as compared to starch extracted by centrifugation. For centrifugation, the average values of yield and protein content, over three steeping timings and three levels of kernels were 51.1 and 3.29% respectively; whereas for sedimentation, corresponding average values were 56.9 and 1.65% respectively.

Lactic acid was used by few authors for steeping maize for achieving higher yields of starch from maize. Steeping maize kernels with lactic acid or sulfurous acid individually did not increase the starch yield significantly, but treatment with both acids increased the yield slightly.[165] In another investigation greater recoveries of starch was obtained by supplementation of lactic acid along with SO_2 during steeping as compared to conventional method where steeping is done in SO_2 solution alone.[166] Supplementation with lactic acid yielded starch with no damaged granules. The presence of lactic acid affected the RVA profiles. No great modification of the thermal properties was observed; only a slight decrease in amylopectin retrogradation and in the melting enthalpy of the amylose-lipid complex was observed.

Matsunaga and Seib[167] studied the effect of aqueous sodium hydroxide on phosphorus content of starch extracted from wheat. He found that stirring wheat starch (30-35%, w/w) in aqueous sodium hydroxide at pH 11.5-12.3 and 25-42°C releases odorous substances and apparently saponifies the lysophospholipids (LPL) in the starch. Saponification of the LPL decreases the pasting temperature of the low-phosphorus starch, and may be helpful when converting wheat starch to starch hydrolysis products. Grant[168] compared the effect of different parameters on gelatinization and regtrogradation properties of extracted starch. He isolated starch from whole wheat and flour of four hard red spring wheat cultivars. He also studied the effect of freeze drying and air drying, and different grinding methods using pestle and mortar and Wiley Jr. Mill. He observed that whole wheat starch isolates had higher peak, lower trough, and lower final viscosities, as determined by starch paste

viscosity analysis, than did starch isolates derived from flour. Major effects of all treatments on differential scanning calorimetry gelatinization properties showed lower onset temperature for flour starch isolates, lower peak temperature for freeze-dried starches, and no effects due to grinding methods.

The procedure to isolate starch from rice is different from that used to extract starch from corn and wheat. This is due to differences in protein content and starch properties in each case. The rice grain contains four types of proteins which are present in the endosperm and accounts for about 7-8% (dry basis) of the milled rice kernel. They are water soluble albumin (5-11%), salt soluble globulin (7-15%), alkali-soluble glutelin (80%) and alcohol-soluble prolamin (2-4%).[169-170] They are tightly associated with the surface of the starch granule making their detection and removal difficult[170]. Because the biggest protein fraction (glutelin) is soluble in alkali, rice starch is conventionally isolated by an alkaline steeping method.[171-175] Apart from alkaline solvent, surfactants or protein hydrolyzing enzymes are used to remove rice protein from rice flour.[171] Cardoso[175] investigated the effect of concentration of NaOH on rice starch extraction and properties of extracted starch. It was observed that optimal extraction was obtained using NaOH concentrations between 0.15%and0.18%(w/v).With NaOH concentrations higher than 0.24% (w/v), alkaline swelling of the granules occurred resulting in a significant disruption of the granular morphology associated with a decrease of crystallinity and gelatinization enthalpy. Lim et al.[173] compared the protein removal efficiencies and pasting properties of rice starch isolated using various alkaline solutions and surfactants. They used aqueous solutions of sodium hydroxide (0.1 and 0.2%), sodium lauryl sulfate (SLS, 1.2%) containing sodium sulfite (0.12%), and dodecylbenzene sulfonate (DoBS, 1.2%) containing sodium sulfite (0.12%). It was observed that more than 80% of the flour protein was extracted in 1h by stirring the dispersion (1:3, w/v) at room temperature. Repeating the extractions (1 or 2h for each step) with fresh solution significantly increased the protein removal efficiency. Raising the extraction temperature slightly increased the protein solubility, but starch loss also became significant. Among the solutions, DoBS was most effective in removing rice protein whereas SLS was least. The residual protein content of the isolated starch showed a negative correlation to the peak viscosity and a positive correlation to the pasting temperature.

The isolation of starches from legume seeds is difficult due to the presence of insoluble flocculent protein and fine fibre, which decreases sedimentation and co-settles with the starch to give a brownish deposit.[176] Legume starches are isolated using aqueous techniques as well as pin milling and air classification.[177] Lee et al.[178] investigated the effect of temperature (22-40 °C) and pH (8-9.5, adjusted using NaOH) on extraction and properties of starch from flours of two Australian lentil varieties. Extraction at pH 9.5, for all temperatures, achieved the maximum starch yield (85–95%) for both varieties. Protein yield achieved was relatively low compared to the analysed protein content. The % starch damage was high for both varieties when extracted at higher temperature and pH. The DSC ΔH value increased with increasing pH and temperature.

It was observed that for starch isolated from tubers, yield of starch is dependent on the size of the tubers. Investigations conducted at the Central Potato Research Station, Shimla, India, revealed that the recovery of starch from large potatoes was 67-75% as against 38-67% from small tubers. There is no difference in the starch recovery from green or non-green potatoes.[179] It was further reported that excessive maceration of tuber tissue for longer duration significantly lowered the starch recovery, while potassium content of starch increased.

Isolation of starch is more complicated when the extraction of starch is carried out from dried tubers as compared to the fresh tubers. Meuser et al.[180] studied the extraction of starch from cassava chips, pellets and roots. It was observed that extraction of starch was more difficult from the dried materials than from fresh roots. Impurities were difficult to remove from chips and pellets and mineral substances were found in the isolated starch reducing its quality.

Extraction of starch from cassava is simple and the isolated starch is pure white in colour and relatively free from other chemical impurities, whereas this is not so with other tuber starches like yam or taro due to high viscosity of the slurry caused by non-starch polysaccharides like mucilage and latex, leading not only to loss of starch, but also lowering of the quality of extracted starch. Work has been carried out on the use of various chemicals in increasing the yield of starch from various tubers.[33] Moorthy[181] used 0.03M ammonia solution for extraction of starch from cassava, taro, tannia, yam and sweet potato. He found that there was noticeable improvement in the yield of starch from taro (6-16%), while it fell for sweet potato starch and remained almost the same for the other tubers. Total amyloses of all starches were unaffected

while the 'soluble amylose' was slightly suppressed for taro starch extracted with ammonia solution. Peak viscosity was found to be increased to a large extent for taro and yam starches by ammonia extraction, while it was lowered for sweet potato starch. Lactic and citric acids improved the yield and colour of starch from sweet potato tubers.[182]

Daiuto et al.[25] investigated the effect of different methods on extraction of starch from yam tubers. It was observed that when the tubers were digested with an aqueous oxalic acid/ ammonium oxalate (OA/AO) 1/1 solution, it was easier to separate the starch slurry from residual mass, because viscosity was reduced. Also, the largest nitrogen reduction was observed with OA/AO followed by the control (water). A continuous bubble separation process for separating and recovering starch and mucilage from yam tubers in the absence of undesirable chemical treatment was developed.[183]

1.7.1 Enzymatic extraction of starch

Enzymatic treatment has been shown to give higher recovery of various food components like oils, juices, proteins etc.[184-196]

Enzymes are frequently used to assist starch separation from cereals in wet milling systems. The effect of various parameters including type of enzyme on enzymatic starch extraction was studied by various authors. Spanheimer et al.[197] studied the effect of different proteases and chemicals on prime starch yield from corn grits. They also investigated the effect of moisture content, incubation period and enzyme dosage on enzyme activity. They found that protein hydrolysis was more extensive with bromelain than other proteases. Moisture content was varied from 30 to 40% with an increment of 5% for bromelain. It was found that bromelain was more effective at 35% moisture than at 30%. In the presence of excess moisture, less protein was solubilized compared to grit moisture was 35%. This apparently was a function of effective enzyme concentration. With the large excess of moisture (40%), some of the enzyme may never have come in to contact with the substrate. Incubation was varied from 4 to 64 hours with bromelain at 30% moisture. It was found that prime starch yield was greatest with longest holding time. In wheat starch processing, utilization of xylanase reduced slurry viscosity and improved starch-gluten separation and yields, when compared to conventional wet-milling procedure.[198]

Roushdi et al.[199] studied the effects of alcalase and neutrase (both protease) on steeping of intact, scratched, and broken maize kernels. Treating broken kernels with these enzymes increased water-solubility of maize protein, improved starch recovery and decreased effective steeping time by 50%. No effects, however, were observed with scratched or intact kernels. The intact bran was believed to be impermeable to proteases. Steinke and Johnson[200] studied the effect of multiple enzymes on starch yield and protein content of starch from corn. The yields and protein contents of most fractions recovered from maize steeped for 24 hours with either treatment of multiple enzymes and sulfur dioxide were similar to those of maize steeped with 0.20 % sulphur dioxide alone for 48 hours and were significantly superior to those from maize steeped for only 24 hours with 0.20% sulphur dioxide alone. Zheng and Bhatty[201] studied enzyme assisted wet separation of starch from other seed components of four varieties hull-less barley. They used the multiple enzyme cocktail (Roxazyme-G) derived from *Tricoderma viride* containing cellulase, endoglucanase and xylanase. Compared to conventional procedure, enzyme assisted wet extraction reduced slurry viscosity by 50 to 90%, amount of water and ethanol used in and β-glucan precipitation by 30 to 60% and screening time by 20 to 80%. The enzyme assisted extraction resulting in a 10% increase in average starch extraction efficiency through reduction in starch contents and yields of tailings and bran fractions.

Radosavljevic et al.[202] investigated the effect of diluted alkaline protease treatment on recovery and properties of amaranth starch. Starch recovery was much higher with protease treatment than steeping with NaOH alone (from 79.4 to 83.5%). The method requires much less NaOH for processing, which reduces production costs of starches. The method also produces better quality of starch. Wang et al.[203] tested a multiple enzyme cocktail with pectolytic, cellulolytic, hemicellulolytic and proteolytic activities for wet-milling of sorghum. They found that the enzymes slightly increased starch yield and produced a more refined starch when compared with grains steeped with SO_2. Johnston and Singh[204] demonstrated that the use of proteolytic and cell wall degrading enzyme (CWDE) complex during wet-milling of maize significantly lowered SO_2 steeping requirements while maintaining similar starch yields. They also found that use of proteases reduced steeping time and SO_2 requirements in a two-stage wet-milling process. Serna-Saldívar and Mezo-Villanueva[205] studied the effect of cell-wall-degrading enzyme complex on starch

recovery and steeping requirements of sorghum and maize. When steep timings were compared, grains soaked for 48 hours produced 1.7% higher starch yields than counterparts treated for 24 hours. CWDE complex significantly increased starch yields and recoveries. Enzyme-treated grains yielded 2.5% more starch than counterparts steeped regularly. For both grains, the best wet milling conditions to obtain the highest amount of starch were 48 hours of steeping and CWDE addition. Johnston and Singh[206] obtained starch yields equivalent to conventional yields by addition of bromelain (0.4g protein/kg of corn) during steeping of maize. Proteases have been frequently used for isolation of rice starch from rice kernels as the conventional alkaline process produces highly loaded alkaline effluents. Lumdubwong and Seib[172] achieved 10% increase in yield of starch from wet milled rice flour using alkaline protease. Puchongkavarin et al.[207] developed a rice starch isolation process from polished rice grain by first application of cellulase under slightly acidic conditions in order to degrade the cellular tissue, followed by protease (Corolase 7089 or papain) under neutral conditions in order to loosen the protein bodies that are associated with starch granules. In comparison with the alkaline process, the enzyme process provided rice starch with a slightly elevated protein content, but less damaged starch. No differences were found between the two proteases used. Li et al.[208] studied the effect of alcalase and protease N treatments on properties of rice starch. The rice starches produced from protease N exhibited higher pasting viscosities than those produced from alcalase. No differences were found in the crystalline pattern, thermal properties, granules appearance, and average molecular weight (MW) of the rice starches between the two protease treatments.

The success of starch extraction from roots and tubers depends on complete rupture of the cell walls thereby releasing the starch granules.[209] Cell wall degrading enzymes can be used to disintegrate the cell walls thereby releasing the starch granules resulting in less mechanical disintegration and higher recovery. George et al.[210] investigated the effect of fermentation on starch extractability from two varieties viz., M4 and H-1687, of cassava tuber. The extracted starch increased from 43.33% in the conventional process to 74.73% by fermentation within a period of 24 hour in the case of M4, and from 51.5% to 70.3% in the case of H-1687. There was also reduction in the waste material produced. Padmanabhan et al.[211] studied enzymatic treatment for recovery of starch from slurry of cassava meal with conventional process. In the conventional process the recovery of starch was 61% and 68%

respectively in static and agitated conditions. In enzymatic treatment, the extent of starch release was dependent on the type and concentration of the enzymes as well as conditions of enzymatic treatment. Complete starch was recovered when 0.1% of pectinase and 0.1% of cellulase were used for 24 hours at 30±1°C under agitated conditions. Kallabinski and Balagopalan[159] studied the enzymatic starch extraction of starch from tropical root and tuber crops. The treatment of ground cassava roots with pectinolytic enzymes (0.1ml/100g fresh cassava root) increases starch recovery by 3.06, 9.70 and 10.10% after 2 h and 4.12, 12.95 and 16.40% after 4 h incubation at 45°C for different pectinases viz., Pectinex Ultra SP-L (containing pectinesterase, polygalactouronase, pectin lyase and hemicellulase), Pectinase 3XL AP-18 (containing pectin lyase, polygalactouronase, pectinesterase, and hemicellulase) and Rohament P (containing pectinesterase, polygalactouronase and pectin lyase) respectively. The cellulase (Celluclast 1.5L containing cellulase and pectinglycosidase) treatment (0.1ml/100g) increased starch recovery by 16.14% after 2 h and 15.70% after 4 h. The combined use of both (Rohament P and Celluclast 1.5L) resulted in increase up to 36.02% starch after 3 h of incubation. The results were confirmed in experiments with sweet potato also. Gayal and Hadge[212] used cellulase from *Penicillium funiculosum* for isolation of starch from potato. Hydrolysis of cellulose and release of starch were assessed at various enzyme concentrations, with different incubation periods. About 68% starch was recovered in 6 h and the recovery was increased to 90% in 2 h by supplementing with pectinase. Dzogbefia et al.[213] observed that an enzyme dosage of 0.02% of crude pectinase from *Saccharomyces cerevisiae* ATCC 52712 with a reaction time of 30 min gave the optimum increase in rate of starch extraction (60%) and starch yield extracted (53%) from cassava tubers.

1.7.2 Ultrasound extraction of starch

Ultrasound is defined as sound waves at a frequency above the upper value of normal human hearing range (>20 kHz). When ultrasound is applied to a liquid it propagates via a series of compression and rarefaction waves induced in the molecules of the medium through which it passes. Cavitation, a phenomenon caused by ultrasonication when the amplitude is high enough, results in the formation of bubbles in the liquid. These bubbles can explosively collapse and generate localized

pressure fluctuations. This localized pressure gradients causes high local velocities of liquid layer in the vicinity which in turn cause shear forces that have no significant influence on small molecules but are capable of breaking chains of polymer, provided they are longer than a certain limiting value.[214] This mechanical action of sonication has been used to disrupt cell membrane and to break the cell wall for enhancing various biological and biochemical separation processes. Ying et al.[215] obtained higher yield of polysaccharides from mulberry leaves using ultrasound assisted extraction. Hromádková et al.[216] was able to reduce the time of extraction of water soluble hemicelluloses from wheat bran using ultrasound. Li et al.[217] used ultrasound for extraction of oil from soybean and were able to reduce the time of oil extraction compared to conventional process.

Ultrasound has been used for increasing the recovery of starch from cereals like maize and rice or to modify the properties of isolated starch. Wang and Wang[218] evaluated high-intensity ultrasound as an alternative method to isolate rice starch without the use of chemicals as in the traditional alkaline steeping method. Surfactants, including sodium dodecyl sulphate (SDS), sodium stearoyl lactylate (SSL), and Tween 80, combined with high-intensity ultrasound were also investigated for rice starch isolation. They observed that ultrasonication for 20 min yielded 76.4% starch and the combination of 0.5% SDS and high-intensity ultrasound improved the starch yield to 84.9% with low residual protein. The thermal properties of the isolated starch were not changed by sonication and the amylose content remained unchanged. The surface of the isolated starch was not damaged by sonication. Wang and Wang[219] also investigated the efficacy of a neutral protease and combinations of neutral protease and high-intensity ultrasound for isolation of rice starch. The combination of neutral protease and high intensity ultrasound was an effective technique for isolation of rice starch without generating high salt wastes. Zhang et al.[220] used ultrasonication for enhancing corn starch separation. Starch yields from ultrasound treatments varied from 66.93 to 68.72% and were comparable to conventional wet milling (68.92%). The ultrasound treated samples produced 6.35 to 7.02% more starch compared to the milling-only corn and also, the ultrasound-produced starches showed a significant increase in whiteness and decrease in yellowness that are comparable to starches produced by conventional wet milling. Park et al.[221] developed a method for isolation of sorghum and other cereal starches using ultrasonication. Starches separated by ultrasonication method showed significantly less protein content and b values

(yellowness) compared with starches separated by enzymatic methods or methods using NaCl solutions and protein extraction buffers with multiple washing steps, both of which take several hours to complete. A rapid small-scale starch isolation technique for isolation of starch from sorghum and maize in the laboratory involving a combination of dry grinding of grain, suspension of the resulting flour in extraction buffer, application of ultrasonic sonication, then separation by sucrose density centrifugation was proposed by Benmoussa and Hamaker[222]. Ultrasonication yielded higher starch compared to conventional method. Combination of ultrasonication and sucrose density separation yielded 61% starch from sorghum and 63% from maize. The isolated starch showed lower starch damage and proteins content than by the conventional method.

1.8 Scope and objectives of the present investigation

Worldwide, the major sources of industrial starch are maize, cassava, sweet potato, potato and wheat. In developing countries, root and tuber crops are relatively more important as sources of starch than cereal crops. Asia contributes about 37% of world starch production. While approximately 70% of world starch production was derived from maize, maize accounted for only 45% of starch production in Asia. Root and tuber crops supplied more than half of Asia's starch needs, especially cassava (24.7%), sweetpotato (23.5%) and potato (6.0%).[223]

Indian starch industry is at a nascent stage with per capita consumption of starch in the country being 1.3kg compared with 64.5kg in the USA and over 10kg in many comparable Asian countries. Out of more than 1,000 downstream applications of starch, only 40 have been commercialised in India. However, the same is likely to improve in the coming years, as starch finds diverse applications in the food and beverage, paper, pharmaceutical, textile and animal feed industries. Currently Indian starch and starch derivatives sector is witnessing healthy 20% growth.[224] In India starch is mainly extracted from maize and cassava. Most of the cassava starch produced is utilized for production of sago pearls. Therefore, the Indian starch Industry is predominantly based on corn. But in the current years the prices of starch and its derivative are easing, and the prices of maize, which is the major input, has been rising, and leading to squeeze in margins. Therefore it is necessary to search for alternate sources of starch for the growing starch market.

Taro has a great potential as a source of starch and could replace many commercial starches in many industrial applications.[225] The potential of this crop is high in humid and sub humid tropics where cereal production is costly or not suitable.[9] Taro is found throughout India with greater diversity in North-Eastern, Eastern and Southern India.[2] Taro is abundantly produced in India and particularly North-Eastern region of India. It is grown in an unplanned manner by small farmers and their economic importance is also not exploited. Moreover, North-East India is thought to be one of the centers of origin for *Colocasia*.[7, 8] Information related to the physicochemical and functional properties of the starches of taro cultivars of North-East India are not available in literature.

The starch extraction process from roots and tubers consists of grating the raw material, in order to break vegetal cells and release the starch. This step is followed by passing the fibre through sieves of different mesh sizes and subsequent slurry concentration by decantation or centrifugation.[25] Complete disintegration of the cell walls to release the starch granules is not possible in grating, thereby decreasing the recovery of starch. Alternate methods for extraction of starch from taro tubers are required to increase recovery of starch from taro tubers. In the light of the above discussion the present investigation was undertaken with the following objectives:

- To examine the physicochemical, functional, textural and colour characteristics of starch from different cultivars of taro available in Assam
- To study the effect of incorporation of taro starch on quality of tomato ketchup
- To optimize the enzymatic starch extraction process from taro tubers
- To compare the functional properties of the starch extracted by enzymatic process with that of conventional process
- To study the effect of ultrasound on yield and functional properties of taro starch
- To study the effect of combination of ultrasound and enzymatic pretreatment on yield and functional properties of taro starch

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Chapter 2

Physicochemical, Functional, Textural and Colour Characteristics of Starches Isolated from Different Cultivars of Taro of Assam and Their Comparison to Starches from Rice, Maize and Potato

2.1 Introduction

Taro a member of the *Araceae* family is an important tuber crop largely grown for its underground corms and consumed in tropical and sub-tropical areas of the world, primarily as a root vegetable and secondarily as a leaf vegetable. The post harvest losses of taro are high due to its high moisture content and large size of corms. Cold storages are also not available for this crop. These losses can be minimized by converting them into non-perishable forms by drying or extracting the starch and other components from the corms which might also reduce the storage space required for storing these tubers.[1]Taro has a great potential as a source of starch and could replace many commercial starches in many industrial applications.[2] The potential of this crop is high in humid and sub humid tropics where cereal production is not suitable.[3] Before accepting taro as potential sources of starch for food application, it is necessary to characterize their physicochemical, functional and sensory properties. Therefore it is evident that a significant amount of work needs to be done on the properties of native taro starch for it to become competitive with other commercial starches.

Cultivated taro is classified as *Colocasia esculenta*, but the species is polymorphic. There are two botanical varieties: *Colocasia esculenta* (L.) Schott var. *esculenta* and *Colocasia esculenta* (L.) Schott var. *antiquorum*.[3] Colocasia is found throughout India with greater diversity in North-Eastern, Eastern and Southern India.[4] North-East India is also thought to be one of the centres of origin for *Colocasia*.[5, 6] The starch content of taro varies from 12 – 25% for different varieties available in India.[4] Taro has been reported to have 70–80% starch with small granules.[7] Taro starch is used directly in different ways or as a raw material for further processing. It is considered to be easily digestible because of the small sizes of its starch granules; hence it is widely used in baby foods and the diets of people allergic to cereals and children sensitive to milk.[8-10] The *in vivo* digestibility of taro starch was found to be comparable to corn starch[11] and was more susceptible to pancreatin hydrolysis than other tuber and root starches.[12] Owing to ease of assimilation, taro starch can be used by infants and persons with digestive problems.[13] Due to its small granule size, it is suitable for many industrial applications[14] and form smooth textural gel.[15] Its starch could be used in the preparation of biodegradable polyethylene film[14, 16] and as a fat substitute due to its small granule size. They could also be used for entrapment of flavouring compounds like vanillin.[17, 18] The small size of granules makes it ideal in

cosmetic formulations like face powder and in dusting preparations that use aerosol dispensing systems.[14] The physicochemical properties of taro starch vary with location and variety.[2, 19-22] Similarly, amylose content, water absorption capacity, water solubility index and other properties of the taro starch also vary significantly with variety and location.[2, 7, 20, 23] The application of a given starch is decided by its physicochemical and functional properties. Starches from different sources differ in their physical and chemical properties.[24] Factors such as farming practices and differences in cultivar among taro might affect their chemical composition and hence, the physicochemical properties of the starches.[25] Although, some studies on properties of taro flour from some Indian varieties have been reported,[26, 27] however, no major study on the properties of isolated starch was investigated for the Indian varieties.

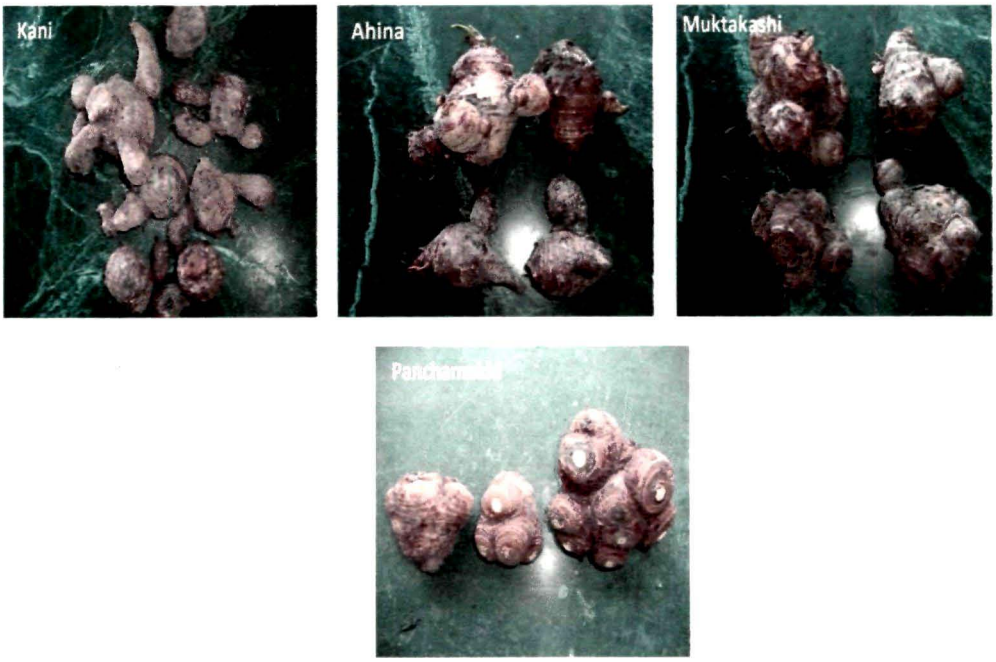
Taro is abundantly produced in North-Eastern region of India, particularly Assam. Information related to the physicochemical and functional properties of the starches of taro cultivars of North-East India are not available in literature. Therefore, the present study was taken up to evaluate the physicochemical, functional, textural and colour characteristics of starches from seven cultivars of taro of this region. The properties of the taro starches were further compared to rice, maize and potato starches which are main commercial sources of starch, so that the industrial application of taro starch might be exploited.

2.2 Materials and methods

2.2.1 Sample collection

Tubers of seven cultivars of taro were used in the present investigation. The cultivars collected were: *Kani*, *Ahina*, *Muktakashi*, *Panchamukhi*, *Garu*, *JCC37* and *JCC57*. Among the seven cultivars *Kani*, *Ahina*, *Muktakashi* and *Panchamukhi* were "eddoe" type taro i.e. *Colocasia esculenta* var. *antiquorum*, and *Garu*, *JCC37* and *JCC57* were "dasheen" type taro i.e. *Colocasia esculenta* var. *esculenta* (Fig. 2.1). *Ahina*, *Muktakashi*, *Garu*, *JCC37* and *JCC57* were obtained from research farm of Assam Agricultural University, Assam, India, while *Kani* and *Panchamukhi* were collected from a local farm near Tezpur University, Assam, India. Freshly harvested tubers were collected after 8 months from cultivation. Starches from potato variety *Chandramukhi* (*Solanum tuberosum*) and rice variety *Mahsuri (Oryza sativa)*, locally

known as *Aijong rice* were used for comparison. Maize starch was purchased from HiMedia Laboratory, India and also used for comparison.



A)

B)

Fig. 2.1 Images of tubers of various taro cultivars investigated: A) Cultivars belonging to 'eddoe' type taro, and B) Cultivars belonging to 'dasheen' type taro

2.2.2 Starch isolation

Starch from taro and potato was extracted as per the method described by Benesi et al.[10] Tubers were washed under tap water, peeled and cut into cubes of approximately 1 cm. The cubes were ground using a high speed laboratory blender (Philips HL 1632, India) for two minutes. The slurry was mixed with 10 times its volumes of distilled water. The suspension was filtered through double fold cheese cloth and the filtrate was kept for sedimentation for 6 hours. The supernatant was discarded and the sediment thus obtained was washed with distilled water for two times. The final sediment was dried at 45 °C for 24 h in drying oven. The dried starch was ground and passed through 100 mesh sieve and kept in air tight plastic containers for further analysis. Starch from rice was extracted as per method described by Wang and Wang.[28]

2.2.3 Physicochemical properties

2.2.3.1. Chemical composition and amylose content

The moisture, fat, ash and crude fibre content of the isolated starches were determined by AOAC methods.[29] Protein content (N×6.5) was determined by Kjeldahl method.[30] The amylose content was determined by colorimetric method.[31] The standard curve was prepared using pure potato amylose type III (HiMedia, India).

2.2.3.2 Granule size and shape

Shape and size of starch granules were evaluated using scanning electron microscope (JEOL JSM 6390 LV, Singapore). A thin layer of starch granule was mounted on the aluminium specimen holder by double-sided tape. The samples were coated with platinum and examined under the microscope at an accelerating voltage of 15 kV with magnification of 4000X. Size of the starch granules were determined by measuring the diameters of 30 randomly selected granules from the micrographs.

2.2.3.3 XRD analysis

XRD analysis of the starch samples were carried out using X-ray diffractometer (Miniflex, Japan). The samples were exposed to X-ray beam at 15 mA and 30 kV. Data were recorded over a diffraction angle (2θ) range of 5° to 50° with a

step angle of 0.05°. Percent crystallinity was determined by calculating the percentage ratio of diffraction peak area to the total diffraction area.

2.2.3.4 Fourier transform infrared (FT-IR) analysis

In order to spectral features of the taro starches, FT-IR spectra were obtained using FT-IR (Spectrum 100, Perkin Elmer, SA). The spectra were recorded in absorbance mode from 4,000 to 400 cm^{-1} (mid-infrared region) at a resolution of 4 cm^{-1}. Samples were thoroughly grounded with exhaustively dried pure KBr (1:100, w/w) and pellets were prepared by compression and analyzed. Background value from pure KBr was acquired before each sample was scanned.

2.2.4 Functional properties

2.2.4.1 Swelling and solubility

Swelling power and solubility of the starches were determined by modified method of Torruco-Uco and Betancur-Ancona.[32] Starch (0.5 g) was dispersed in 20 ml distilled water in a pre-weighed 50 ml centrifuge tubes and kept in shaking water bath at 60, 70, 80 and 90 °C for 30 min. The suspension was then centrifuged at 12,000 × *g* for 10 min. The supernatant was carefully decanted in a Petri dish and dried at 103 °C for 12 h. After decantation the weight swollen granules were taken. The swelling power and percentage solubility were calculated using the following formulas:

Swelling Power = Weight of swollen granules × 100 / (Weight of sample – Weight of dissolved starch)

% Solubility = Weight of dried starch in Petri dish × 100 / Sample weight

2.2.4.2 Clarity and stability

Clarity and stability of the starches were measured following the method described by Sandhu and Singh.[33] Aqueous starch suspension was prepared by heating 0.2 g starch in 20 ml water in shaking water bath at 90 °C for 1 h. The starch paste was cooled to room temperature. The starch pastes were stored at 4 °C in refrigerator and the absorbance was measured at 640 nm in spectrophotometer (Spectrascan UV-2600, Thermo Fisher Scientific, India). The absorbance was measured after every 24 h for seven days to determine the stability of the pastes.

2.2.4.3 Freeze-thaw stability

The freeze-thaw stability was determined according to the method of Singhal and Kulkarni.[34] Starch (5% dry basis, w/v) was dissolved in distilled water at 95 °C for 30 minutes with constant stirring. Ten ml of paste was transferred to weighed centrifuge tubes. The weight of the paste was then determined. This was subjected to alternate freezing and thawing cycles (22 h freezing at -20°C followed by 2 h thawing at 30°C) for 5 days, centrifuged at 5000 × g for 10 minutes after each cycle and the percentage syneresis was determined as weight of exudates to the weight of paste.

2.2.4.4 Thermal properties

Gelatinization properties of the starches were determined by differential scanning calorimeter (DSC-60, Shimadzu, Singapore) by the method described by Jiranuntakul et al.[35] with some modifications. Starch (3 mg) was weighed in the aluminum pans and water was added in the ratio of 1:3 for starch: water. The pans were sealed and allowed to stand for 12 h at 4°C for moisture equilibration. The samples were scanned from 25 -120°C at 10°C/min. The onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and gelatinization temperature range (T_c-T_o) were noted from the graphs. The enthalpy of gelatinization was estimated as Joules per gram of dry starch.

2.2.4.5 Pasting properties

Pasting properties of the starches were evaluated using Rapid Visco-Analyzer (RVA), model StarchMaster2 from Newport Scientific, Australia. Viscosity profiles were recorded using 12.5 % starch slurry in distilled water (total weight 28 g). A heating and cooling cycle of 13 min was used where the samples were heated from 50°C to 95°C in 5 min, held at 95°C for 2 min, cooled from 95 °C to 50 °C in 4 min and held at 50°C for 2 min. Pasting temperature (PT), peak viscosity (PV), hold viscosity (HV), final viscosity (FV), breakdown viscosity (BV) and setback viscosity (SV) were recorded from the graph.

2.2.5 Textural and colour characteristics

2.2.5.1 Texture analysis of starch pastes

Starch pastes were prepared by heating an aqueous suspension of starch (1 g starch in 50 ml distilled water) in a shaking water bath at 100 °C for 30 min. The starch pastes were cooled to 25 °C by keeping the starch pastes in cooling water bath maintained at 25 °C for 1 h. Textural properties such as firmness, consistency and cohesiveness of starch pastes were determined by back extrusion method in Texture Analyzer, TA.HDplus (Stable Micro Systems, UK) using a cylindrical probe (P-35). The probe was allowed to penetrate 20 mm from the surface of the sample at a speed of 1 mm/s. Firmness, cohesiveness, consistency and index of viscosity were calculated from the graphs using the software Exponent Lite 32 provided with the instrument.

2.2.5.2 Colour analysis of starch pastes

Colour parameters of the starch pastes containing 2 % starch were measured using colorimeter (Ultrascan VIS, Hunterlab, USA). Results were obtained in terms of L* (lightness), ranging from 0 (black) to 100 (white), a* (redness), ranging from +60 (red) to −60 (green), and b* (yellowness), ranging from +60 (yellow) to −60 (blue) values.

2.2.5.3 Colour of starch (dry powder)

Colour of starch (dry powder) was measured using colorimeter (Ultrascan VIS, Hunterlab, USA). L, a* and b* values were noted. L is for lightness, a* for redness and b* yellowness.

2.2.6 Statistical analysis

The data were subjected to single factor analysis of variance (ANOVA) using 'Data Analysis Tool' of 'Microsoft Excel'. Fisher's 'Least Significant Difference (LSD)' method was used to determine the statistical difference between the results obtained.

2.3 Results and discussion

2.3.1 Physicochemical properties of taro starches

2.3.1.1 Chemical composition and amylose content

The chemical composition and amylose content of the isolated starches are shown in Table 2.1. The moisture content of the starches varied from 8.96 to 11.93% which were within safe limit for storage of starches without deterioration in quality of starches.[2] The ash contents of the starch samples varied from 0.07 to 0.77%. The lowest ash content was observed for maize starch whereas the highest was observed for potato starch. The higher ash content of potato starch might be attributed to the presence of phosphates in potato starch. The ash content of the starches from various taro cultivars varied from 0.09 to 0.71%. The lowest ash content was observed for *Panchamukhi* starch. Nand et al.[22] found that the ash contents of starch obtained from the cassava and taro samples ranged between 0.08 - 0.20 %. Mweta et al.[36] also found that ash content of the taro and cassava starches ranged from 0.10-0.20%. The protein content of the taro starches ranged from 0.33 to 0.71%, and that of rice, maize and potato starches were 1.26, 0.64 and 0.39% respectively. The reason for higher protein content of rice starch might attributed to the structure of the starch granules in cereals which are embedded in protein matrix, so it is difficult to separate protein from the granules and might have contributed to the protein content of rice starch. The results were in accordance with the results obtained by Peroni et al.[37] for cassava, arrowroot and sweet potato starches whose protein content varied from 0.08-0.35%. The lipid content of taro starches ranged from 0.11 to 0.43% for the various taro cultivars. Jane et al.[7] reported that the lipid content in taro starches ranged between 0.08 to 0.12 % (dry basis) which is in agreement with the present findings. Peroni et al.[37] also found that tuber starches contain lipids in the range 0.10-.24%. Lower lipid content of starches indicates a high purity of extracted starches. The differences in the composition of the starches among the taro cultivars might be attributed to the differences in mucilage content of the taro tubers. Tubers with higher mucilage contributed to the impurities of the starch samples as separation of starch granules from other components of the starch slurry with higher mucilage was difficult.

Table 2.1. Chemical composition and amylose content of starches from various taro cultivars, rice, maize and potato

Sample[1,2,3]	Moisture, %	Protein, %	Fat, %	Fibre, %	Ash, %	Starch, %	Amylose, %
Kani	11.27±1.02^{ab}	0.71±0.23^{b}	0.30±0.05^{abc}	0.15±0.02^{ab}	0.71±0.20^{ab}	96.25±1.15^{ab}	16.19±1.52^{cd}
Ahina	9.36±2.12^{bc}	0.62±0.12^{bc}	0.33±0.09^{abc}	0.21±0.06^{ab}	0.52±0.21^{ab}	94.18±1.62^{b}	15.79±2.23^{cde}
Muktakashi	10.12±1.36^{abc}	0.54±0.21^{bcd}	0.21±0.20^{cd}	0.19±0.09^{ab}	0.43±0.15^{b}	95.95±1.32^{ab}	14.78±1.27^{de}
Panchamukhi	9.22± 0.10^{bc}	0.34±0.05^{d}	0.11± 0.007^{d}	0.09±0.03^{b}	0.09± 0.002^{c}	95.87± 1.52^{ab}	18.14 ± 1.23^{c}
Garu	10.36±0.89^{abc}	0.52±0.06^{bcd}	0.41±0.10^{ab}	0.19±0.05^{ab}	0.65±0.11^{ab}	96.01±1.25^{ab}	13.21±0.98^{ef}
JCC37	9.57±1.54^{bc}	0.33±0.09^{d}	0.43±0.14^{a}	0.25±0.11^{a}	0.57±0.17^{ab}	96.69±0.94^{ab}	11.87±1.24^{f}
JCC57	8.96±0.98^{c}	0.42±0.15^{cd}	0.36±0.07^{abc}	0.17±0.08^{ab}	0.43±0.25^{b}	95.12±2.12^{ab}	13.18±0.57^{ef}
Rice	9.96±1.54^{abc}	1.26±.26^{a}	0.31±0.04^{abc}	0.23±0.03^{a}	0.58±0.09^{a}	95.69±1.56^{ab}	21.57±2.08^{b}
Maize	11.93±0.23^{a}	0.64±0.07^{b}	0.24±0.006^{bcd}	0.16±0.08^{ab}	0.07±0.004^{c}	96.30±1.96^{ab}	24.20±1.51^{ab}
Potato	10.64±1.23^{abc}	0.39±0.11^{cd}	0.19±0.09^{cd}	0.10±0.02^{b}	0.77±0.36^{a}	97.13±0.88^{a}	25.75±2.12^{a}

[1]Values reported as Mean ± S. D. of three replications and expressed in dry basis except for moisture which is expressed in wet basis
[2]Meanss followed by same superscript small letters within a column are not significantly different ($p>0.05$)
[3]Amylose content calculated as amount of amylose present in pure starch; and amylopectin, % = (100 – amylose %)

The purity of the isolated starches from taro cultivars varied from 94.18 to 96.69%, which were less as compared to potato starch with a purity of 97.13%, but were closer to the purity of rice and maize starches with purity of 95.69 and 96.30%. This might be due to high amount of mucilage in taro tubers compared to potato, and separation of starch from starch water containing mucilage becomes difficult, but the values were not significantly different from each other.

The amylose content of the taro starches ranged between 11.87 to 18.14%, which were significantly lower than rice, maize and potato starches with amylose content of 21.57, 24.20 and 25.75% respectively. The amylose content of *Kani*, *Ahina*, *Muktakashi* and *Panchamukhi* cultivars were found to be more compared to *Garu*, *JCC37* and *JCC57* cultivars. This might be due to the fact that *Kani*, *Ahina Muktakashi* and *Panchamukhi* belonged to same variety i.e. *antiquorum* whereas *Garu*, *JCC37* and *JCC57* belonged to var. *esculenta*. The highest amylose content was observed for *Panchamukhi* starch. The results were in agreement with the findings of Mweta et al.[2] for different varieties of taro starches which varied from 10.6 to 21.0 %. Singh et al.[38] reported amylose content of native potato starches from 25.6 to 30.4 % and for rice starch, amylose content varied from 21.88 to 31.6 %.[39, 40] The amylose content of the taro starches was found to be lower than potato and rice starches reported by other authors.[38, 39] The low level of amylose makes taro starches more hydrolysable as starch with high level of amylose hydrolyzed more slowly than starch with low level of amylose owing to the double helical form of amylose molecule which is not easily accessible by enzymes.

2.3.1.2 Granule size and shape

Scanning electron micrographs (Fig. 2.2) of the taro cultivars clearly showed a mixture of various shapes and sizes of the starch granules. The starch granules were dome shaped, polygonal, split and irregular in shape. The average size of the starch granules varied from 2.22 to 3.29 μm for the different taro cultivars (Table 2.2). Significant differences were not observed between the sizes of the starch granules, although the *JCC37* was found to have largest granule size amongst the seven cultivars investigated. Similar values for granule sizes of taro starch from different regions were observed by, Mweta et al.,[2] Jane et al.,[7] Mepba et al.,[25] and Agama-Acevedo et al.[41] The average sizes of rice, maize and potato starch granules were

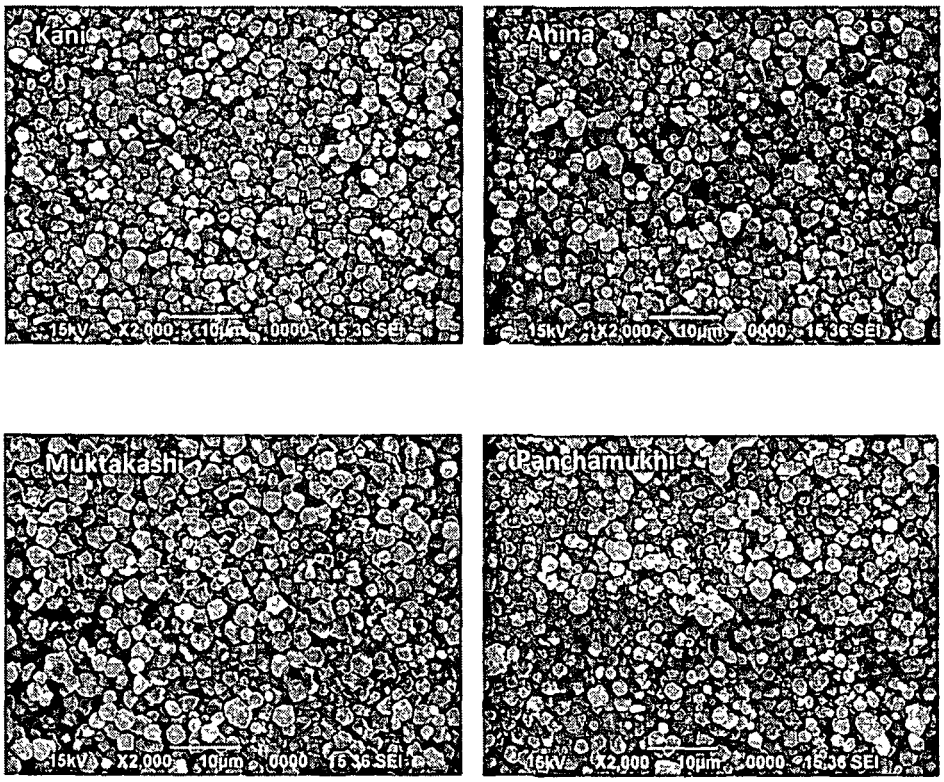


Fig. 2.2 a) Scanning electron micrographs of starch granules of taro cultivars *Kani*, *Ahina*, *Muktakashi* and *Panchamukhi* belonging to *Colocasia esculenta* var. *antiquorum*

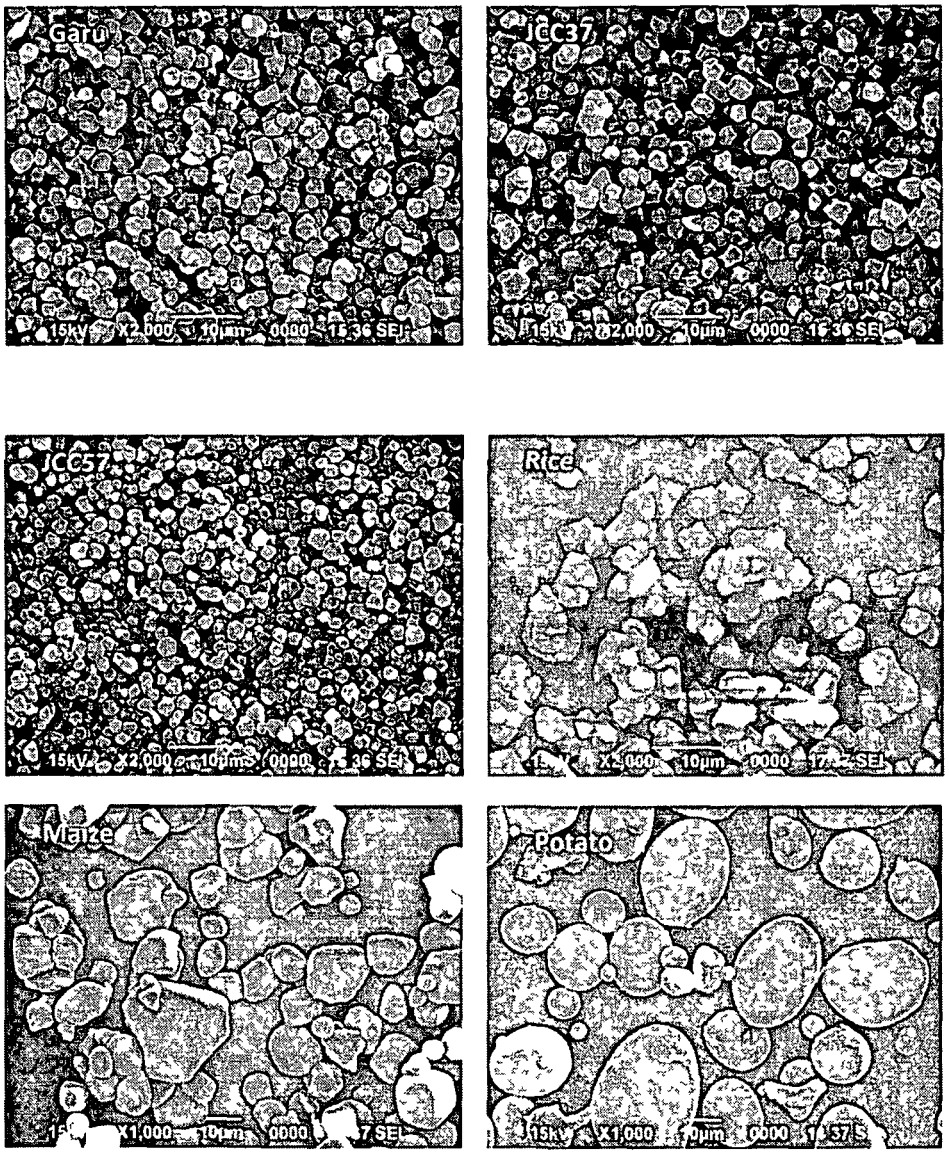


Fig. 2.2 b) Scanning electron micrographs of starch granules of taro cultivars *Garu*, *JCC37* and *JCC57* belonging to *Colocasia esculenta* var *esculenta* and of rice, maize and potato

4.31, 11.71 and 20.50 μm. The shape of rice starch granules was similar to the taro starch granules. Maize starch was found to have irregular, round or oval shapes, while potato starch was found to have round and oval shapes. It was evident from the micrographs that taro starch granules were much smaller than starch granules of maize or potato, and were comparable with that of rice starch in size and shape of granules. Maize and potato starch granules also presented broader range of the sizes of the starch granules. Shape and size of starch granules influence many physicochemical and functional properties of starches like paste viscosity[41] and swelling volume and solubility.[20, 32]

2.3.1.3 XRD pattern

The position of strong and weak peaks and % crystallinity of the starches of the taro cultivars investigated were presented in Table 2.2. The positions of the peaks were found to be similar for the taro starches. It was observed that all the taro cultivars and rice and maize displayed A-type XRD pattern (Fig. 2.3), which is a characteristic of cereal starches. Potato starch presented B-type pattern. Generally tuber starches have B or C-type pattern.[42] Strong peaks were observed near 15°, 17°, 18° and 23° 2θ for starches isolated from the different taro cultivars and the starches isolated from rice and maize. For potato starch strong peaks were observed near 6° and 17° 2θ, which is a characteristic of B-type starches. The % crystallinity of the taro starches were higher than rice or potato starch and were closer to that of maize starch. Many properties like digestibility and retrogradation are affected by XRD pattern and the degree of crystallinity of the starches.[7, 43]

2.3.1.4 FT-IR spectra

FT-IR spectra of the starch samples in the 4,000–400 cm^{-1} region are shown in Fig. 2.4. The spectra obtained for the ten starch samples were similar in the form and intensity of the major peaks. The spectra showed high absorption near the wave numbers 574, 930, 1016-1022, 1048, 1080, 1154, 1365, 1420, 1644, 2930 and 3380cm^{-1} confirming the carbohydrate nature of the samples. The peaks at 3,400 cm^{-1} and 2,930 cm^{-1} could be attributed to O–H and H-C-H bond stretching, respectively, while the peak at 1644 may be attributed to COO- stretching vibration in a carbohydrate group.[44, 45] Peaks at 1,420 cm^{-1} and 1,365 cm^{-1} were attributable to the bending modes of H–C–H and C–H symmetric bending of CH_3.[44, 46-48] C-C and C-O

Table 2.2 Granule size distribution, % crystallinity and position of peaks in X-ray diffractogram of starch samples

Sample	Granule size distribution		% Crystallinity	Position of strong peaks (2θ)	Position of weak peaks (2θ)
	Range, μm	Average granule size[1,2], μm			
Kani	1.07 – 3.33	2.22±0.73[b]	40.26	15.4, 17.25, 18.00, 23.4	26.80, 30.45, 33.45
Ahina	1.19 – 4.17	2.51±0.79[b]	34.97	15.15, 17.45, 18.15, 23.35	26.35, 30.85, 32.20
Muktakashi	1.43 – 3.87	2.95±0.73[ab]	39.24	15.30,17.4,18.05, 23.25	26.40,31.65,33.05
Panchamukhi	2.19 – 4.28	2.77±1.12[a]	44.73	15.15, 17.15, 17.80, 23.10	26.35, 30.35, 33.05
Garu	1.91 – 4.17	3.12±0.75[b]	39.14	15.45, 17.35, 18.1, 23.30	26.45, 31.1, 33.65
JCC37	2.14 – 4.88	3.29±0.86[a]	42.13	15.35,17.55,18.35,23.25	26.30, 31.45, 33.10
JCC57	1.43 – 3.45	2.49±0.53[b]	45.11	15.60,17.49, 18.6,23.70	26.75,30.75,33.55
Rice	2.26 – 6.55	4.31±1.15[b]	31.55	15.25, 17.05, 17.95, 23.15	26.70, 30.45, 33.95
Maize	7.38 - 16.67	11.71±3.93[b]	43.59	15.30, 17.60, 18.45, 23.10.	26.15, 30.50, 33.75
Potato	7.65 – 47.63	20.50±11.57[a]	23.57	5.60, 17.15	11.65, 15.00, 22.30, 24.00, 34.35

[1]Values reported as Mean ± S. D. of three replications

[2]Meanss followed by same superscript small letters within the column are not significantly different (p>0.05)

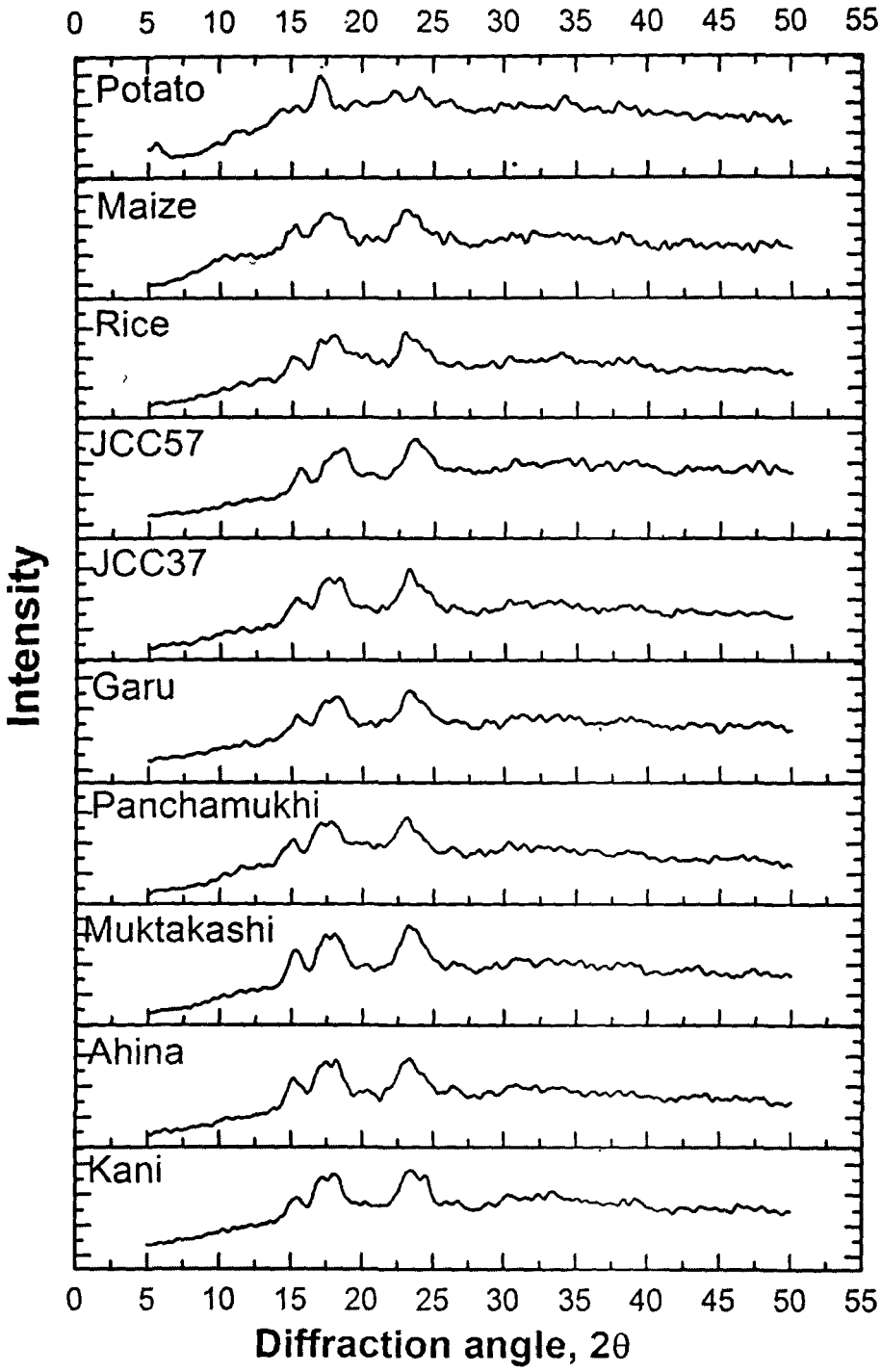


Fig. 2.3 XRD pattern of starches of various taro cultivars, rice, maize and potato

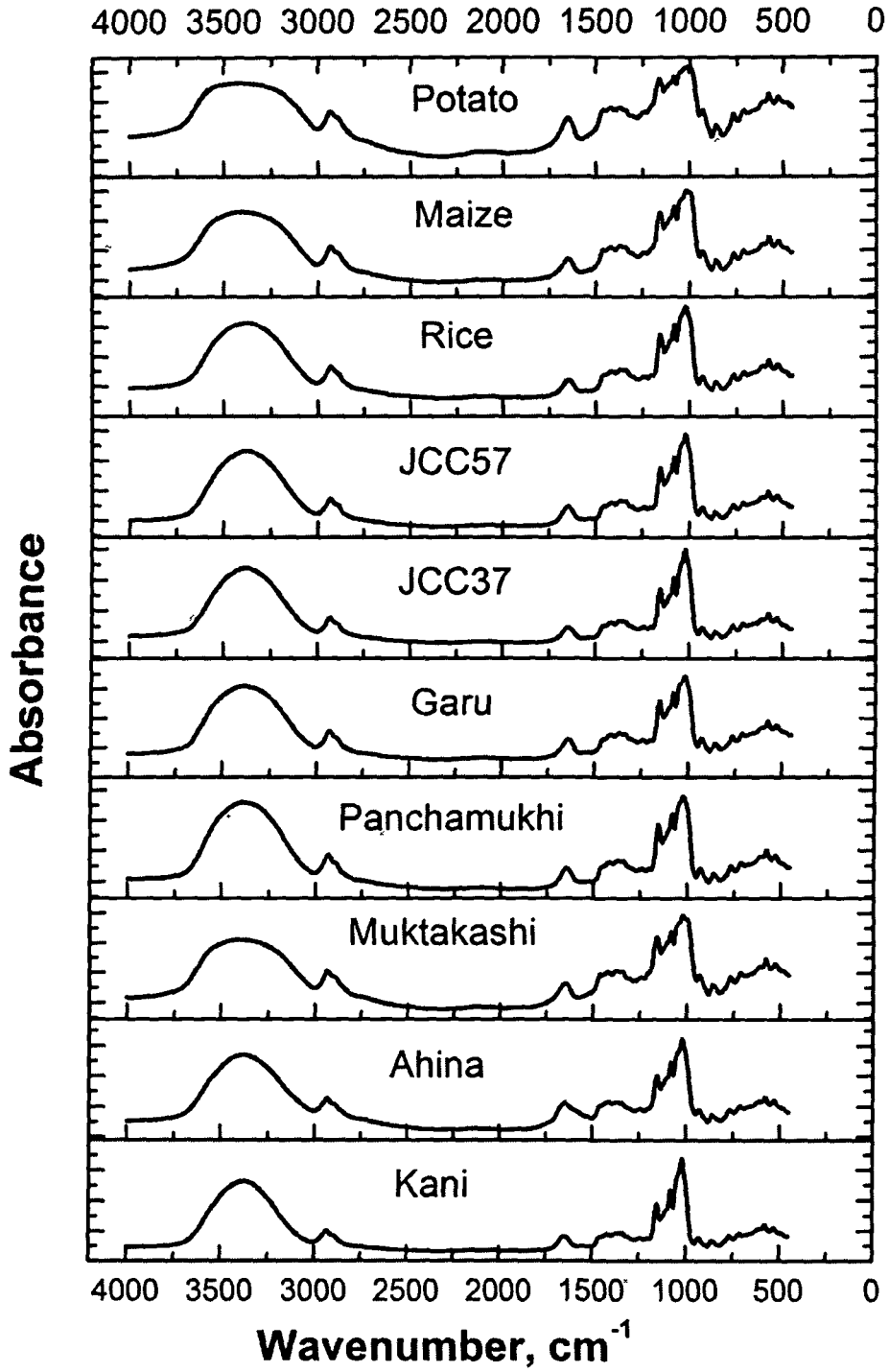


Fig. 2.4 FT-IR spectra of starches from various taro cultivars, rice, maize and potato

bond stretching could be assigned to the peak at 1154 cm^{-1} [44, 46]. The peak at 1080 cm^{-1} was attributed to C–O–H bending.[49] The bands at 1,047 and 1,022 cm^{-1} were associated with the crystalline and amorphous structures of starch, respectively.[44, 45, 50] The ratio of absorbance at 1047 and 1022 cm^{-1} indicate the degree of order in starch samples [48, 49, 50].[50-52] The bands at 930 cm^{-1} and 574 cm^{-1} describe the D-glucopyranosyl ring vibrational modes and skeletal modes of pyranose ring, respectively. The FT-IR spectra obtained in the present work are not much different from the spectra obtained by Aboubakar et al.[53] for Cameroonian taro starches and that of rice starches reported by Fan et al.[45]

2.3.2 Functional characteristics

2.3.2.1 Swelling and solubility

The swelling and solubility of the starches increased with the increase in temperature (Fig. 2.5). The solubilities at 60°C were lower and were not significantly different among the seven cultivars and the starches from rice maize and potato, although the solubility of *Panchamukhi* starch was highest at this temperature. At 70°C and 80°C the solubility of *Panchamukhi* taro starch was significantly higher than the other starches. At 90°C highest solubility was observed for *Kani* starch and was found to be close to that of *Panchamukhi* starch and the differences in solubility of these two starches were not significantly different. It was further observed that *Ahina* and *Muktakashi* which belonged to same variety as that of *Kani* and *Panchamukhi* i.e. var. *antiquorum*, showed lower solubilities compared to *JCC37* and *JCC57* at 80 and 90°C, but were higher than that of *Garu* starch at 60, 70 and 80 °C. The low solubility of starches at low temperatures might be due to the semi-crystalline structure of the starch granules and the hydrogen bonds formed between hydroxyl groups within the starch molecules. As the temperature increased, the solubility increased due to breaking of starch granules and exposure of hydrophilic groups to water.[54] Swelling power indicates the water holding capacity of starch granules and is affected by the extent of chemical cross bonding within the granules.[55] It was observed that at 60 °C the differences in swelling power of all the starches were not significantly different. But as the temperature was increased potato starches swelled significantly more as compared to other starches. Swelling of taro starches were not significantly different from each other at all temperatures and were comparable with that of rice and maize starch. The reason for higher swelling power

of potato starch may be attributed to the large size of the starch granules and higher amount of phosphate present in potato starch.[56]

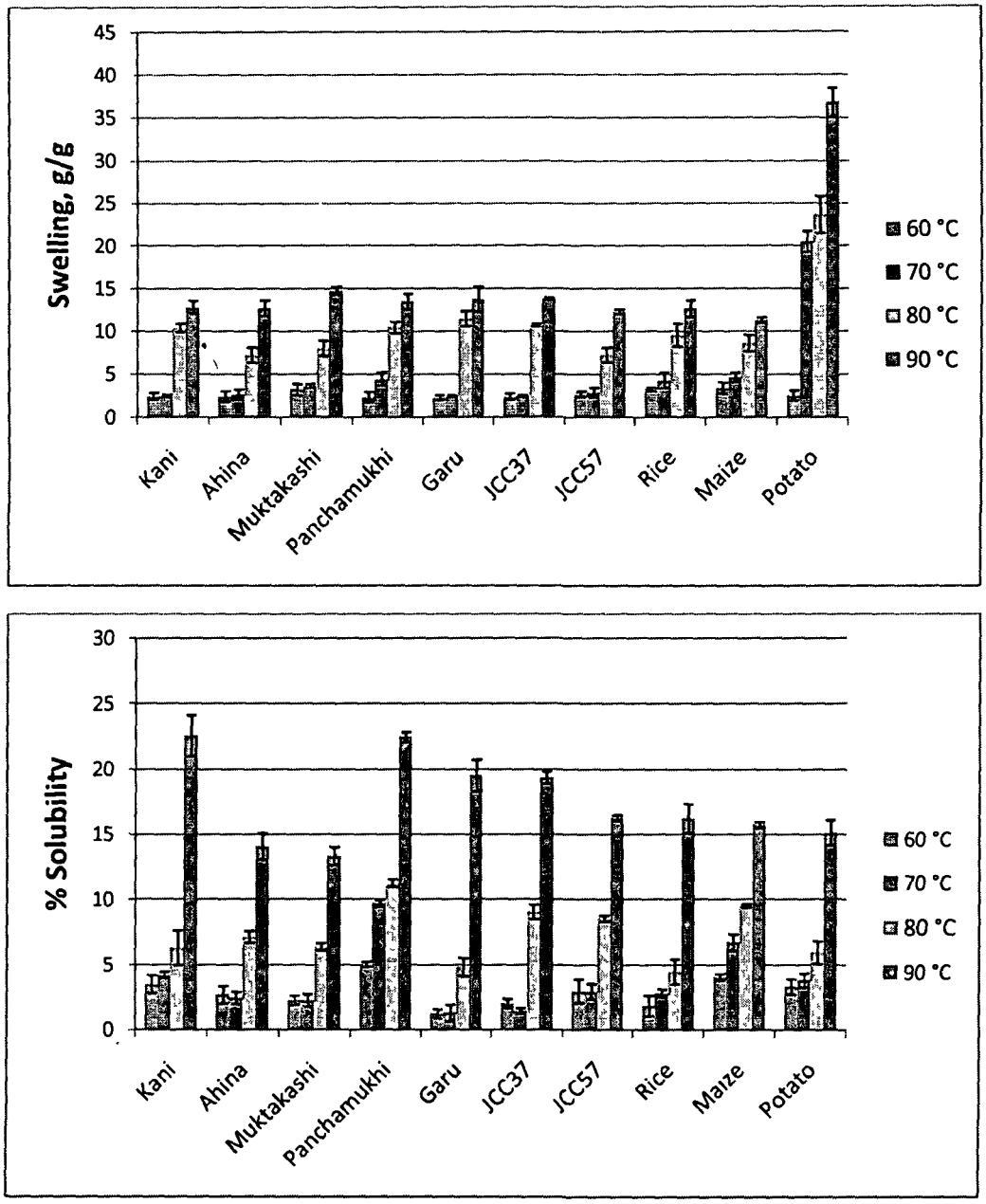


Fig. 2.5 Swelling and solubility of starches of various taro cultivars, rice, maize and potato

Vertical error bars above the columns represent standard deviation (S. D.) of three replications

2.3.2.2 Paste clarity and stability

Clarity (% transmittance) of the starch pastes of the starches from the different taro cultivars and that of rice, maize and potato for a period of seven days are shown in Fig. 2.6. The clarity of starch paste from *Panchamukhi* was significantly higher than other taro starches during the initial and final days. The stability of *Panchamukhi* starch paste was also found to be better in comparison to the starch pastes from other taro cultivars as the variation in clarity during 7 days of storage was less. Potato starch showed the highest light transmittance as compared to other starch pastes. The clarity of the rice and maize starch paste was also significantly more than the taro starches. The stability of potato starch paste was also more than other starches as % light transmittance did not differ significantly for the storage period. Clarity of the taro starch pastes were the lowest and found least stable except for *Panchamukhi* taro which was closer to clarity and stability of rice starch paste. The higher clarity and stability of potato starch paste may be due to the large size of the potato starch granules because of which less number of starch particles was present in the solution thereby scattering less amount of light.[38] Taro starches being smaller in size scattered more amount of light. In addition, potato starch has B-type crystallinity and B-type starches exhibit better clarity than A-type starches.[11] The lower stability of taro starch paste can be attributed to lower swelling of taro starch granules, as starch with higher swelling power are less susceptible to retrogradation which determines the stability of starch pastes.[24] The clarity of the starch pastes increased up to the 3rd day, remained stable till 5th day, and then started decreasing. Similar pattern of starch paste stability was observed by Mweta et al.[36] for taro and cassava starches. The reason for increase in clarity in the initial period might be settling of the larger molecules or impurities present in the starch paste, thereby increasing the clarity. The decrease in clarity after a certain period of storage may be attributed to retrogradation, where molecules comprising gelatinized starch reassociate into an ordered structure to retrieve a crystalline order, and during this process, the starch paste becomes more opaque.[57]

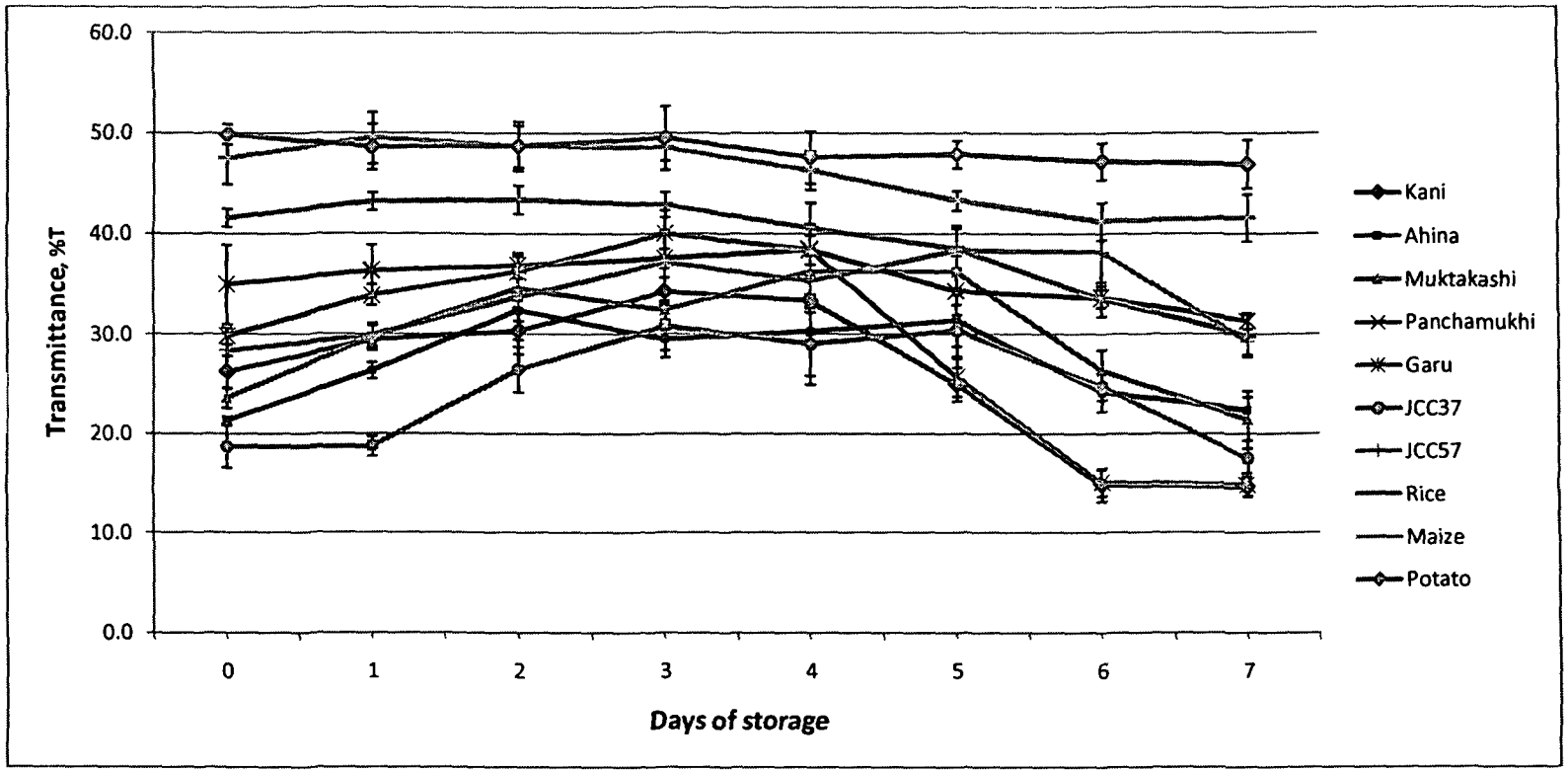


Fig. 2.6 Clarity and stability of starch pastes from various taro cultivars, rice, maize and potato
Vertical error bars represent standard deviation (S. D.) of three replications

2.3.2.3 Thermal properties

The gelatinization properties of the starches are presented in Table 2.3. The peak gelatinization temperatures (T_p) of the taro cultivars were significantly different from each other. The T_p of starches for the taro cultivars ranged from 70.61 and 83.33°C. The T_p of *JCC37* was the highest while that of *Muktakashi* starch was the lowest among the taro cultivars.

The T_p for rice and maize starches were 72.53 and 69.70°C and was found to be closer to the taro starches except for *JCC37* and *JCC57* starches. The T_p of potato starch was observed to be 65.55°C which was much lower compared to taro and rice starches. Wang et al.[40] and Park et al.[58] reported similar values for rice starches, Jiranuntakul et al.[35] for rice and potato starches, and Mweta et al.[2] for native taro starches of South Africa. The enthalpy of gelatinization (ΔH) of potato starch (15.27 J g^{-1}) was the highest among the starches followed by maize starch with ΔH of 15.24 J g^{-1}. The difference in the thermal behavior of the starches might be attributed to the amylose content[59] and crystallinity of the starch granules[60] as taro, maize and rice starches presented A-type XRD patterns. Potato starches also exhibited broader range of gelatinization compared to taro and rice starches, which might be attributed to the higher amylose content of the potato starches.[61] It was observed that the gelatinization behaviour of the taro starches were comparable to rice and maize starch, but diffred much from potato starch.

2.3.2.4 Pasting properties

The pasting profile of the starches from the different taro cultivars, rice, maize and potato is presented in Table 2.4. Pasting temperature is the temperature at which the viscosity of the starch pastes begins to rise. The pasting temperatures of all the taro cultivars were found to be high and ranged from 82.1 to 88.3°C. *Kani*, *Ahina*, *Muktakashi* and *JCC57* showed higher pasting temperature than the other three cultivars. The pasting temperatures of the North-East Indian varieties were found to be higher than the Mexican taro variety[41] or the Australian variety reported by Aprianita et al.[62] The pasting temperatures of the taro starches were observed to be higher than the T_p values in DSC study, except for *JCC37* where pasting temperature and T_p values were closer. This might be attributed to the moisture equilibration process carried out before scanning the samples in DSC. Starch granules of taro being smaller in size, absorbed moisture at faster rate during equilibration

Table 2.3 Gelatinization properties of starch samples

Sample[1,2]	T_o, °C	T_p, °C	T_c, °C	T_c-T_o, °C	ΔH, J/g
Kani	66.13±0.08^{e}	74.67±0.09^{c}	77.94±0.12^{e}	11.81±0.09^{c}	13.36±0.15^{de}
Ahina	66.23±0.09^{e}	73.33±0.11^{f}	78.05±0.03^{e}	11.82±0.07^{c}	12.58±0.19^{f}
Muktakashi	64.18±0.15^{f}	70.61±0.13^{h}	75.27±0.07^{g}	11.08±0.06^{e}	13.59±0.23^{cd}
Panchamukhi	66.94±0.08^{d}	74.36±0.08^{d}	78.96±0.13^{c}	12.02±0.11^{b}	13.98±0.15^{bc}
Garu	67.25±0.13^{c}	74.10±0.07^{b}	78.59±0.14^{d}	11.34±0.07^{d}	12.24±0.27^{f}
JCC37	77.24±0.21^{a}	83.33±0.22^{a}	86.99±0.05^{a}	9.75±0.12^{g}	14.25±0.41^{b}
JCC57	74.29±0.11^{b}	80.01±0.14^{b}	84.67±0.08^{b}	10.38±0.13^{f}	13.15±0.24^{e}
Rice	66.31±0.21^{e}	72.53±0.12^{g}	77.67±0.09^{f}	11.36±0.17^{d}	14.94±0.18^{a}
Maize	62.19±0.07^{g}	69.70±0.11^{i}	74.87±0.14^{h}	12.68±0.09^{a}	15.24±0.18^{a}
Potato	58.62±0.12^{h}	65.55±0.09^{j}	71.32±0.17^{i}	12.70±0.15^{a}	15.27±0.21^{a}

[1] Values reported as Mean ± S. D. of three replications

[2] Means followed by same small letter superscripts within a column are not significantly different (p>0.05)

Table 2.4 Pasting properties of the starch samples

Sample[1,2]	Pasting Temperature, °C	Peak Viscosity, cP	Hold Viscosity, cP	Final Viscosity, cP	Breakdown Viscosity, cP	Setback Viscosity, cP
Kani	88.3±1.02^{a}	2129±32.3^{i}	1575±15.6^{h}	2203±98.6^{f}	554±46.3^{i}	628±94.3^{g}
Ahina	87.20±0.21^{ab}	3379±22.2^{h}	1875±34.1^{g}	2871±16.4^{e}	1504±22.2^{h}	996±29.2^{d}
Muktakashi	87.00±0.67^{ab}	2175±10.4^{i}	1553±21.3^{h}	2034±59.6^{g}	622±31.1^{i}	481±34.4^{h}
Panchamukhi	84.1±1.12^{c}	4301±31.3^{f}	2114±18.6^{f}	2940±48.6^{e}	2187±46.3^{f}	826±24.3^{ef}
Garu	82.1±1.15^{d}	4736±45.6^{e}	2217±23.2^{e}	2982±103.2^{e}	2519±54.3^{d}	765±106.4^{f}
JCC37	83.30±1.08^{cd}	4868±31.2^{d}	2241±15.3^{e}	3160±29.6^{d}	2627±21.3^{c}	919±25.9^{de}
JCC57	86.10±0.34^{b}	5704±16.9^{b}	2680±11.7^{c}	3834±47.2^{c}	3024±17.6^{b}	1154±54.4^{c}
Rice	78.4±1.06^{e}	5130±36.4^{c}	2757±21.1^{b}	4741±69.4^{b}	2373±36.5^{e}	1984±82.2^{a}
Maize	78.1±0.75^{e}	3974±25.6^{g}	2331±26.5^{d}	3878±56.9^{c}	1661±52.6^{g}	1529±29.4^{b}
Potato	68.8±0.96^{f}	10571±29.2^{a}	6082±16.3^{a}	6380±156.3^{a}	4489±78.2^{a}	298±54.2^{i}

[1] Values reported as Mean ± S. D. of three replications

[2] Means followed by same small letter superscripts within a column are not significantly different (p>0.05)

which facilitated in reaching the gelatinization temperature at an early stage. Starch granules of *JCC37* being larger in size as compared to the other cultivars might not have absorbed moisture at the same rate unlike other cultivars, thereby showing closer values of pasting temperature and T_p. Pasting temperatures were also observed to be higher than rice, maize and potato starches investigated. The results corroborates with the findings of other authors.[39, 63] The pasting temperatures obtained in the present study were found to be closer to corn starches reported by others.[33, 64] The peak, hold, final, breakdown and setback viscosities of *Garu*, *JCC37* and *JCC57* were much higher compared to the viscosities of other cultivars (Fig. 2.7) particularly *Kani* and *Muktakashi*. These differences might be due to amylose content of the starches and the differences in variety of taro. The viscosities observed in the present study were much higher than those observed by Lu et al.[21] and were similar to those observed by Agama-Acevedo et al.[41] It was seen that for all the starches the final viscosity was lower than peak viscosity. These results support the observations made by Mepba et al.[25] and Agama-Acevedo et al.,[41] but were different than those observed by Nwokocha et al.[24] and Aprianita et al.[62] where final viscosity was found to be higher than peak viscosity. The viscosities of rice and maize starch were found to be closer to that of the taro starches particularly *Garu*, *JCC37*, *JCC57* and *Panchamukhi* starches. The final and setback viscosities of taro starches were lower than rice and maize starches, which makes them better suited for application requiring constant stirring during heating and cooling. It was further observed that the peak, hold and final viscosities of potato starch were much higher than the other starches studied. This might be due to the large size of the starch granules and higher swelling of the potato starch. The viscosity values of the taro starches measured by RVA were comparable to rice starches of Indian varieties investigated by Gani et al.[65] and starch from Chinese rice varieties[66] and were found to be lower compared to potato, arrowroot and canna starches.[67]

2.3.2.5 Freeze-thaw stability

Freeze-thaw stability of a starch gel is an important parameter in determining the industrial application of a starch.[68] The amount of water separated (% syneresis) from the starch gels of the seven taro cultivars and rice, amize and potato up to 5 freeze-thaw cycles is shown in Fig. 2.8. It was observed that percent syneresis gradually increased up to 4 freeze thaw cycle and then decreased after the 5th cycle for *Kani*.

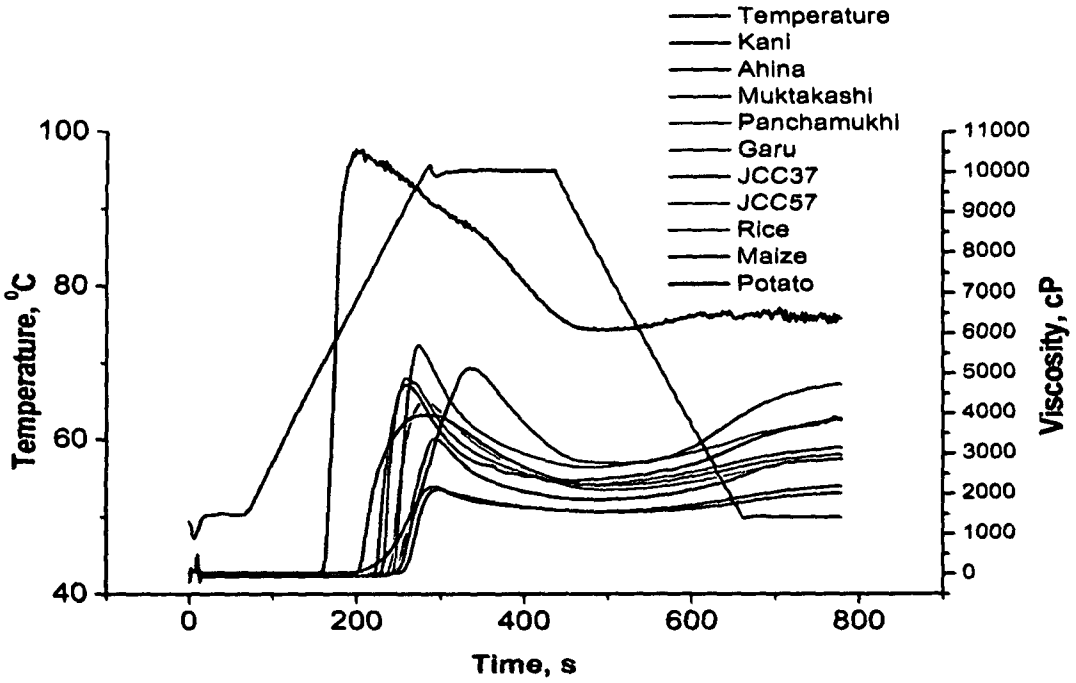


Fig. 2.7 Pasting profiles of starches from various taro cultivars, rice, maize and potato

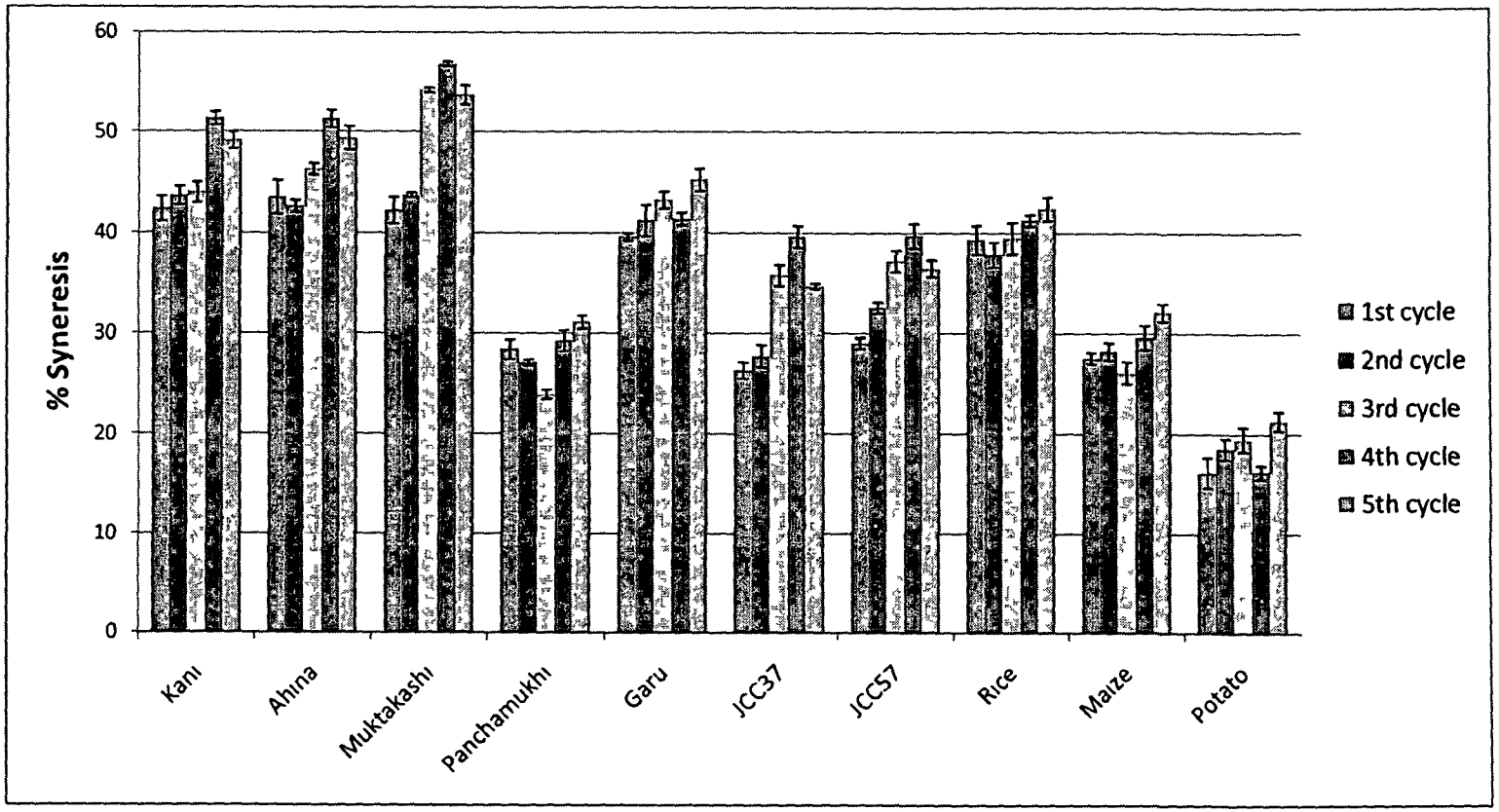


Fig. 2.8 Freeze-thaw stability of starch pastes from various taro cultivars, rice, maize and potato

Vertical error bars above the columns represent standard deviation (S D) of three replications

Ahina, *Muktakashi*, *JCC37* and *JCC57* starches. In case of *Panchamukhi* starch %syneresis decreased up to the 3rd cycle and then gradually increased till 5th cycle. Syneresis in *Garu* and potato starch pastes initially increased up to the 3rd cycle and then decreased in the fourth cycle and then again increased in the 5th cycle. For rice starch syneresis decreased in the second cycle and then gradually increased in the subsequent cycles till the 5th cycle. Syneresis in maize starch paste increased in the second cycle, then decreased in the 3rd cycle and again an increase in the syneresis was observed till the 5th cycle. This decrease in syneresis was likely from the changes in gel structure to sponge-like structure. This structure easily reabsorbed most of the extruded water if the water separation was too slow.[69] Similar observations for amaranth, maize, cowpea, chickpea and rice starch gel have been reported.[64, 68, 70, 71] The %syneresis was highest for *Muktakashi*, whereas it was lowest for *Panchamukhi*. Although, all the starch gels exhibited poor freeze-thaw stability, *Panchamukhi*, *JCC37* and *JCC57* starch pastes were more stable to freeze-thaw compared to *Kani*, *Ahina*, *Muktakashi* and *Garu* starch pastes. Freeze-thaw stability of *Panchamukhi*, *JCC37* and *JCC57* was found to be better compared to rice starch and was comparable to maize starch. Potato starch showed best freeze-thaw stability among the starches, which might be due to the higher swelling of potato starch granules and presence of phosphate groups in the amylopectin molecules. Among the taro cultivars *Panchamukhi* starch showed least syneresis and best freeze-thaw stability. Modification of the taro starches would be required if they are to be used in foods meant for refrigeration.

2.3.3 Textural and colour characteristics of taro starches

2.3.3.1 Texture properties

The texture parameters of the starch pastes are shown in Table 2.5. The firmness of the starch pastes from the taro cultivars varied from 11.17 to 11.93 g. The firmness of taro starch pastes did not differ significantly from each other. The firmness of the taro starch pastes was also not significantly different from the starch pastes of rice and maize with firmness of 11.38 and 11.71 g respectively. The firmness of potato starch paste was considerably higher than the other starch pastes. This might be due to higher swelling of potato starch granules. Similar trend was observed with consistency and cohesiveness of the starch pastes, where potato starch paste had the highest values of consistency and cohesiveness compared to the starch pastes of

various taro cultivars and that of rice and maize. This might be due to larger granule size and higher viscosity of the potato starch as observed in SEM and pasting property analysis respectively. The indexes of viscosity of all the starch pastes were low signifying that the viscosities of the starch pastes change rapidly with change in temperature. The texture of the starch pastes are an important parameter in determining the application of a starch. It was observed that the firmness of the starch pastes were lower which will give a smooth texture to the products in which the taro starches will be incorporated.

2.3.3.2 Colour properties

The L*, a* and b* values of the starch pastes are shown in Table 2.5. It was observed that *Panchamukhi* and *Garu* starch pastes were significantly lighter than starch pastes of other taro cultivars. This might be due to the difference in composition and sizes of the starch granules. *Panchamukhi* starch had lesser impurities compared to other starches whereas the granule size of *Garu* and *JCC37* starches were larger and the lightness of the pastes are related to granule size and swelling power of the starches.[11, 24] Potato starch paste exhibited highest lightness as the granules of potato starch are significantly larger than taro, rice or maize starches and the swelling power of the granules was also very high. The a* and b* values of all the starch pastes were not significantly different from each other and the values were positive, indicating that the starch pastes were slightly reddish and yellowish in nature. Starch paste colour is an important property of starch for industrial application and light coloured pastes are preferred.[72] The colours of the taro starch pastes were found to be comparable to the colour of rice and maize starch pastes. The colour quality of the starch pastes can be improved by application of certain chemicals which affects other functional properties of the starch.[73] However, in certain food applications where light colour is not desirable like ketchups or sauces, native taro starches might find useful applications.

Table 2.5 Texture and colour parameters of starch pastes

Sample[1, 2]	Texture properties				Colour parameters		
	Firmness (g)	Consistency (g.s)	Cohesiveness (g)	Index of viscosity (g.s)	L*	a*	b*
Kani	11.93±0.11^{b}	194.07±1.21^{b}	7.15±0.12^{e}	0.87±0.20^{b}	22.29±2.58^{def}	1.34±0.52^{abc}	3.83±0.85^{bc}
Ahina	11.57±0.56^{bc}	189.67±1.29^{cde}	6.76±0.34^{f}	0.84±0.37^{b}	17.23±1.56^{f}	1.69±0.58^{ab}	2.03±0.54^{d}
Muktakashi	11.17±0.24^{c}	188.87±2.21^{de}	7.88±0.12^{c}	1.04±0.21^{ab}	23.17±2.34^{de}	1.13±0.12^{abc}	3.97±0.23^{b}
Panchamukhi	11.37±0.15^{bc}	188.36±1.04^{e}	7.66±0.18^{cd}	1.02±0.15^{ab}	26.31±2.31^{cd}	1.84±0.96^{a}	3.56±0.24^{bc}
Garu	11.93±0.56^{b}	195.33±0.96^{b}	7.42±0.21^{de}	0.92±0.35^{ab}	27.04±3.21^{cd}	1.69±0.12^{ab}	5.02±0.54^{a}
JCC37	11.64±0.28^{bc}	190.61±2.12^{cde}	7.57±0.26^{cd}	0.98±0.25^{ab}	22.19±3.47^{def}	1.34±0.47^{abc}	3.83±0.71^{bc}
JCC57	11.56±0.09^{bc}	189.93±1.08^{cde}	8.46±0.17^{b}	1.25±0.19^{a}	18.84±2.54^{ef}	1.74±0.36^{ab}	4.32±0.11^{ab}
Rice	11.38±0.11^{bc}	191.59±0.58^{cd}	8.54±0.13^{b}	1.24±0.26^{a}	32.91±1.88^{b}	0.79±0.41^{c}	2.95±0.69^{cd}
Maize	11.71±0.57^{bc}	188.65±1.12^{e}	7.89±0.21^{c}	1.10±0.26^{ab}	29.51±3.14^{bc}	0.93±0.25^{bc}	4.25±0.37^{ab}
Potato	13.49±0.23^{a}	220.44±1.16^{a}	10.32±0.09^{a}	0.99±0.12^{ab}	44.49±5.02^{a}	1.09±0.36^{abc}	5.00±0.81^{a}

[1] Values reported as Mean ± S. D. of three replications
[2] Means followed by same small letter superscripts within a column are not significantly different (p>0.05)

2.3.3.3 Colour of starch (dry powder)

Colour of dry starch is an important characteristic for determination of its quality. The colour parameters of the starch (dry powder) samples are presented in Table 2.6. The isolated starches had high L* values and lower a* and b* values which confirms the high purity of the starches. The L* value of *Panchamukhi* starch (95.88) was the highest and *Ahina* starch (92.29) recorded the lowest. Pérez Sira and Amaiz[74]

Table 2.6 Colour parameters of starch (dry powder)

Sample[1, 2]	L*	a*	b*
Kani	94.38±0.54^{bc}	1.96±0.38^{a}	4.32±0.14^{bc}
Ahina	92.29±0.64^{d}	1.86±0.28^{a}	4.17±0.21^{c}
Muktakashi	92.41±0.51^{d}	1.95±0.54^{a}	4.58±0.15^{ab}
Panchamukhi	95.88±0.91^{b}	1.54±0.42^{a}	2.27±0.24^{g}
Garu	93.26±0.62^{cd}	1.85±0.45^{a}	4.79±0.12^{a}
JCC37	93.27±1.07^{cd}	1.67±0.23^{a}	2.95±0.09^{de}
JCC57	92.89±0.85^{cd}	1.81±0.27^{a}	3.22±0.18^{d}
Rice	97.64±1.56^{a}	1.67±0.43^{a}	2.67±0.19^{ef}
Maize	98.26±1.04^{a}	1.24±0.26^{a}	2.43±0.31^{fg}
Potato	95.19±0.25^{b}	1.71±0.77^{a}	3.13±0.17^{d}

[1]Values reported as Mean ± S. D. of three replications
[2]Means followed by same small letter superscripts within a column are not significantly different ($p>0.05$)

estimated that a value greater than 90 gives a satisfactory lightness for the purity of starch. Present results revealed that a* and b* values of the starches varied from 1.54 to 1.95 and 2.27 to 4.79 respectively for the various taro cultivars. Significant

differences were not observed in the a* values. The b* values of *Kani*, *Ahina*, *Muktakashi* and *Garu* starches were higher compared to *Panchamukhi*, *JCC37* and *JCC57* and the differences were significant indicating that *Kani*, *Ahina*, *Muktakashi* and *Garu* starches were slightly yellowish. The difference in the colour parameters among the taro starches might be attributed to the difference in the chemical composition of the starches. The colour of the dry starches of the taro cultivars were less lighter than the dry starches from rice and maize but were similar to that of potato starch. The high L* values combined with low a* and b* values of the taro starches evinced that it could be conveniently used in products requiring clear and uniform colour.

2.4 Conclusions

The study revealed that the physicochemical, functional, textural and colour properties of starches of various taro cultivars of Assam from North-East India are different from taro varieties available in other regions of the world. The properties of taro starch were found to be closer to rice and maize starch in many respects, evincing its potential as an ingredient in food processing and other industries. The high gelatinization and pasting temperatures coupled with low viscosity make them suitable for applications in food products which are subjected to heating at higher temperature, wherein change in viscosity is not desirable during heating and cooling. However, the poor freeze-thaw stability of these starches makes them inadequate as thickener and stabilizer for refigerated and frozen foods. It addition it would be prudent to mention that starch from *Panchamukhi* taro has better functional properties like better clarity and stability, pasting properties, and freeze-thaw stability as compared to the starches from the other taro cultivars investigated.

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Chapter 3

Effect of Incorporation of Taro and Maize Starch on Various Quality Parameters of Tomato Ketchup

3.1 Introduction

Starch is the most widely used thickening and gelling agent in the food industry as it can provide wide variety of texture and mouthful sensations in the food product. Starch is an important ingredient for the preparation of foodstuffs such as sauces, soups, and many other processed foods. Properties of starch in a food system depend on the botanical source of the starch, whether the starch has been modified or not (modified or native starch), the concentration of starch, the cooking procedure (temperature, pH, heating time, shearing time and intensity, among others) and the presence of other ingredients or additives.[1]

Industrial applications of native starches are limited their thermal and shearing stability is poor. Native starches tend to retrograde during cooling or/and freezing, which decreases the food product. In current times there is a tendency towards natural and clean-label food products which has promoted the use of native starches.[1] Tomatoes are one of the most important vegetables and are widely grown in tropical and sub-tropical regions of the world. It is mainly marketed as a processed product, in the form of pastes, concentrates ketchup, salsa, etc.[2, 3] The term ketchup is used to describe a number of different food products, which consist of various pulps, strained and seasoned fruits. Among the different ketchups tomato ketchup is the most popular condiment.[4] It is a heterogeneous, spiced product, produced basically from either cold or hot extracted tomatoes; or directly from concentrates, purees and tomato paste. Consistency/viscosity of ketchup is an important attribute from the manufacturing and consumer viewpoints.[5-7]

Thick products are preferred by consumers, therefore, tomato ketchup is now prepared with the addition of thickeners[8] like native starches from potato or corn,[9] modified starches,[10] various hydrocolloids, e.g. carboxymethylcellulose, xanthan gum, locust been gum, guar gum, and traganth gum are now increasingly used.[2] Tomato varieties with less pectin might result in reduced consistency, and other factors such as enzymatic degradations, pectin/ protein interaction, pulp content, homogenization process and concentration may also affect the consistency of tomato products.[6, 11-13] However, the consistency can be maintained by adding polysaccharides such as starch, gum, etc.[14]

Industrial starch is produced from a variety of agricultural products, from cereals to roots and tubers. Maize supplies more than 80% of global starch needs.[15, 16] But when functionality (i.e., the specific quality characteristic of starch) is important,

then other sources of starch might be used.[15] No major studies on incorporation of taro starch on textural, and colour properties of tomato ketchup have been carried out. Therefore, the present study was carried out with the objective to study the effect of incorporation of taro starch and maize starch on sensory properties of tomato ketchup, so that the industrial application of taro starch might be established.

3.2 Materials and methods

3.2.1 Materials

Local cultivar of taro known as *Panchamukhi* taro (*Colocasia esculenta* var. *antiquorum*) was collected from an agricultural farm near Tezpur University, Assam, India. *Panchamukhi* taro was selected because the functional properties of *Panchamukhi* taro like solubility, freeze-thaw stability etc, were found to be better compared to the other cultivars of taro. Starch was isolated from the tubers by method of Benesi et al.[17] Taro tubers were washed under tap water and were hand peeled and cut into approximately 1 cm^3 using stainless steel knife. The cubes were ground using a commercial blender (Philips HL 1632, India) for two minutes. The resulting slurry was suspended in 10 times its volume of distilled water and stirred for 5 min. The suspension was filtered through double fold muslin cloth and the filtrate was kept for sedimentation for 6 h. The supernatant was discarded and the sediment was washed with distilled water for two times. The final sediment was dried at 45 °C for 24 h in drying oven. The dried starch was ground with mortar and pestle and kept in air tight plastic containers for further analysis. Maize starch was purchased from HiMedia Laboratory, India for comparison.

Tomatoes (*Lycopersicon esculentum*) of even ripeness and colour were purchased from local market. The initial total soluble solids (TSS) of the tomato pulp was 3.5±0.29 °Brix. All other ingredient for preparation including spices was also purchased from local market of Tezpur University.

3.2.2 Preparation of tomato ketchup

Ketchup was prepared as per the method described by Srivastava and Kumar.[18] In brief, 500 g pulp was prepared from tomatoes by hot pulping method by heating the pieces of tomato at 70°C for 2-3 min. Sugar (7.5 % w/w of initial weight of pulp) and salt (1% w/w of initial weight of pulp) were added to the pulp and the

pulp was cooked at 100±2°C till the TSS reached 25° Brix. Starch from taro and maize were added 10 min before the end of cooking at a rate of 1 and 2% by weight of the amount of initial pulp taken. The pulp was continuously stirred with stainless steel ladle during cooking. To prepare the control ketchup sample the pulp was continuously stirred till the end of cooking without adding any starch. Acetic acid (5 ml per 1 kg of tomato pulp) was added to the ketchup at the end of cooking. The total cooking time was approximately 40 min and the final volume of the ketchup samples obtained were approximately 220 g from 500 g of pulp.

3.2.3 Serum loss

Serum loss from the tomato ketchups prepared was measured according to the method described by Gujral et al.[19] Tomato ketchup (20 g) was taken in a centrifuge tube and then centrifuged at 5000 rpm for 10 min. The supernatant was discarded and the remaining ketchup was weighed.

% Serum loss = (Weight of serum removed/ weight of ketchup taken) × 100

3.2.4 Textural properties of ketchup

The textural properties of the ketchup samples were analysed by a texture analyser TA.HDplus (Stable Micro Systems, UK). Back extrusion was done to determine textural properties such as firmness, consistency and cohesiveness with a back extrusion cell using a long probe adaptor along with a 35 mm compression disc. For measuring the textural properties, ketchup samples were filled into the glass beakers provided by the manufacturer up to a height of 5 cm. The probe was allowed to penetrate 20 mm from the surface of the sample at a speed of 1 mm / s. Firmness, cohesiveness, consistency and index of viscosity were calculated from the graphs using the software 'Exponent Lite 32'.

3.2.5 Colour parameters of ketchup

The colour parameters of the ketchup samples were analysed by a colorimeter (Ultrascan VIS, Hunterlab, USA). L, a* and b* values were noted. L is for lightness, a* for redness and b* for yellowness.

3.2.6 Sensory evaluation

Sensory evaluation of ketchup samples were conducted using a 9-point hedonic scale. The colour was judged visually. The smoothness was judged through the sense of touch. The flavour was judged by the sense of smell and taste. The mouth feel was judged by the tactile character and eating quality. The samples were presented individually to 15 semi-trained panellists. Water was used for mouth rinsing before and after testing of each sample.

3.2.7 Statistical analysis

The data were subjected to single factor analysis of variance (ANOVA) using 'Data Analysis Tool' of 'Microsoft Excel'. Fisher's 'Least Significant Difference (LSD)' method was used to determine the statistical difference between the results obtained.

3.3 Results and discussion

3.3.1 Effect of starch concentration and storage time on serum loss from tomato ketchup

The stability of tomato ketchup during storage is indicated by the amount of serum separated from the ketchup after storage. Lower serum loss i.e. ability to hold more water signifies better stability of ketchup during storage. Ketchups prepared by addition of starch from *Panchamukhi* taro and maize at 1 and 2% concentrations significantly lowered the serum loss as compared to the control sample throughout the storage period (Fig. 3.1). The serum loss increased with increase in storage period for all the ketchup samples. Significant differences were observed in the values of serum loss in the control sample after 15 and 30 days of storage. Ketchups prepared using 1% *Panchamukhi* taro starch had higher serum loss after 15 and 30 days than ketchup prepared by incorporating 1% maize starch, although significant differences were not observed on 0 day i.e. before storage. Stability of ketchups containing 2% starches were found to be better than ketchups containing 1% starches. The stability of ketchup sample prepared using 2% *Panchamukhi* starch was found to be better compared to that prepared by using 2% maize starch, as the difference in serum loss in ketchup containing 2% *Panchamukhi* starch at 0, 15 and 30 days after storage were very less and statistically non-significant.

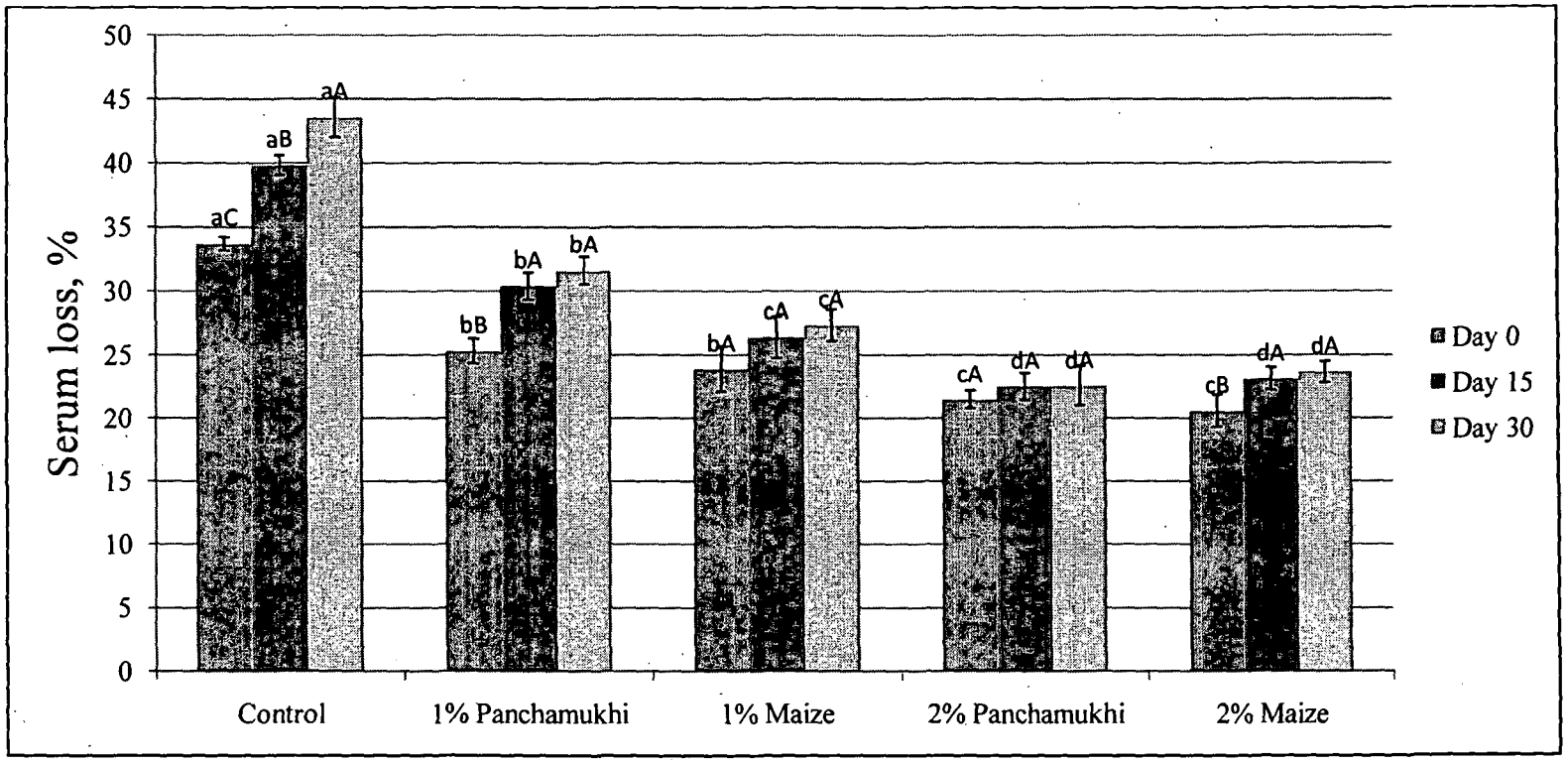

Fig. 3.1 Serum loss during storage from the ketchup samples prepared by incorporation of Panchamukhi and maize starch at different concentrations

Vertical error bars above the columns represent standard deviation (S. D.) of three replications

Similar small letters above the bars denote values are not significantly different ($p>0.05$) between different ketchup samples for same storage period

Similar capital letters above the bars denote values are not significantly different ($p>0.05$) between different storage period for the same ketchup sample

3.3.2 Effect of starch concentration and storage time on texture properties of tomato ketchup

The maximum force experienced by the probe during penetration is taken as the measurement of firmness of the sample and is used as an index of textural quality.[20] The firmness of the different ketchup samples prepared by incorporating *Panchamukhi* and maize starch is given in Fig. 3.2. The firmness of the ketchups significantly increased due to incorporation of starch which acts as a thickening agent. The firmness also increased as the concentration of the starch in the ketchup samples were increased from 1 to 2%. The firmness of the ketchups containing maize starch was much higher compared to that of *Panchamukhi* starch at same concentration. This might be due to the higher final viscosity of maize starch as observed in RVA profile. Not much difference was observed in the firmness of the ketchups containing starch as when compared to the control sample. The firmness of the control sample decreased significantly after 15 days of storage, which might be due to loss of serum from the ketchup as in the absence of any binding agent (stabilizer or thickener) the solids in the control sample were not able to hold the water and it separated out.[19] No significant differences were observed up to 30 days of storage with the ketchups prepared with starch, indicating that both *Panchamukhi* and maize starch can be used as a thickener in ketchups.

Consistency is another important parameter in determining the quality of ketchup. If the consistency is very high the ketchup might not flow properly from the container, and if it is very low ketchup might not give the desired mouth feel. Fig.3.3 shows that consistencies of the ketchups prepared using starch were significantly higher than that of control. Consistency increased with increase in concentration for ketchups prepared using *Panchamukhi* starch, but for maize starch both the concentration gave similar consistencies and was close to that of ketchup containing 2% *Panchamukhi* starch. The consistency of the control ketchup decreased significantly after 15 days, but significant differences were not observed in the other ketchup samples.

The cohesiveness and viscosity of the ketchup samples also increased due to incorporation of starch indicating that more force is required to overcome the

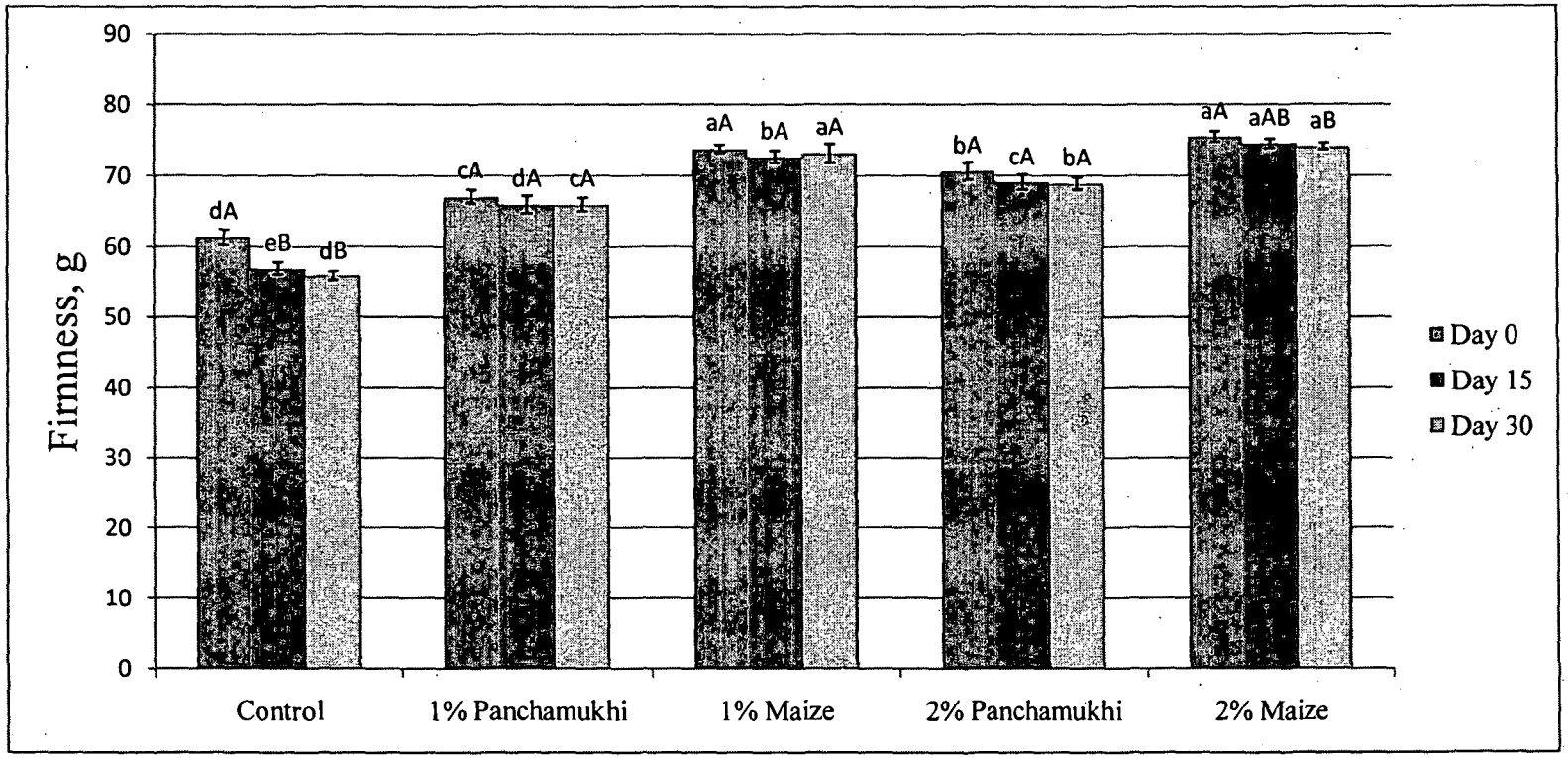

Fig. 3.2 Variation in firmness of different ketchup samples with days of storage

Vertical error bars above the columns represent standard deviation (S. D.) of three replications

Similar small letters above the bars denote values are not significantly different ($p>0.05$) between different ketchup samples for same storage period

Similar capital letters above the bars denote values are not significantly different ($p>0.05$) between different storage period for the same ketchup sample

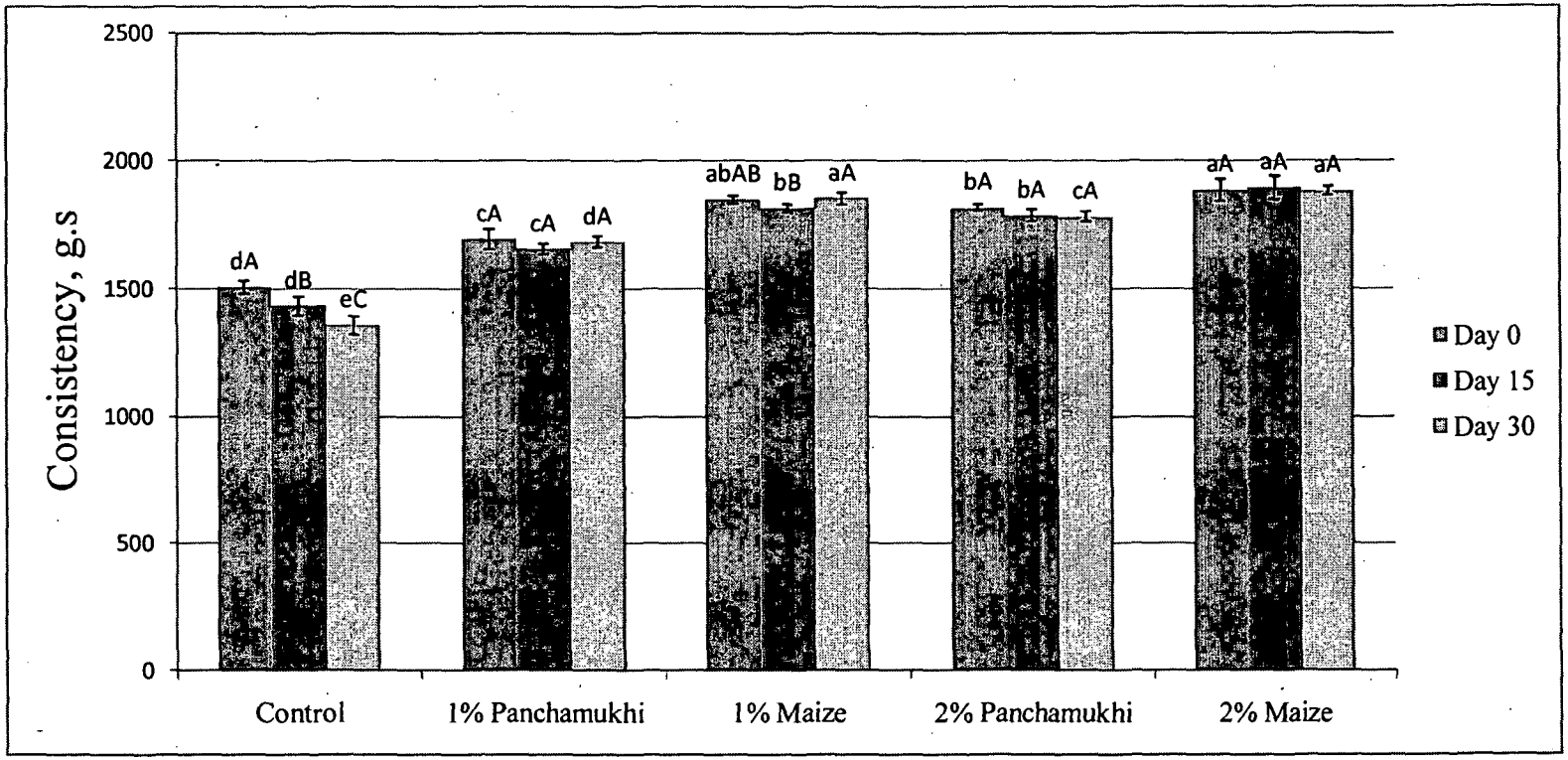

Fig. 3.3 Variation in consistency of different ketchup samples with days of storage

Vertical error bars above the columns represent standard deviation (S. D.) of three replications

Similar small letters above the bars denote values are not significantly different ($p>0.05$) between different ketchup samples for same storage period

Similar capital letters above the bars denote values are not significantly different ($p>0.05$) between different storage period for the same ketchup sample

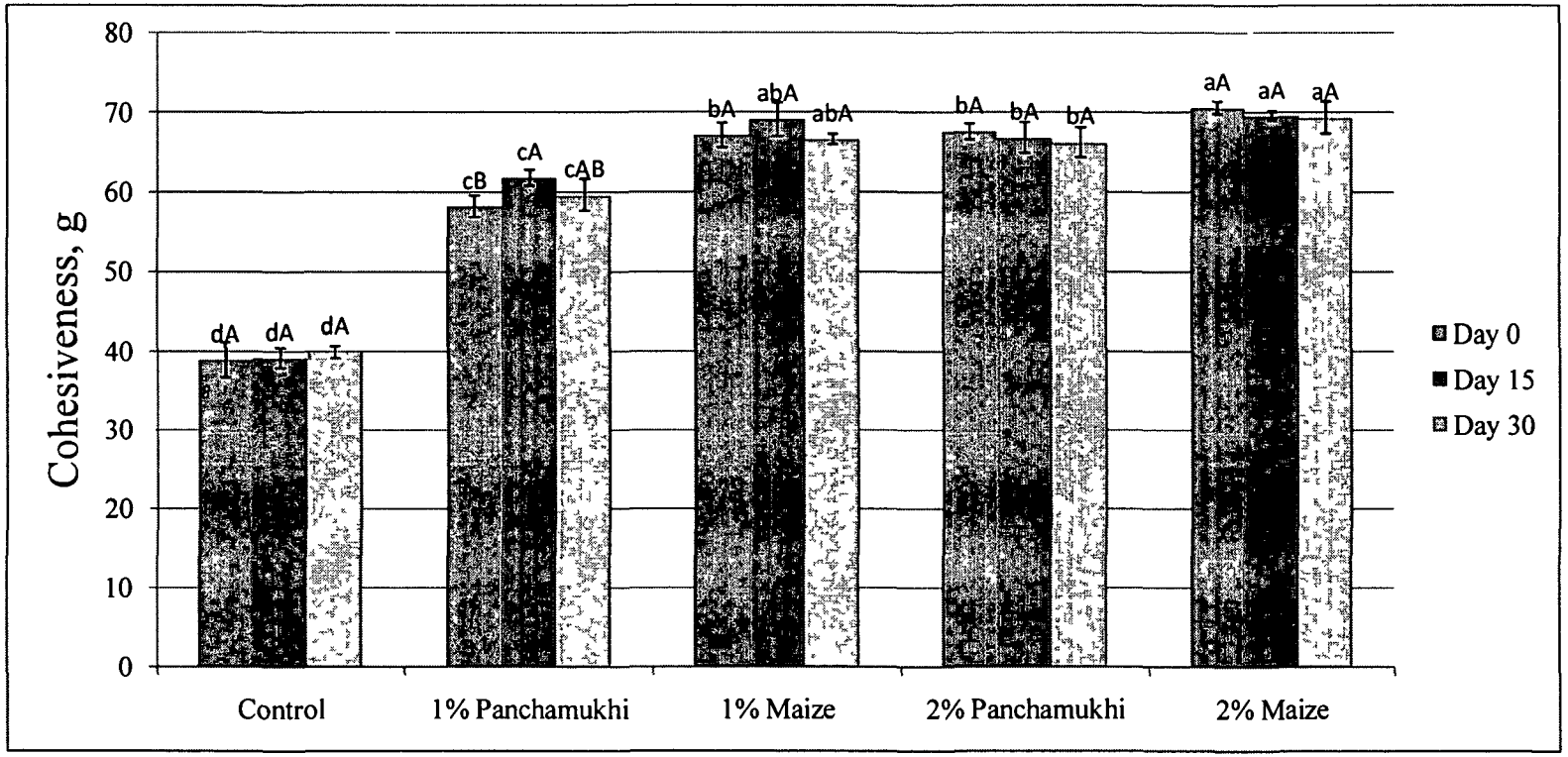

Fig. 3.4 Variation in cohesiveness of different ketchup samples with days of storage

Vertical error bars above the columns represent standard deviation (S D) of three replications

Similar small letters above the bars denote values are not significantly different (p>0 05) between different ketchup samples for same storage period

Similar capital letters above the bars denote values are not significantly different (p>0 05) between different storage period for the same ketchup sample

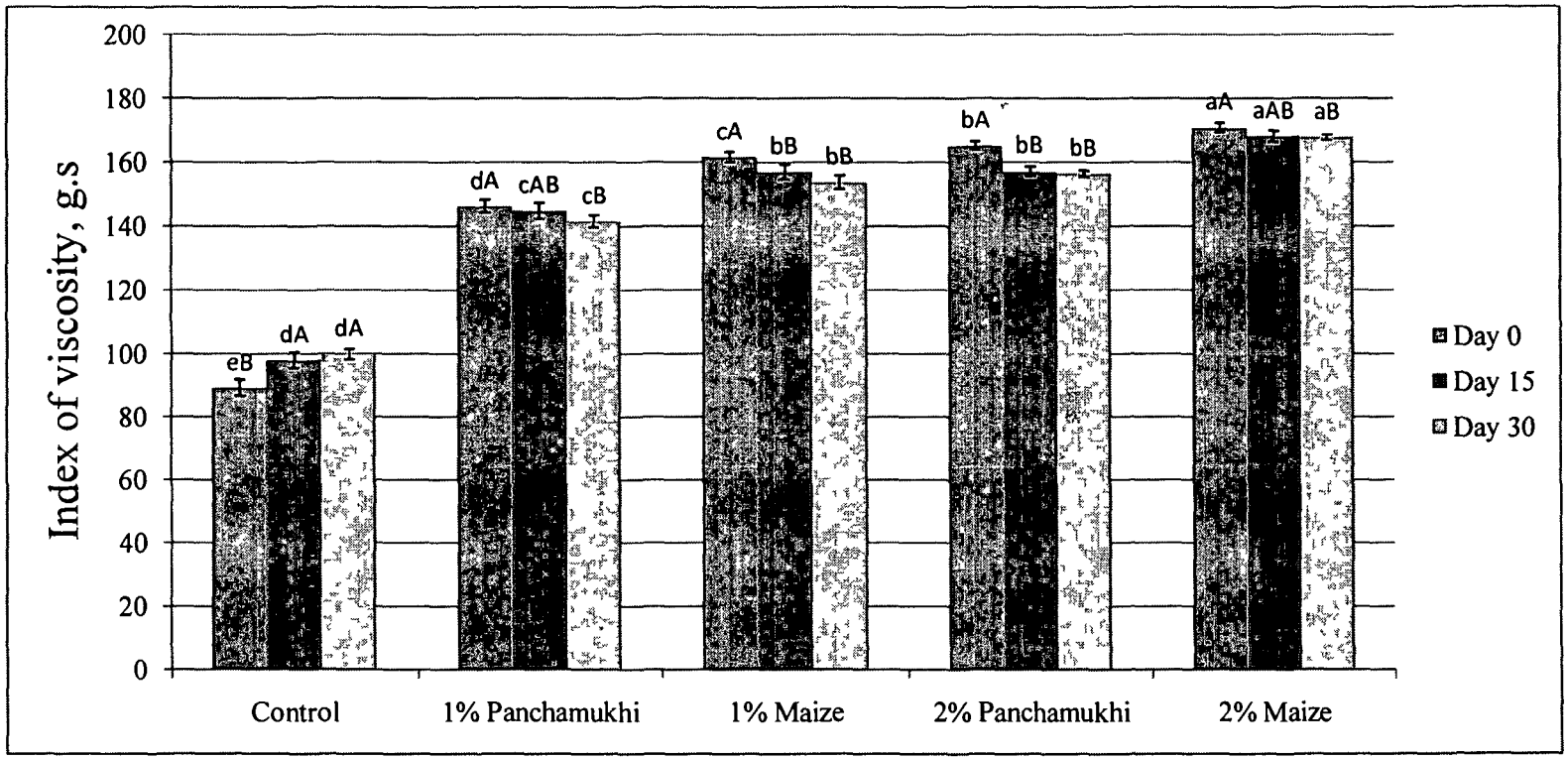

Fig. 3.5 Variation in index of viscosity of different ketchup samples with days of storage

Vertical error bars above the columns represent standard deviation (S D) of three replications

Similar small letters above the bars denote values are not significantly different (p>0 05) between different ketchup samples for same storage period

Similar capital letters above the bars denote values are not significantly different (p>0 05) between different storage period for the same ketchup sample

resistance during pulling out the sample.[21] The variation in cohesiveness and index of viscosity with storage time for the different ketchup samples is given in Fig. 3.4 and Fig. 3.5 respectively. Cohesiveness values of the ketchup samples prepared by incorporation of the starches were significantly higher compared to the control sample for all storage periods. The cohesiveness of the of the ketchups containing 2% starch were higher than that containing 1% starch for a particular type of starch, although the differences between ketchups containing 1% and 2% maize starch were less as compared to between 1% and 2% *Panchamukhi* starch incorporated ketchups. Further it was observed that cohesiveness of ketchup containing 2% *Panchamukhi* starch was not significantly different form that containing 1% and 2% maize starch. The results suggest that increasing the concentration of *Panchamukhi* starch is more effective in controlling the texture of tomato ketchup compared to maize starch. No significant differences were observed in the cohesiveness of a particular ketchup sample during storage. Significant increase in index of viscosity was observed for the control sample after 15 days. This might be attributed to higher serum loss from the control sample and aggregation of the solids which resulted in higher force during back extrusion. The index of viscosities of ketchup samples containing 2% starches were more compared to those containing 1% starches, which shows that change in viscosity with temperature is more in ketchups containing 2% starches.

3.3.3 Effect of starch concentration and storage time on colour of tomato ketchup

The L*, a* and b* values of the ketchups prepared using *Panchamukhi* and miaze starch were not found to be significantly different from the control ketchup (Table 3.1). Although, L*, a* and b* values increased when both the starches were incorporated at 2% concentration. Statistically significant differences were not observed in the ketchup samples up to 30 days of storage. The results revealed that addition of starch up to 2% concentration might not affect the colour of tomato ketchups.

3.3.4 Effect of starch concentration on sensory quality of tomato ketchup

Sensory scores (Table 3.2) revealed that statistically significant differences from control were not present in colour, and flavour of tomato ketchup prepared by incorporation of *Panchamukhi* taro or maize starch. Ketchup containing 1%

Table 3.1 Change in colour of ketchups incorporated with *Panchamukhi* taro and maize starch during storage

Ketchup Sample[1, 2, 3]	L*			a*			b*		
	0 Day	15 Day	30 Day	0 Day	15 Day	30 Day	0 Day	15 Day	30 Day
Control	31.63±1.26^{aA}	31.64±1.56^{aA}	31.58±0.99^{aA}	12.68±0.96^{aA}	12.84±1.06^{aA}	12.90±0.98^{aA}	9.95±0.59^{aA}	9.65±0.57^{aA}	9.72±0.62^{aA}
1% *Panchamukhi*	31.91±1.23^{aA}	31.82±1.12^{aA}	31.88±1.07^{aA}	12.50±0.59^{aA}	12.28±0.78^{aA}	12.30±0.54^{aA}	9.85±0.87^{aA}	9.56±0.97^{aA}	9.03±0.49^{aA}
2% *Panchamukhi*	32.79±0.69^{aA}	32.87±0.98^{aA}	32.80±0.84^{aA}	12.95±0.96^{aA}	13.09±.83^{aA}	13.74±1.12^{aA}	10.02±1.02^{aA}	10.21±0.67^{aA}	10.12±0.96^{aA}
1% Maize	31.68±0.98^{aA}	31.64±0.59^{aA}	31.54±0.67^{aA}	12.09±1.02^{aA}	12.47±0.42^{aA}	12.53±0.58^{aA}	9.57±0.49^{aA}	9.52±0.59^{aA}	9.21±0.59^{aA}
2% Maize	32.72±1.09^{aA}	32.51±1.42^{aA}	32.30±1.16^{aA}	13.18±0.77^{aA}	13.16±0.69^{aA}	13.25±1.07^{aA}	10.11±0.67^{aA}	10.04±1.02^{aA}	9.71±0.52^{aA}

[1]Values reported as Mean ± S. D. of three replications
[2]Means followed by same superscript capital letters within a row are not significantly different for a particular colour parameter ($p>0.05$)
[3]Means followed by same superscript small letters within a column are not significantly different ($p>0.05$)

Table 3.2 Sensory scores of ketchups prepared using starch

Ketchup Samples[1,2]	Colour	Smoothness	Flavor	Mouthfeel
Control (Without Starch)	7.43±0.16[a]	7.24±0.21[b]	7.20±1.05[a]	7.28±0.06[d]
1% *Panchamukhi*	7.35±0.13[a]	7.59± 0.17[a]	7.15±0.60[a]	8.34±0.12[a]
1% Maize	7.31±0.10[a]	7.54±.19[a]	7.19±01.25[a]	8.29±0.13[a]
2% *Panchamukhi*	7.39±0.17[a]	7.14±0.15[b]	7.09±0.09[a]	8.26±0.[15ab]
2% Maize	7.11±0.09[b]	7.20±0.08[b]	7.12±0.29[a]	7.75±0.18[c]

[1]Scores reported as Mean ± S. D. of 15 semi-trained panelists
[2]Means followed by same superscript capital letters within a column are not significantly different ($p<0.05$)

Panchamukhi taro and maize starch were smoother compared to control or ketchup containing 2% starch from *Panchamukhi* and maize. The scores for mouthfeel of tomato kethchups containing 1 and 2% *Panchamukhi* taro starch and 1% maize starch were significantly higher compared to control sample and ketchup prepared using 2% maize starch and the differences were statistically significant. Ketchup containing 2% maize starch was felt more firm by the panellists and was not liked in terms of mouthfeel. *Panchamukhi* starch might be used in preparation of tomato ketchup for improving the texture and mouthfeel without affecting the acceptability.

3.4 Conclusions

Incorporation of *Panchamukhi* taro and maize starch as thickeners in preparation of tomato ketchup significantly reduced the serum loss compared to control sample, thereby increasing the stability of the tomato ketchups. Texture, colour and sensory evaluation of the ketchups incorporated by *Panchamukhi* taro and maize starch showed significant improvement in the texture properties like firmness and consistency without affecting the colour. The ketchup containing 2% *Panchamukhi* taro starch was found to be more acceptable to the consumers than the other ketchup samples indicating that *Panchamukhi taro* starch has potential for other food and non-food applications also.

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Chapter 4

Optimization of Starch Isolation from Taro Using Enzymes and Comparison of Properties of Starches Isolated by Enzymatic and Conventional Methods

4.1 Introduction

The starch granules present in the roots and tubers are embedded in cellulosic fibers and held together by pectin substrates.[1] The high water content and other morphological similarities of tuber crops require a familiar technological process of starch extraction from these crops.[2] The starch extraction process from roots and tubers involves of grating the raw material, in order to break vegetal cells and release the starch. This step is followed by passing the fiber through sieves of different mesh sizes and subsequent slurry concentration by decantation or centrifugation.[3] The success of starch extraction from tubers depends on complete rupture of the cell walls and thereby releasing the starch granules.[4]

The cell walls of raw taro corms contain hemicelluloses like xylan, though it is in small amounts, apart from cellulose,[5, 6] but helps in maintaining the rigidity of the cell walls. Breakdown of both cellulose and hemicelluloses are necessary for recovery of intracellular materials like starch. Enzymatic methods have been used by several researchers to increase the recovery of starch from roots and tubers.[7-11] Most of the methods relied on use of cellulases and pectinases, but till now no investigation on the use of xylanase has been reported. Therefore the present study was taken up to investigate the effect of cellulase and xylanase on recovery of starch from taro corms and optimize the enzymatic starch isolation process, and to compare the functional properties of starch extracted by enzymatic method with that of conventional method.

4.2 Materials and methods

4.2.1 Starch isolation using enzymes

Taro tubers locally known as *Panchamukhi* (*Colocasia esculenta* var. *antiquorum*) was collected from an agricultural farm near Tezpur University, Assam, India. Tubers were washed under tap water, peeled and cut into cubes of approximately 1 cm. Cubes (100 g) were weighed and ground using a laboratory blender (Philips HL 1632, India) for 1 min 30 s. The slurry was mixed with 100 ml distilled water and transferred to a 250 ml beaker. The slurry was subjected to enzymatic treatment using cellulase from *Aspergillus niger* (Sigma-Aldrich, 0.3 U/mg) and xylanase from *Thermomyces lanuginosus* (Sigma-Aldrich, 2500 U/g) and their combination. The slurry was incubated with different concentrations of cellulase and xylanase for varying time at different temperatures as per the experimental design in incubator shaker (CERTOMAT® IS, Sartorius Stedim Biotech, Goettingen,

Germany) at 150 rpm. After incubation the suspension was filtered through double fold cheese cloth and the filtrate was centrifuged at 3000 × g for 10 min. The supernatant was discarded and sediment was washed twice with distilled water. The final sediment was dried at 45°C for 24 h in hot air drying oven. The dried starch was ground and passed through 100 mesh sieve and kept in air tight plastic containers for further analysis. For conventional method the incubation step with enzymes was omitted. Starch slurry after grinding was mixed with 100 ml distilled water and was filtered through double fold cheese cloth and the filtrate was centrifuged at 3000 × g for 10 min. The rest of the procedure was similar to the enzymatic process.

Moisture and starch contents of the dried samples were determined to calculate the yield of pure starch. Moisture content was determined by hot air oven method[12] and starch content was determined by acid hydrolysis method using perchloric acid which hydrolysed the starch to glucose and dehydrated it to hydroxylmethyl furfural which was then measured by anthrone reagent.[13] The starch content of the taro tubers were 21.96±0.42 g (n=5) per 100 g fresh tuber (21.96 % wb).

Yield was obtained by calculating the amount of pure starch (db) recovered from 100 g of fresh taro sample.

4.2.2 Experimental design

A central composite rotatable design (CCRD) with four numerical factors was employed to design the experiments. The numerical factors were cellulase concentration (C), xylanase concentration (X), temperature of incubation (T) and incubation time (t). The cellulase and xylanase concentration were varied from 0 to 400 U/ 100 g taro tuber, incubation time was varied from 1 to 5 h and temperature was varied from 30°C to 50°C. A total of 30 experiments were performed (Table 4.1). Six replicates at the centre points of the design were performed to allow the estimation of pure error. All experiments were carried out in a randomized order to minimize the effect of external factors.[14]

4.2.3 Properties of starches isolated by enzymatic and conventional methods

After optimization of the enzymatic method, starch was isolated from taro tubers using the optimized condition. The functional properties of the starch isolated

Table 4.1 Starch yield for different combinations of experimental conditions

Experiment No.	Time (t), h	Temp (T), °C	Cellulase (C), U/100g tuber	Xylanase (X), U/100g tuber	Yield,%
1	1(-2)[1]	40(0)	200(0)	200(0)	14.46
2	2(-1)	35(-1)	100(-1)	100(-1)	13.56
3	2(-1)	35(-1)	100(-1)	300(1)	15.67
4	2(-1)	35(-1)	300(1)	100(-1)	15.99
5	2(-1)	35(-1)	300(1)	300(1)	17.49
6	2(-1)	45(1)	100(-1)	100(-1)	13.17
7	2(-1)	45(1)	100(-1)	300(1)	15.16
8	2(-1)	45(1)	300(1)	100(-1)	15.48
9	2(-1)	45(1)	300(1)	300(1)	15.94
10	3(0)	30(-2)	200(0)	200(0)	16.57
11	3(0)	40(0)	0(-2)	200(0)	13.99
12	3(0)	40(0)	200(0)	0(-2)	14.29
13	3(0)	40(0)	200(0)	200(0)	15.19
14	3(0)	40(0)	200(0)	200(0)	15.31
15	3(0)	40(0)	200(0)	200(0)	15.11
16	3(0)	40(0)	200(0)	200(0)	14.96
17	3(0)	40(0)	200(0)	200(0)	15.24
18	3(0)	40(0)	200(0)	200(0)	15.38
19	3(0)	40(0)	200(0)	400(2)	16.02
20	3(0)	40(0)	400(2)	200(0)	16.69
21	3(0)	50(2)	200(0)	200(0)	15.05
22	4(1)	35(-1)	100(-1)	100(-1)	14.78
23	4(1)	35(-1)	100(-1)	300(1)	16.02
24	4(1)	35(-1)	300(1)	100(-1)	16.45
25	4(1)	35(-1)	300(1)	300(1)	16.61
26	4(1)	45(1)	100(-1)	100(-1)	14.65
27	4(1)	45(1)	100(-1)	300(1)	15.54
28	4(1)	45(1)	300(1)	100(-1)	15.96
29	4(1)	45(1)	300(1)	300(1)	16.03
30	5(2)	40(0)	200(0)	200(0)	15.21

[1]Numbers in parentheses correspond to coded values of different levels of factors

by the optimized enzymatic method were compared with the starch isolated by conventional method.

4.2.3.1 Freeze-thaw stability

The freeze-thaw stability of the enzymatically and conventionally isolated starches were determined according to the method of Singhal and Kulkarni.[15] Starch (5% (w/v) db) was heated in distilled water at 95°C for 30 minutes with constant stirring. 10 ml of paste was transferred to pre-weighed centrifuge tubes. The weight of the paste was then determined. This was subjected to alternate freezing and thawing cycles (22 h freezing at -20 °C followed by 2 h thawing at 30 °C) for 3 days, centrifuged at 5000 × g for 10 minutes after each cycle and the percentage syneresis was determined as weight of exudates to the weight of paste.

4.2.3.2 Pasting properties

The pasting properties of the starches were evaluated using Rapid Visco-Analyzer (RVA), model StarchMaster2 from Newport Scientific, Australia. Viscosity profiles were recorded using 12.5 % starch slurry in distilled water (total weight 28 g). A heating and cooling cycle of 13 min 30 s was used where the samples were heated from 50 °C to 95 °C in 5 min, held at 95 °C for 2 min, cooled from 95 °C to 50 °C in 4 min and held at 50 °C for 2 min 30 s. Pasting temperature (PT), peak viscosity (PV), hold viscosity (HV), final viscosity (FV), breakdown viscosity (BV) and setback viscosity (SV) were recorded from the graph.

4.2.3.3 Swelling and solubility

The swelling power and solubility of the starches were determined by modified method of Torruco-Uco and Betancur-Ancona.[16] Starch (0.5 g) was dispersed in 20 ml distilled water in a pre-weighed 50 ml centrifuge tubes and kept in shaking water bath at 90 °C for 30 min. The suspension was then centrifuged at 12,000 × *g* for 10 min. The supernatant was carefully decanted in a Petri dish and dried at 103 °C for 12 h. After decantation the weight of swollen granules were taken. The swelling power and percentage solubility were calculated using the following formulas:

Swelling Power = Weight of swollen granules × 100 / (Weight of sample – Weight of dissolved starch)

% Solubility = Weight of dried starch in Petri dish × 100 / Sample weight

4.2.3.4 Clarity of starch pastes

The clarity of the starches was measured following the method described by Sandhu and Singh.[17] Aqueous starch suspension containing 1% starch was prepared by heating 0.2 g starch in 20 ml water in shaking water bath at 90 °C for 1 h. The starch paste was cooled to room temperature and the transmittance was measured at 640 nm in spectrophotometer (Spectrascan UV-2600, Thermo Fisher Scientific, India).

4.2.4 Data analysis and optimization

Design Expert version 8 was used for analysis of data for starch yield and optimization. Experimental data were fitted to a second order polynomial model as follows:

$$Y = \beta_o + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum \sum_{i<j=1}^{3} \beta_{ij} X_i X_j \qquad \text{Eqn. 1}$$

Where Y represents the response i.e. starch recovery, β_o is the constant, β_i, β_{ii} and β_{ij} are the regression coefficients and X_i and X_j are the independent variables in coded values.

Significant terms in the model were found by analysis of variance (ANOVA). Model adequacy was checked by lack of fit test, R^2, predicted R^2, adequacy precision and predicted residual sum of squares (PRESS). A non-significant ($p > 0.05$) lack of fit, predicted R^2 comparable to fitted R^2, low PRESS and adequacy precision higher than 4, shows that the model fitted is adequate to predicting.[18-20] Response surfaces were generated to study the effect of interactions on starch recovery. The optimization of the extraction process was done using desirability function.

Functional properties of the starches were determined for optimized condition for enzymatic method and conventionally extracted starches in replicates of three. Fisher's 'Least Significant Difference (LSD)' method was used to determine the statistical difference between the results obtained.

4.3 Results and discussion

4.3.1 Model fitting

The values of starch yield for different experimental combinations are shown in Table 4.1. Multilinear regression analysis of the data yielded second order polynomial equations for starch yield. Analysis of variance (ANOVA) was performed to determine the significant effects of the process variables on starch yield. Regression coefficients of the different terms in the equations for starch yield in coded factors were obtained (Table 4.2). Model adequacy was checked by lack of fit test and by considering fitted R^2, predicted R^2, PRESS and adequacy precision. A non- significant ($p > 0.05$) lack of fit, predicted R^2 comparable to fitted R^2, low PRESS and adequacy precision higher than 4, implies that the model fitted is adequate to predicting.[18, 19]

It can be observed from Table 2 that the probability (p) values of the model, all the main factors, quadratic term for only temperature (T^2) and interaction of t×C, t×X, and C×X for the response significant and the model has non- significant lack of fit ($p>0.05$) with *p-value* of 0.1404, which is good, and also the adequacy precision for the model was more than 4. The R^2 value of the model was 0.97, whereas the adjusted R^2 (0.95) and predicted R^2 (0.86) were comparable indicating that the model fitted provided appropriate approximation of the true process.

The final equations in terms of coded factors are as follows:

Starch yield, $Y = 15.20 + 0.21t + 0.32T + 0.70C + 0.49X - 0.05t^2 + 0.19T^2 + 0.08C^2 + 0.03X^2 + 0.08t\times T - 0.20t\times C - 0.23t\times X - 0.10T\times C - 0.10T\times X - 0.25C\times X$ Eqn. 2

4.3.2 Effect of interaction of various factors and their interactions on starch yield

The variation in starch yield with time and temperature is shown in Fig. 4.1 a). Starch yield increased with incubation period but decreased as the temperature was increased. Shah[21] also reported similar findings for incubation period in extraction of litchi juice using enzymes and Guan and Yao[22] observed similar changes with temperature for extraction of protein from oat bran using enzymes. The decrease in yield of starch with increase in temperature might be attributed to the loss of activity of the enzymes at higher temperature and resulted in lower breakdown of the cell wall components and thereby releasing lower amount of starch. Another possible reason

Table 4.2 Analysis of variance (ANOVA) for fitted model of starch yield

Source	Sum of Squares	DF	Mean Square	F-Value	p-value
Model	25.45	14	1.82	37.83	< 0.0001*
t	1.08	1	1.08	22.38	0.0003*
T	2.46	1	2.46	51.15	< 0.0001*
C	11.76	1	11.76	244.75	< 0.0001*
X	5.88	1	5.88	122.39	< 0.0001*
t^2	0.07	1	0.07	1.42	0.2527
T^2	1.03	1	1.03	21.47	0.0003*
C^2	0.16	1	0.16	3.34	0.0877
X^2	0.03	1	0.03	0.52	0.4816
t×T	0.10	1	0.10	2.13	0.1650
t×C	0.67	1	0.67	13.99	0.0020*
t×X	0.86	1	0.86	17.81	0.0007*
T×C	0.16	1	0.16	3.41	0.0845
T×X	0.16	1	0.16	3.33	0.0880
C×X	1.02	1	1.02	21.23	0.0003*
Residual	0.72	15	0.05		
Lack of Fit	0.61	10	0.06	2.72	0.1404
Pure Error	0.11	5	0.02		
Correlation Total	26.17	29			
R^2	0.97				
Adjusted R^2	0.95				
Predicted R^2	0.86				
Adequate Precision	26.21				
PRESS	3.67				

t – Time: T – Temperature: C – Cellulase concentration: X – Xylanase concentration

* Terms significant at $p<0.05$

might be that, at higher temperatures some amount of starch might became solubilised in the water and could not be recovered during centrifugation.

Starch yield increased with increase in concentration of cellulase/xylanase for all incubation periods [Fig. 4.1 b) and Fig. 4.1 c)]. No significant variation was observed in starch yield with change in time at higher concentration of the enzymes, but at lower concentration starch yield increased with increase in incubation time, indicating that less time is required to hydrolyze the cell wall components when concentration of the enzymes were high. The starch yield was constant with incubation time at higher concentration of the enzymes. However, it was always found to be higher at high concentration of enzymes than at low concentration, even at higher incubation times. Similar findings were reported for cassava starch yield by Dzogbefia et al.[8] using pectinase enzyme and Shah[21] for litchi juice extraction. Starch yield was found to be higher when the concentration of the enzymes was high. This was observed regardless of incubation time. This shows that higher yield of starch similar to that when enzyme concentrations were high, cannot be achieved by keeping low concentration of the enzymes even when the incubation time is increased. This might be due to inhibition of the enzymes by the degradation products of the enzymes i.e. glucose and xylose. When the enzymes were present at lower concentrations most of the enzymes might be inhibited by the products and therefore could never give similar yield comparable with higher concentration of enzymes even when the incubation time is increased.

From Fig. 4.1 d) and Fig. 4.1 e) it can be observed that starch yield decreased with increase in temperature. At lower temperature the variation in starch yield was more with change in concentration of enzyme as compared to higher temperature. This might be attributed to inactivation of the enzymes or solubilization of some starch at higher temperatures thereby decreasing the yield.

It has already been observed that starch yield increased with increase in concentration of both the enzymes and combination of the two enzymes significantly increased the yield of starch from the tubers [Fig. 4.1 f)]. The effectiveness of xylanase in increasing the recovery of starch from taro tubers clearly indicates that xylan is an important component of the cell walls of taro tubers and degradation of xylan is necessary for releasing starch granules from the cells of taro.

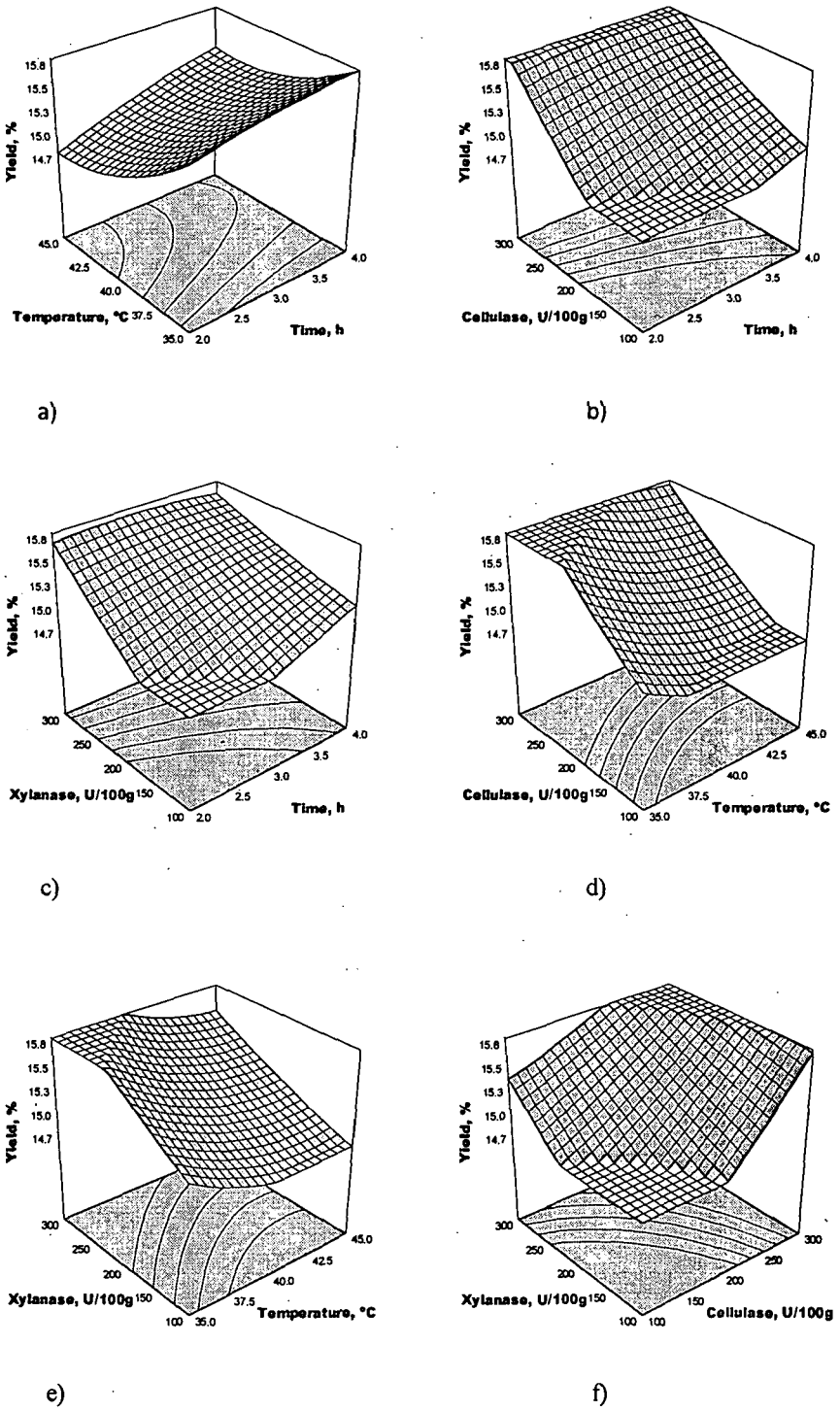

Fig. 4.1 Variation in starch yield due to interaction of a) temperature and incubation time, b) concentration of cellulase and incubation time, c) concentration of xylanase and incubation time, d) concentration of cellulase and temperature, e) concentration of xylanase and temperature, and f) concentration of xylanase and cellulase

4.3.3 Optimization of starch isolation process from taro tuber

Optimization of starch isolation process from taro tubers using cellulase and xylanase was done to obtain maximum yield of starch. The independent parameters selected were cellulase concentration (C), xylanase concentration (X), temperature of incubation (T) and incubation time (t). The cellulase and xylanase concentration were varied from 0 to 100 U/ 100 g taro tuber, incubation time was varied from 1 to 5 h and temperature was varied from 30 °C to 50 °C. Optimization was done using desirability function. One possible solution with highest desirability was selected (Table 4.3). The optimum condition with highest yield of 17.22% was obtained when cellulase and xylanase concentration were 299.86 and 300 U/100g tuber respectively, temperature was 35 °C and incubation time was 2 h.

Table 4.3 Results of optimization of starch recovery by desirability function

Sl. No.	Time, h	Temperature, °C	Cellulase, U/100g tuber	Xylanase, U/100g tuber	Yield, %	Desirability
1	2.00	35.00	299.86	300.00	17.22	0.94

4.3.4 Functional properties of starch isolated by enzymatic and conventional methods

The data for swelling, solubility and clarity of the starch pastes are presented in Table 4.4 and pasting properties is presented in Table 4.5 for starches isolated by enzymatic and conventional methods. It was observed that starch isolated by enzymatic method swelled significantly more as compared to conventional method. On the contrary, starch isolated by conventional method had higher solubility compared to enzymatic method. Dzogbefia et al.[8] reported lower swelling and higher solubility for cassava starch using pectinase as opposed to the present finding, and the variation might be due to the use of different kind of enzyme. Correia et al.[23] and Puchongkavarin et al.[24] also observed similar increase in swelling for chestnut starch and rice starch respectively with enzymatic methods. The higher swelling and lower solubility in the present investigation might be attributed to loss of amylose in enzymatic method which might have leached out during incubation. Amylose hinders swelling and contributes to the soluble portion of the starch and loss of amylose affected the swelling and solubility.

The clarity of the starch pastes extracted by enzymatic method was found to be more as compared to conventional method. The increase in clarity of the starch pastes due to enzymatic treatment might be attributed to the breakdown of the cell wall components like cellulosic and hemicellulosic fibres into soluble fragments which were removed during centrifugation or else it can contribute to the impurities of the isolated starch.

Table 4.4 Swelling, solubility and clarity of starch samples isolated by conventional and enzymatic methods

Isolation method[1,2]	Swelling, g/g	Solubility, %	Clarity, %T
Conventional	13.32±0.43[b]	20.21±0.12[a]	32.67±1.21[b]
Enzymatic	14.95±0.31[a]	19.08±0.27[b]	38.94±1.74[a]

[1]Values reported as Mean ± S. D. of three replications
[2]Means followed by same small letters within a column are not significantly different (p>0.05)

The pasting properties of the starches isolated by the two methods evinced no significant differences in pasting temperature, PV, HV, FV, SV and BV values, although the pasting temperature was slightly higher and viscosities were lower in the starch pastes isolated by enzymatic method. This might be due to loss of amylose in enzymatic method which might have increased the viscosity of the starch pastes. Dzogbefia et al.[8] also did not observe significant differences in pasting properties in cassava starch isolated by enzymatic and conventional methods. The results indicate that enzymatic treatment has no adverse effect on the pasting properties of the starch which could be used to achieve higher recovery of starch.

Table 4.5 Pasting properties of starch isolated by conventional and enzymatic methods

Isolation method[1,2]	Pasting Temperature, °C	Peak Viscosity, cP	Hold Viscosity, cP	Final Viscosity, cP	Breakdown Viscosity, cP	Setback Viscosity, cP
Conventional	84.4±0.30[a]	4333±120[a]	2218±134[a]	2868±97[a]	2115±89[a]	650±34[a]
Enzymatic	84.6±0.40[a]	4272±147[a]	2126±156[a]	2697±108[b]	2146±67[a]	571±53[b]

[1]Values reported as Mean ± S. D. of three replications
[2]Means followed by same small letters within a column are not significantly different (p>0.05)

The freeze-thaw stability of the starch gels were evaluated up to three freeze-thaw cycles and are shown in Figure 2. The freeze-thaw stability of the starch pastes isolated by enzymatic method was more stable as the % syneresis was lower and the differences were less among the three freeze-thaw cycles. Differences in freeze-thaw stability among different types of starches might be due to a variety of factors, most notably, amylose content.[25] The better freeze thaw stability of the enzymatically isolated starch might be attributed to loss of amylose during incubation, and lower amylose corresponds to lower retrogradation tendency, thereby lowering syneresis.[26]

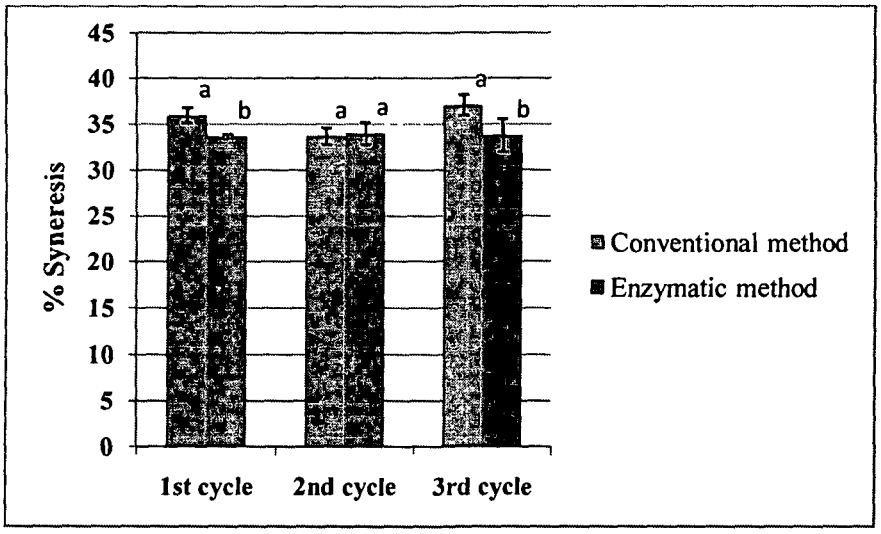

Fig. 4.2 Freeze-thaw behaviour of starches isolated by conventional and enzymatic methods

Vertical error bars above the columns represent standard deviation (S. D.) of three replications
Columns separated by same small letters within a cycle are not significantly different ($p>0.05$)

4.4 Conclusions

The study revealed that higher yield of starch from taro tubers could be achieved using cellulase and xylanase. However, combination of both the enzymes yielded significantly higher amount of starch. Effectiveness of xylanase indicated that xylan is an important component of the cell walls of taro tubers and removal of xylan from the cell walls of taro is necessary in achieving higher yield of starch from taro. The highest yield of starch was achieved when both time and temperature were low and

the properties of starch is possible as both time and temperature are low. The study further revealed that higher yield of starch from taro tubers could be obtained using enzymes without much affecting the properties of native starch.

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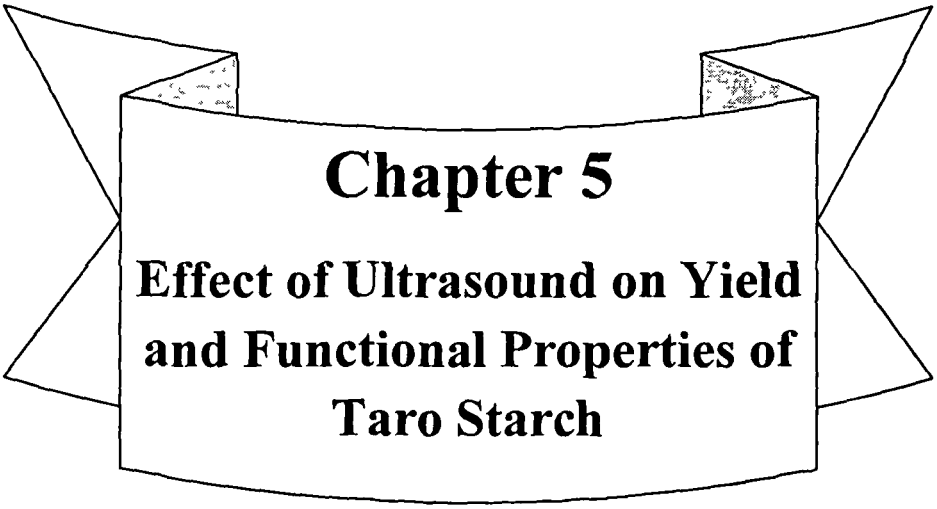

Chapter 5

Effect of Ultrasound on Yield and Functional Properties of Taro Starch

5.1 Introduction

The mechanical action of sonication has been used to disrupt cell membrane and to break the cell wall for enhancing various biological and biochemical separation processes. Ying et al.[1] obtained higher yield of polysaccharides from mulberry leaves using ultrasound assisted extraction. Hromádková et al.[2] was able to reduce the time of extraction of water soluble hemicelluloses from wheat bran using ultrasound. Li et al.[3] used ultrasound for extraction of oil from soyabean and were able to reduce the time of oil extraction compared to conventional process.

Ultrasound has been used for increasing the recovery of starch from cereals like maize,[4-10] but its effect on the recovery of starch from tubers has not been reported yet. Therefore, in the present study the effect of ultrasonication time, amplitude and cycle (pulsating effect) on the yield and properties of taro starch was investigated.

5.2 Materials and methods

5.2.1 Starch isolation using ultrasound

Panchamukhi (*Colocasia esculenta* var. *antiquorum*) taro tubers were collected from an agricultural farm near Tezpur University, Assam, India. Tubers were washed under tap water, peeled and cut into cubes of approximately 1 cm. 100 g of cubes were weighed and ground using a high speed laboratory blender (Philips HL 1632, India) for two minutes. The slurry was mixed with 100 ml distilled water and transferred to a 250 ml beaker. Ultrasound was applied to the suspension using horn type ultrasonic processor of 30 kHz, 100 W and 125 μm amplitude (UP100H, Hielscher, Teltow, Germany). Ultrasonication was carried out using a probe/horn of 7 mm diameter by immersing it in the slurry at a depth of 30 mm from the surface of slurry. The experiments were carried out during winter when the room temperature was 21±1 °C and the slurry temperature was 20±0.5 °C. There was no significant variation in the temperature of the slurry as the maximum ultrasound treatment time was 10 min only. After ultrasound treatment the suspension was filtered through double fold cheese cloth and the filtrate was centrifuged at 3000 × g for 10 min in refrigerated centrifuge at 20 °C (Sigma- K 15, Sigma-SVI Biosolutions Pvt. Ltd., Delhi, India). For control method the slurry after mixing with 100 ml water was immediately filtered through double fold cheese cloth and the filtrate was centrifuged at 3000 × g for 10 min. The supernatant was discarded and sediment was washed

twice with distilled water. The final sediment was dried at 45°C for 24 h in hot air drying oven. The dried starch was ground and passed through 100 mesh sieve and kept in air tight plastic containers for further analysis.

Moisture and starch contents of the dried samples were determined to calculate the yield of pure starch. Moisture content was determined by hot air oven method[11] and starch content was determined by acid hydrolysis method using perchloric acid which hydrolysed the starch to glucose and dehydrated it to hydroxylmethyl furfural which was then measured by anthrone reagent.[12] The starch content of the taro tubers were 21.13±0.21 g (n=5) per 100 g fresh tuber (21.13 % wb).

Yield was obtained by calculating the amount of pure starch (db) recovered from 100 g of fresh taro sample.

5.2.2 Functional properties of isolated starch

5.2.2.1 Swelling and solubility

Swelling power and solubility of the starches were determined by modified method of Torruco-Uco and Betancur-Ancona.[13] Starch (0.5 g) was dispersed in 20 ml distilled water in a pre-weighed 50 ml centrifuge tubes and kept in shaking water bath at 90 °C for 30 min. The suspension was then centrifuged at 12,000 × *g* for 10 min. The supernatant was carefully decanted in a Petri dish and dried at 103 °C for 12 h. After decantation the weight of swollen granules were taken. The swelling power and percentage solubility were calculated using the following formulas:

Swelling Power = Weight of swollen granules × 100 / (Weight of sample – Weight of dissolved starch)

% Solubility = Weight of dried starch in Petri dish × 100 / Sample weight

5.2.2.2 Clarity of starch pastes

Clarity of the starches was measured following the method described by Sandhu and Singh.[14] Aqueous starch suspension containing 1% starch was prepared by heating 0.2 g starch in 20 ml water in shaking water bath at 90 °C for 1 h. The starch paste was cooled to room temperature and the transmittance was measured at 640 nm in spectrophotometer (Spectrascan UV-2600, Thermo Fisher Scientific, India).

5.2.2.3 Colour of starch

Colour parameters of the starch (dry powder) were measured using colorimeter (Ultrascan VIS, Hunterlab, USA). Results were obtained in terms of L* (lightness), ranging from 0 (black) to 100 (white), a* (redness), ranging from +60 (red) to −60 (green), and b* (yellowness), ranging from +60 (yellow) to −60 (blue) values.

5.2.2.4 Pasting Properties

Pasting properties of the starches were evaluated using Rapid Visco-Analyzer (RVA), model StarchMaster2 from Newport Scientific, Australia. Viscosity profiles were recorded using 12.5 % starch slurry in distilled water (total weight 28 g). A heating and cooling cycle of 13 min 30 s was used where the samples were heated from 50 °C to 95 °C in 5 min, held at 95 °C for 2 min, cooled from 95 °C to 50 °C in 4 min and held at 50 °C for 2 min 30 s. Pasting temperature (PT), peak viscosity (PV), hold viscosity (HV), final viscosity (FV), breakdown viscosity (BV) and setback viscosity (SV) were recorded from the graph.

5.2.2.5 Texture analysis

Starch pastes were prepared by heating a 2% aqueous suspension of starch (1 g starch in 50 ml distilled water) in a shaking water bath at 100°C for 30 min. The starch pastes were cooled to 25°C by keeping the starch pastes in cooling water bath maintained at 25°C for 1 h. Textural properties such as firmness, consistency and cohesiveness of starch pastes were determined by back extrusion method in Texture Analyzer, TA.HDplus (Stable Micro Systems, UK) using a cylindrical probe (P-35). Starch pastes were prepared by heating a 2 % aqueous suspension of starch (1 g starch in 50 ml distilled water) in a shaking water bath at 100°C and were cooled to 25°C for determining the textural properties. The probe was allowed to penetrate 20 mm from the surface of the sample at a speed of 1 mm/s. Firmness, cohesiveness, consistency and index of viscosity were calculated from the graphs using the software Exponent Lite 32 provided with the instrument.

5.2.2.6 Freeze-thaw stability

The freeze-thaw stability was determined according to the method of Singhal and Kulkarni.[15] Starch (5% w/v (db)) was heated in distilled water at 95°C for 30

minutes with constant stirring. Paste (10 ml) was transferred to weighed centrifuge tubes. The weight of the paste was then determined. This was subjected to alternate freezing and thawing cycles (22 h freezing at -20°C followed by 2 h thawing at 30°C) for 3 days, centrifuged at 5000 × g for 10 minutes after each cycle and the percentage syneresis was determined as weight of exudates to the weight of paste.

5.2.3 Experimental design and statistical analysis

A three factor two level full factorial design was employed to study the effect of ultrasound treatment on yield of taro starch. The factors were ultrasonication time (5 and 10 min), amplitude of ultrasound (50 and 100%) and ultrasonication cycle (0.5 and 1). A total of nine treatments were performed including control (Table 5.1). Control i.e. without ultrasound treatment, was used to compare ultrasonically extracted starches as the initial levels i.e. 0 cycle or 0 min time or 0% amplitude corresponds to no ultrasonic treatment. Three replications were carried out for each treatment. Analysis of variance (ANOVA) was performed to observe the significance of the ultrasound treatment parameters on yield of starch using Design Expert Version 8 Software (Stat Ease, Inc., Minneapolis, USA). Fisher's 'Least Significant Difference (LSD)' method was used to determine the statistical difference between the results obtained.

Table 5.1 Treatment combinations and yield of starch at different ultrasonic treatment conditions

Treatment	Time, min	Cycle	Amplitude, %	Yield[1,2], %
Control	0	0	0	15.29±0.19[d]
T1	5	0.5	50	17.77± 0.19[c]
T2	10	0.5	50	18.97±0.26[a]
T3	5	0.5	100	17.64± 0.19[c]
T4	10	0.5	100	18.24±0.30[b]
T5	5	1	50	18.23± 0.29[b]
T6	10	1	50	18.54± 0.14[b]
T7	5	1	100	17.45± 0.03[c]
T8	10	1	100	17.65 ±0.04[c]

T1 to T8: Treatment conditions

[1] Starch yield reported as Mean ± S. D. of three replications

[2]Means followed by same small letter superscripts within a column are not significantly different (p>0.05)

5.3 Results and discussion

5.3.1 Effect of sonication parameters on yield of starch

Starch yield from taro for various ultrasound treatment conditions are given in Table 5.1. It was observed that starch yield significantly increased from control for all ultrasonic treatment combinations. Benmoussa and Hamaker[5] also reported higher yield of starch using ultrasonication from maize and sorghum starch compared to conventional method. Starch yield increased with increase in treatment time when both cycle and amplitude were constant, which might be due to higher breakdown of cellulosic materials with increased time and thereby releasing more starch from the cells. Park et al.[16] also obtained similar results for sorghum starch. Significant differences were not observed when the treatments were carried out in full cycle. It was also found that starch yield was more when ultrasonic probe was operated at half cycle and 50 % amplitude. Wang and Wang[6] also observed higher yield of rice starch at lower amplitude. This may be attributed to disintegration of starch molecules apart from breakdown of cellulosic materials which might have solubilized some amount of starch in water as more energy was dissipated in full cycle and 100 % amplitude, and could not be recovered during extraction by centrifugation. When the probe operates in half cycle, localized pressure gradient remains for short duration compared to when the probe operates in full cycle, and less energy is transferred to the starch granules, as the energy is utilized for breakdown of cellulosic materials surrounding the granules thereby degrading less amount of starch. The highest yield was obtained for the treatment combination T2.

Analysis of variance (ANOVA) for 2 level factorial design with three ultrasonic processing parameters on starch yield is presented in Table 5.2. It was found that the model was significant with a non-significant lack of fit, which is desirable. It can be observed that the effect of all the three parameters and their interactions were significant on yield of starch at $p<0.05$, except for interaction of Time×Amplitude which is significant at $p<0.10$. The effect of time and amplitude was more compared to the effect of cycle as can be seen from the values of their coefficients. Cycle and amplitude had negative coefficients implying that starch yield decreases with increase in cycle and amplitude, which may be attributed to degradation and solubilization of some portion of starch along with cellulosic materials due to dissipation of higher energy and thereby decreasing the recovery,

while time has positive coefficient indicating that yield increases with increase in time.

Table 5.2 Analysis of variance (ANOVA) for yield of starch for two factor interaction model

Terms	Coefficient	Sum of squares	Degree of freedom	Mean square	F- value	*p-value*
Constant	18.06	5.67	6.00	0.94	21.19	< 0.0001*
Time	0.29	2.01	1.00	2.01	45.16	< 0.0001*
Cycle	-0.09	0.21	1.00	0.21	4.73	0.0440*
Amplitude	-0.32	2.40	1.00	2.40	53.86	< 0.0001*
Time×Cycle	-0.16	0.61	1.00	0.61	13.72	0.0018*
Time×Amplitude	-0.09	0.19	1.00	0.19	4.24	0.0551
Cycle×Amplitude	-0.10	0.24	1.00	0.24	5.43	0.0324*
Residual		0.76	17.00	0.04		
Lack of Fit		0.09	1.00	0.09	2.23	0.1552
Pure Error		0.67	16.00	0.04		
Cor Total		6.42	23.00			

*Terms significant at $p<0.05$

5.3.2 Effect of ultrasound on solubility and swelling of starch

The solubility and swelling of the extracted starches increased significantly from control with ultrasound pretreatment (Table 5.3). The differences were statistically significant except for solubility at T2 and swelling at T2, T4 and T6, which might be due to lower cycle or amplitude of operation which resulted in lesser degradation of starch molecules. The increase in solubility and swelling may be attributed to the breakdown of the starch structure thereby exposing the hydrophilic

Table 5.3 Solubility, swelling and clarity of starch pastes for different ultrasonic treatment conditions

Treatment[1, 2]	Solubility, %	Swelling, g/g	Clarity, %T
Control	20.14± 0.21^{g}	14.48±0.14 ^{f}	25.94±1.21 ^{a}
T1	22.30 ±0.27^{b}	15.48 ±0.21^{c}	25.82 ±1.11^{a}
T2	20.24 ±f0.31^{g}	14.64 ±0.23$^{e f}$	25.35 ±0.96^{ab}
T3	23.05 ±0.35^{a}	16.50 ±0.09^{a}	25.70 ±0.84^{a}
T4	21.03 ±0.29^{d}	14.59 ±0.12$^{e f}$	23.88 ±0.15^{c}
T5	21.53 ±0.18^{c}	14.76 ±0.12^{e}	24.32 ±0.63^{bc}
T6	20.90 ±0.14$^{d e}$	14.66 ±0.15$^{e f}$	23.66 ±0.25^{c}
T7	22.38 ±0.18^{b}	15.21 ±0.11^{d}	24.32 ±0.34^{bc}
T8	20.60 ±0.22$^{e f}$	15.79 ±0.18^{b}	23.12 ±0.54^{c}

T1 to T8: Treatment conditions as described in Table 5.1
[1]Values reported as Mean ± S. D. of three replications
[2]Means followed by same small letter superscripts within a column are not significantly different (p>0.05)

groups to water and leading to higher water uptake and retention.[9, 17] Similar increase in starch swelling and solubility due to ultrasonication has been reported by Chan et al.[7] for mung bean and sago starch, and Jambrak et al.[9] for corn starch. The solubility and swelling increased when time of ultrasound treatment was less. This might be due to the fact that with increase in time the degradation of starch was more and some degraded soluble starch might have been lost during recovery. Similar trend was also observed with treatment cycle and evinced that some soluble starch might have been lost during treatment with full (continuous) cycle. Solubility and swelling increased with increase in amplitude as more energy was available for starch degradation at higher amplitude which might have modified the structure of the starch and allowed more water to be absorbed.

5.3.3 Effect of ultrasound on clarity of starch pastes

The clarity of the pastes prepared using starch extracted by ultrasonic pretreatment was observed to be lower compared to that extracted by conventional

method, but were not much statistically different (Table 5.3). The results were not in agreement with the results obtained by Jambrak et al.,[9] Zheng et al.[10] and Sujka and Jamroz[18] for various starches treated with ultrasound where the clarity of the starch pastes increased after ultrasound treatment. They also obtained lower viscosity values in pasting property which may explain for increase in clarity. In the present study the decrease in clarity was found to be consistent with increased viscosity values in pasting profile. It can be seen that clarity decreased with increase in time, cycle as well as amplitude. This can be attributed to the higher disintegration of the starch granules with more time, continuous cycle and higher amplitude which allowed more water to be absorbed and consequently the granules swelled more making the starch paste more viscous thereby decreasing the transmittance. Another possible reason might be during pretreatment with ultrasound the starch molecules disintegrated and some bonds became exposed which might allowed some impurities to bond with the starch molecules and thereby decreasing the clarity of the starch pastes. The lowest clarity was observed when the pretreatment was carried out for higher time and the probe was set in continuous cycle at 100 % amplitude which might have caused highest disintegration.

5.3.4 Effect of ultrasound on pasting properties of starch

Table 5.4 shows the pasting profile of the starches extracted by different ultrasound treatments. The pasting temperature, peak viscosity, hold viscosity, final viscosity and setback viscosity of the starches extracted by ultrasound pretreatment were significantly higher compared to control, while the breakdown viscosity was found to be lower. The results obtained were found to be different from those obtained by Wang and Wang[6] for rice starch and Luo et al.[8] for maize starches where viscosities decreased due to ultrasonication, but were in accordance with those obtained by Zhang et al.[4] for ultrasonically separated corn starch. Li et al.[19] also reported increase in peak, hold and final viscosities of rice starch treated with high hydrostatic pressure up to 480 MPa due to rupture in crystalline structure of starch granules. The increase in pasting temperature indicates that starch granules after ultrasound treatment continue to swell at higher temperatures compared to control sample before further distortion or disruption of the granular structure and leading to reduction of viscosity. Some amount of disruption might have already taken place during ultrasonication which allowed water to be absorbed at higher temperatures.

Table 5.4 Pasting properties of starch pastes for different ultrasonic treatment conditions

Treatment[1,2]	Pasting Temperature, °C	Peak Viscosity, cP	Hold Viscosity, cP	Final Viscosity, cP	Breakdown Viscosity, cP	Setback Viscosity, cP
Control	84.1± 0.03^{f}	4301± 45^{f}	2114± 23^{f}	2940± 36^{g}	2187±19^{b}	826 ±11^{f}
T1	85.3 ±0.05^{b}	4726 ±42^{c}	2626 ±25^{b}	3628 ±34$^{b\,c}$	2100 ±14$^{c\,d}$	1002±12 ^{d}
T2	85.3 ±0.02^{b}	4595 ±41^{e}	2572 ±31^{c}	3665 ±29^{b}	2023 ±12^{e}	1093 ±09^{b}
T3	84.8 ±0.05^{d}	5092 ±21^{a}	2706 ±27^{a}	3863 ±28^{a}	2386 ±17^{a}	1157 ±17^{a}
T4	84.9 ±0.02^{c}	4947 ±49^{b}	2584 ±11^{c}	3638 ±27^{b}	2363 ±20^{a}	1054 ±12^{c}
T5	85.7 ±0.03^{a}	4702 ±59$^{c\,d}$	2577 ±17^{c}	3583 ±42^{c}	2125 ±14^{c}	1006 ±15^{d}
T6	84.5 ±0.03^{e}	4639 ±38$^{d\,e}$	2559 ±18^{c}	3487 ±19^{d}	2080 ±16^{d}	928 ±18^{e}
T7	83.7 ±0.04^{h}	4104 ±37^{h}	2327 ±19^{d}	3136 ±36^{e}	1777 ±14^{f}	809 f± 09^{g}
T8	84.0 ±0.01^{g}	4224 ±24^{g}	2212 ±28^{e}	3011 ±24^{f}	2012 ±11^{e}	799 ±08^{g}

T1 to T8: Treatment conditions as described in Table 5.1

[1] Values reported as Mean ± Std. Dev. of three replications

[2] Means followed by same small letter superscripts within a column are not significantly different (p>0.05)

Increased values of peak viscosity, hold viscosity and final viscosity and lower values of breakdown viscosity suggest that the starch paste obtained after treatment with ultrasound could be more resistant to shearing and form a more rigid gel after cooling. High setback viscosity of the ultrasonically extracted starches implies that solubilized starch after collapses of the swollen granules were more capable of reassociation during cooling.[7] The viscosities of the starch with treatment T3 (less time and cycle and high amplitude) were found to be highest (Fig. 5.1). It was observed that the peak, hold and final viscosities were more when time of treatment was more as disruption of starch structure could be more which increased swelling and therefore viscosity also increased. Peak Viscosity was found to increase with amplitude for same treatment time at half cycle, but decreased for full cycle. This might be attributed to loss of some soluble portion of starch during extraction at full cycle operation which might have otherwise contributed to the viscosity and the viscosity decreased due to loss of this soluble starch. A strong relationship was found between the pasting profile and swelling and solubility of starches.

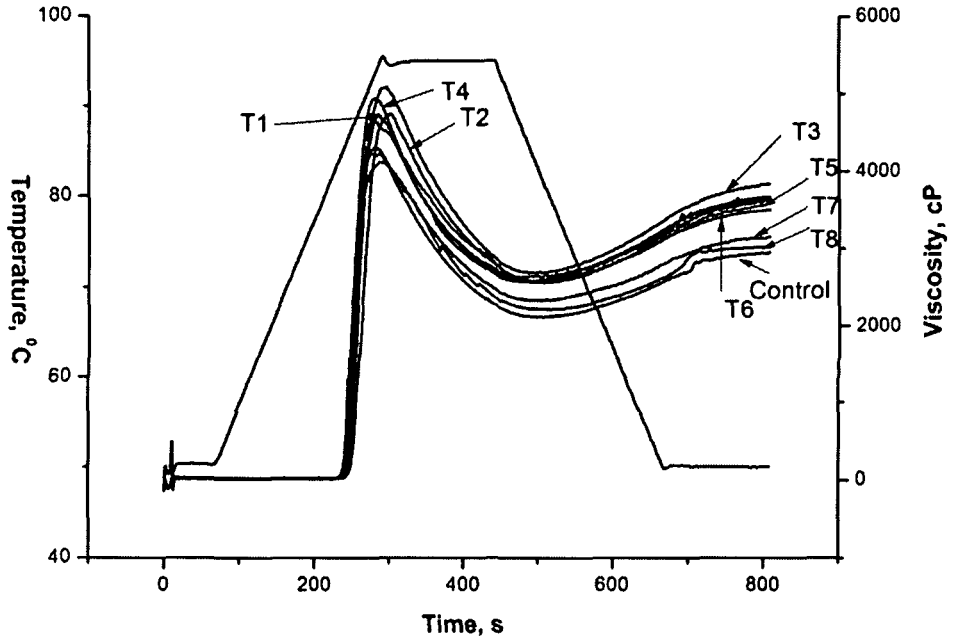

Fig. 5.1 Pasting profiles of starch pastes for different ultrasonic treatment conditions
T1 to T8: Treatment conditions as described in Table 5.1

5.3.5 Effect of ultrasound on texture of starch pastes

The textural attributes of the starch pastes are shown in Table 5.5. The firmness and consistency of the ultrasonically treated starch pastes varied from 10.89 g to 11.89 g and 164.24 to 170.53 respectively and were significantly higher compared to the untreated starch paste with firmness of 9.43 g and consistency of 157.57 g.s. Similar increase in hardness, cohesiveness and adhesiveness of ultrasonically treated corn starch gels were observed by Herceg et al..[20] The increase in firmness and consistency of the treated starch pastes might be attributed to the higher swelling of the starch granules due to partial disruption of the starch structure which increased the viscosity. The index of viscosity of the treated samples also increased implying that the treated starch paste samples are more susceptible to viscosity change with temperature compared to the control and was confirmed from the values of breakdown viscosities and setback viscosities in pasting profile. The increase in firmness and consistency of starch pastes may be desirable for certain products and are important criteria in determining the application of a starch.

Table 5.5 Texture properties of starch pastes for different ultrasonic treatment conditions

Treatment[1, 2]	Firmness, g	Consistency, g.s	Cohesiveness, g	Index of Viscosity, g.s
Control	9.43± 0.05^{e}	157.57 ±1.21^{d}	5.82 ±0.04$^{b c}$	0.84 ±0.01^{f}
T1	11.20± 0.02^{c}	166.12 ±0.90$^{b c}$	5.87 ±0.05^{b}	1.02 ±0.01^{b}
T2	11.42 ±0.12^{b}	169.07 ±1.12^{a}	5.7 ±0.04^{d}	0.93 ±0.01^{d}
T3	10.89 ±0.13^{d}	164.73 ±0.89$^{b c}$	5.24± 0.03^{f}	0.85 ±0.01$^{e f}$
T4	11.52 ±0.07^{b}	165.93 ±1.23$^{b c}$	5.96 ±0.02^{a}	1.00 ±0.02^{c}
T5	10.99 ±0.11^{d}	164.24 ±1.57^{c}	5.47 ±0.08^{e}	0.86 ±0.02^{e}
T6	11.41 ±0.08^{b}	165.17 ±0.99$^{b c}$	5.8 ±0.01^{c}	0.94 ±0.01^{d}
T7	11.89 ±0.09^{a}	170.53 ±1.17^{a}	5.54± 0.01^{e}	0.87 ±0.02^{e}
T8	11.38 ±0.05^{b}	166.46 ±1.48^{b}	5.85 ±0.03^{b}	1.07 ±0.01^{a}

T1 to T8: Treatment conditions as described in Table 5.1

[1]Values reported as Mean ± Std. Dev. of three replications

[2]Means followed by same small letter superscripts within a column are not significantly different (p>0.05)

5.3.6 Effect of ultrasound on freeze-thaw stability of starch gels

The percent syneresis of the starch gels recovered by sonication treatments were found to be substantially lower than the control starch gel during all the three freeze-thaw cycles (Fig. 5.2). Also ultrasonically extracted starches were observed to be more stable under repeated freeze-thaw cycles as less difference was noticed in % syneresis between the freeze-thaw cycles. Because of ultrasonic treatment breakage of starch chains in the amorphous region caused extensive reordering of the chain segments. Therefore, amount of water expelled during thawing was less in sonicated starches compared to control sample as due to breakage and reordering a greater number of hydrophilic bonds were exposed which could hold more water during thawing and thereby reducing syneresis. A similar observation on freeze-thaw behavior of ultrasonically treated maize starch was made by Luo et al.[8]. Retrogradation of native starch makes them unacceptable in many food applications.[21] Better freeze-thaw stability of the ultrasonically extracted starches make taro starch more acceptable for application in food meant for refrigeration.

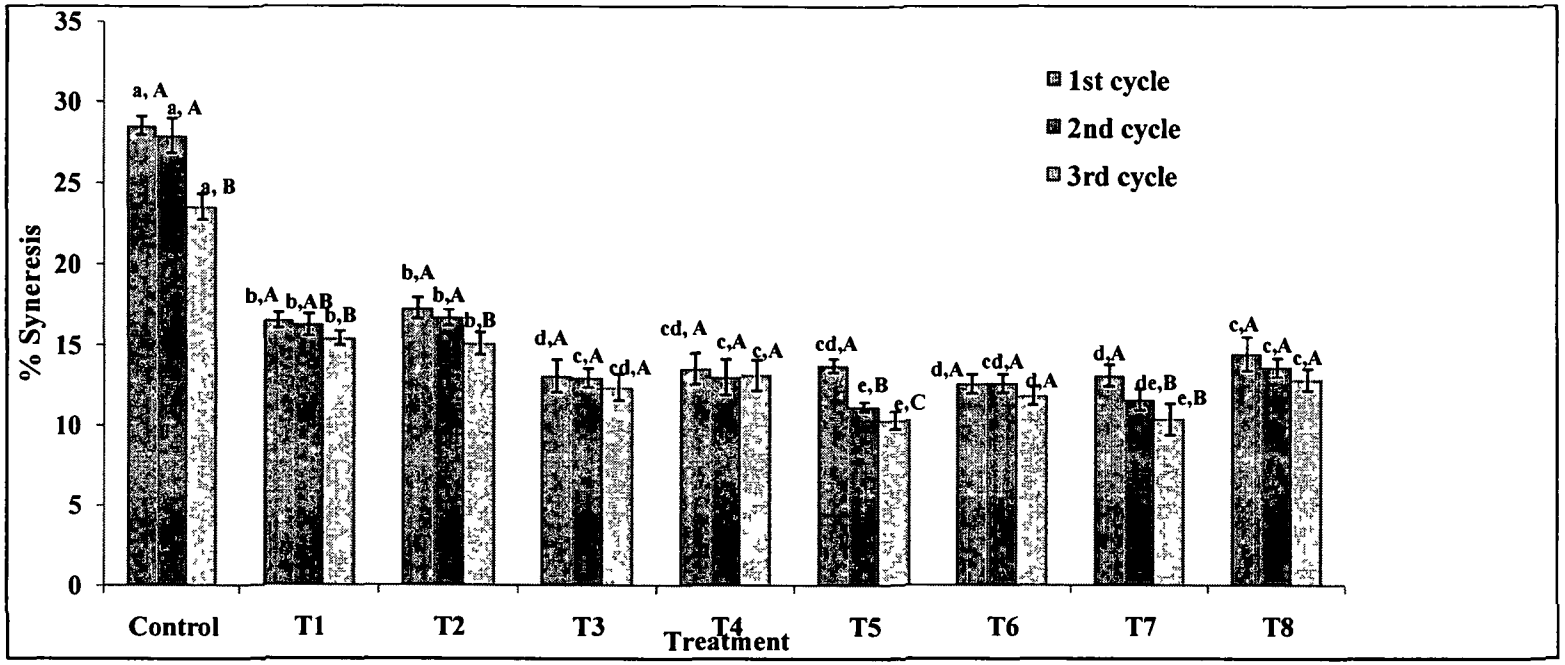

Fig. 5.2 Freeze-thaw behavior of starch gels up to 3 cycles for different ultrasonic treatment conditions

T1 to T8: Treatment conditions as described in Table 5.1

Vertical error bars above the columns represent standard deviation (S. D.) of three replications

Similar small letters above the bars denote values are not significantly different ($p>0.05$) between treatments for same freeze-thaw cycle

Similar capital letters above the bars denote values are not significantly different ($p>0.05$) between different freeze-thaw cycles for the same treatment

5.3.7 Effect of ultrasound on colour of starch (dry powder)

Colour is an important characteristic for determination of starch quality. The colour parameters of the starch (dry powder) samples are presented in Table 6. The isolated starches had high L* values and lower a* and b* values which confirm the high purity of the starches. The L* values of ultrasonically extracted starch ranged from 93.4 to 95.45, while that of conventionally extracted starch was 96.24. Pérez Sira and Amaiz[22] estimated that a value greater than 90 gives a satisfactory lightness for the purity of starch. The whiteness of the starches extracted by ultrasonication was lower compared to starch extracted by conventional method although the differences were not significant. Ultrasonically extracted starches also exhibited more redness and yellowness compared to conventionally extracted starch. This might be attributed to disintegration of starch molecules during ultrasonication which might have allowed some impurities to bond with the starch molecules and thereby reducing the whiteness and increasing the redness and yellowness.

Table 5.6 Colour of starch (dry powder) for different ultrasonic treatment conditions

Treatment[1,2]	L*	a*	b*
Control	96.24 ±1.25[a]	1.41 ±0.02[f]	2.20 ±0.04[i]
T1	94.45±1.58[a]	2.07 ±0.02[b]	3.43 ±0.05[b]
T2	95.27 ±0.98[a]	1.74 ±0.05[d]	2.91 ±0.04[e]
T3	93.4 ±1.57[a]	2.42 ±0.02[a]	3.79 ±0.04[a]
T4	95.15± 2.12[a]	1.77 ±0.03[d]	3.34 ±0.03[c]
T5	95.39 ±1.32[a]	1.89 ±0.04[c]	2.66 ±0.01[g]
T6	95.45 ±2.01[a]	1.58 ±0.01[e]	2.35 ±0.05[h]
T7	95.31 ±1.27[a]	1.85 ±0.02[c]	2.80 ±0.01[f]
T8	95.12 ±1.09[a]	1.76 ±0.02[d]	3.27 ±0.02[d]

T1 to T8: Treatment conditions as described in Table 5.1
[1]Values reported as Mean ± Std. Dev. of three replications
[2]Means followed by same small letter superscripts within a column are not significantly different (p>0.05)

5.4 Conclusions

Application of ultrasound presents a novel technique to isolate starch from tubers. It uses much less energy and has potential to increase the yield of starch from taro and

pretreatment of ground tuber using power ultrasound significantly increased the yield of starch from taro compared to conventional method. Lower amplitude and lower cycle (pulsating effect) was found to give the highest yield while the properties were also not much altered. Longer time, higher amplitude and full cycle yielded comparatively less starch with better functional properties. Increase in freeze-thaw stability and change in pasting and texture properties of the starch pastes suggest that starch extracted by ultrasonic pretreatment can be used in products subjected to refrigeration or requiring high viscosity. Future investigations by combining ultrasound with other treatments like enzymatic or other mechanical or chemical treatments may be carried out to study their effect on yield and properties of starch from various sources.

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Chapter 6

Effect of Combination of Ultrasound and Enzymatic Pretreatment on Yield and Properties of Taro Starch

6.1 Introduction

It was already observed that both ultrasound and enzymatic method gives higher yield of starch from taro tubers. But the effect of ultrasound or combination of ultrasound and enzymatic methods for isolation of tuber starches were not studied earlier. Combined effect of ultrasound and protease treatment has been studied by Wang and Wang[1] for isolation of rice starch. Therefore, the present study was taken up to investigate the effect of combination of ultrasound with different enzymatic pretreatments on yield and functional properties of taro starch.

6.2 Materials and methods

6.2.1 Starch isolation using ultrasound and enzymes

Taro tubers locally known as *Panchamukhi* (*Colocasia esculenta* var. *antiquorum*) was collected from an agricultural farm near Tezpur University, Assam, India. Tubers were washed under tap water, peeled and cut into cubes of approximately 1 cm. 100 g of cubes were weighed and ground using a laboratory blender (Philips HL 1632, India) for two minutes. The slurry was mixed with 100 ml distilled water and transferred to a 250 ml beaker. Ultrasound was applied to the suspension using ultrasonic processor of 30 kHz and 100 W (UP100H, Hielscher, Teltow, Germany). Ultrasonication was carried out at 80% amplitude correspond to 100 μm. After ultrasonication the slurry was subjected to enzymatic treatment using cellulase from *Aspergillus niger* (Sigma-Aldrich, 0.3 U/mg) and xylanase from *Thermomyces lanuginosus* (Sigma-Aldrich, 2500 U/g) and their combination. The slurry was incubated at 40°C for 2 h in incubator shaker (CERTOMAT® IS, Sartorius Stedim Biotech, Goettingen, Germany) at 150 rpm. After incubation the suspension was filtered through double fold cheese cloth and the filtrate was centrifuged at 3000 × g for 10 min. The supernatant was discarded and sediment was washed twice with distilled water. The final sediment was dried at 45 °C for 24 h in hot air drying oven. The dried starch was ground and passed through 100 mesh sieve and kept in air tight plastic containers for further analysis.

Moisture and starch contents of the dried samples were determined to calculate the yield of pure starch. Moisture content was determined by hot air oven method[2] and starch content was determined by acid hydrolysis method using perchloric acid which hydrolysed the starch to glucose and dehydrated it to hydroxylmethyl furfural which was then measured by anthrone reagent.[3] The starch

content of the taro tubers were 21.96±0.42 g (n=5) per 100 g fresh tuber (21.96 % wb).

Yield was obtained by calculating the amount of pure starch (db) recovered from 100 g of fresh taro sample.

6.2.2 Freeze-thaw stability

The freeze-thaw stability was determined according to the method of Singhal and Kulkarni.[4] Starch (5% w/v (db)) was heated in distilled water at 95°C for 30 minutes with constant stirring. 10 ml of paste was transferred to pre-weighed centrifuge tubes. The weight of the paste was then determined. This was subjected to alternate freezing and thawing cycles (22 h freezing at -20 °C followed by 2 h thawing at 30 °C) for 3 days, centrifuged at 5000 × g for 10 minutes after each cycle and the percentage syneresis was determined as weight of exudates to the weight of paste.

6.2.3 Pasting properties

Pasting properties of the starches were evaluated using Rapid Visco-Analyzer (RVA), model StarchMaster2 from Newport Scientific, Australia. Viscosity profiles were recorded using 12.5 % starch slurry in distilled water (total weight 28 g). A heating and cooling cycle of 13 min 30 s was used where the samples were heated from 50 °C to 95 °C in 5 min, held at 95 °C for 2 min, cooled from 95 °C to 50 °C in 4 min and held at 50 °C for 2 min 30 s. Pasting temperature (PT), peak viscosity (PV), hold viscosity (HV), final viscosity (FV), breakdown viscosity (BV) and setback viscosity (SV) were recorded from the graph.

6.2.4 Swelling and solubility

Swelling power and solubility of the starches were determined by modified method of Torruco-Uco and Betancur-Ancona.[5] Starch (0.5 g) was dispersed in 20 ml distilled water in a pre-weighed 50 ml centrifuge tubes and kept in shaking water bath at 90 °C for 30 min. The suspension was then centrifuged at 12,000 × *g* for 10 min. The supernatant was carefully decanted in a Petri dish and dried at 103 °C for 12 h. After decantation the weight of swollen granules were taken. The swelling power and percentage solubility were calculated using the following formulas:

Swelling Power = Weight of swollen granules × 100 / (Weight of sample – Weight of dissolved starch)

% Solubility = Weight of dried starch in Petri dish × 100 / Sample weight

6.2.5 Clarity of starch pastes

Clarity of the starches was measured following the method described by Sandhu and Singh.[6] Aqueous starch suspension containing 1% starch was prepared by heating 0.2 g starch in 20 ml water in shaking water bath at 90 °C for 1 h. The starch paste was cooled to room temperature and the transmittance was measured at 640 nm in spectrophotometer (Spectrascan UV-2600, Thermo Fisher Scientific, India).

6.2.6 Experimental design and statistical analysis

A three factor two level full factorial design was employed to study the effect of ultrasound treatment on yield of taro starch. The factors were ultrasonication time (0, 10 and 20 min), concentration of cellulase (0 and 100 U/100 g fresh tuber) and concentration of xylanase (0 and 100 U/100 g fresh tuber). A total of twelve treatments were performed including control (Table 1). Three replications were carried out for each treatment. Analysis of variance (ANOVA) was performed to observe the significance of the ultrasound treatment parameters on yield of starch using Design Expert Version 8 Software (Stat Ease, Inc., Minneapolis, USA). Functional properties of the starches were determined for each treatment and their replicates. Fisher's 'Least Significant Difference (LSD)' method was used to determine the statistical difference between the results obtained.

6.3 Results and discussion

6.3.1 Effect of ultrasound and enzymatic pretreatment on yield of starch

The study investigated the effect of ultrasonication and enzymatic pretreatment on yield of starch from taro. The yield of starch for the various treatment combinations is presented in Table 6.1. It was observed that significant increase in yield of starch was obtained when ultrasound was applied as pretreatment. Significant difference was not observed when ultrasound time was increased from 10 to 20 min. Starch yield also increased from control with the application of cellulase or xylanase alone and when they were applied in combination. The yield obtained using cellulase was slightly higher compared to that obtained using xylanase, but the differences

Table 6.1 Treatment combinations and yield of starch at different treatment conditions

Treatment[1, 2]	Sonication Time, St, min	Concentration of Cellulase, Cc, U/100 g tuber	Concentration of Xylanase, Xc, U/100 g tuber	Yield, % starch (db)/100 g tuber
C1	0	0	0	14.60 ± 0.02^{h}
C2	0	100	0	17.56 ± 0.06^{f}
C3	0	0	100	17.37 ± 0.06^{f}
C4	0	100	100	18.44 ± 0.13^{e}
C5	10	0	0	16.16 ± 0.50^{g}
C6	10	100	0	18.89 ± 0.13^{d}
C7	10	0	100	18.68 ± 0.02^{de}
C8	10	100	100	20.31 ± 0.03^{b}
C9	20	0	0	16.22 ± 0.61^{g}
C10	20	100	0	19.63 ± 0.08^{c}
C11	20	0	100	19.34 ± 0.15^{c}
C12	20	100	100	20.81 ± 0.06^{a}

C1 to C12: Treatment conditions

[1]Starch yield reported as Mean ± S. D. of three replications

[2]Means followed by same small letter superscripts within a column are not significantly different ($p>0.05$)

were not significant. The reason for higher yield using cellulase might be attributed to the fact that cellulose is the major cell wall polysaccharide and degradation of cellulose by cellulase yielded higher starch compared to xylanase, as xylan is present in very small fraction in the cell walls of taro tuber. Combination of both the enzymes yielded significantly higher starch, implying that xylan, which is present in small amounts in the cell walls, is an essential component and helps in maintaining the rigidity of the cell wall and breakdown of xylan is important for releasing the intracellular components like starch. Treatment with single enzyme or their combination after ultrasonication of the starch slurry yielded significantly higher starch compared to ultrasonication or enzymatic pretreatment alone. Application of ultrasound partially broke the cell walls which made the access of the enzymes to their respective substrates easier, thereby releasing more amount of starch. Highest yield of starch was obtained when ultrasound was applied for 20 min followed by

incubation with both cellulase and xylanase. Wang and Wang[1] obtained higher yield of rice starch using different combinations of ultrasound and protease treatment, as rice starch granules are enclosed in protein matrix.

Analysis of variance (ANOVA) for the 3 × 2 × 2 factorial design on starch yield is presented in Table 6.2. It was found that the model was significant with a non-significant lack of fit, which is desirable. It can be observed that the effect of all the three parameters and the interaction of cellulase and xylanase were significant on yield of starch. The interaction of ultrasonication time with any of the enzymes were not significant, implying that increasing the ultrasonication time may not be required if ultrasound is followed by enzymatic treatment and 10 min is sufficient to partially break the cell walls which helps in better accessibility of the enzymes to the substrates.

Table 6.2 Analysis of variance (ANOVA) for yield of starch for two factor interaction model

Source	Sum of Squares	DF	Mean Square	F- Value	*p-value*
Model	112.218	9	12.46867	186.5738	< 0.0001*
St	26.25172	2	13.12586	196.4076	< 0.0001*
Cc	44.0896	1	44.0896	659.7306	< 0.0001*
Xc	35.36284	1	35.36284	529.1486	< 0.0001*
St×Cc	0.279717	2	0.139858	2.092757	0.1436
St×Xc	0.149706	2	0.074853	1.120053	0.3415
Cc×Xc	6.084444	1	6.084444	91.04402	< 0.0001*
Lack of Fit	0.338772	2	0.169386	2.906253	0.0741

St – Sonication time: Cc – Cellulase concentration: Xc – Xylanase concentration
*Terms significant at $p<0.05$

6.3.2 Freeze-thaw stability of starch gels

The percent syneresis of the starch gels during three freeze-thaw cycles is shown in Fig. 6.1. It was observed that % syneresis of the freeze-thawed gels ultrasonically extracted starches were less compared to control and starch isolated by enzymatic alone. No significant differences were observed between the control and starch extracted by enzymatic methods. Ultrasonically extracted starches were also observed to be more stable under repeated freeze-thaw cycles as less difference was

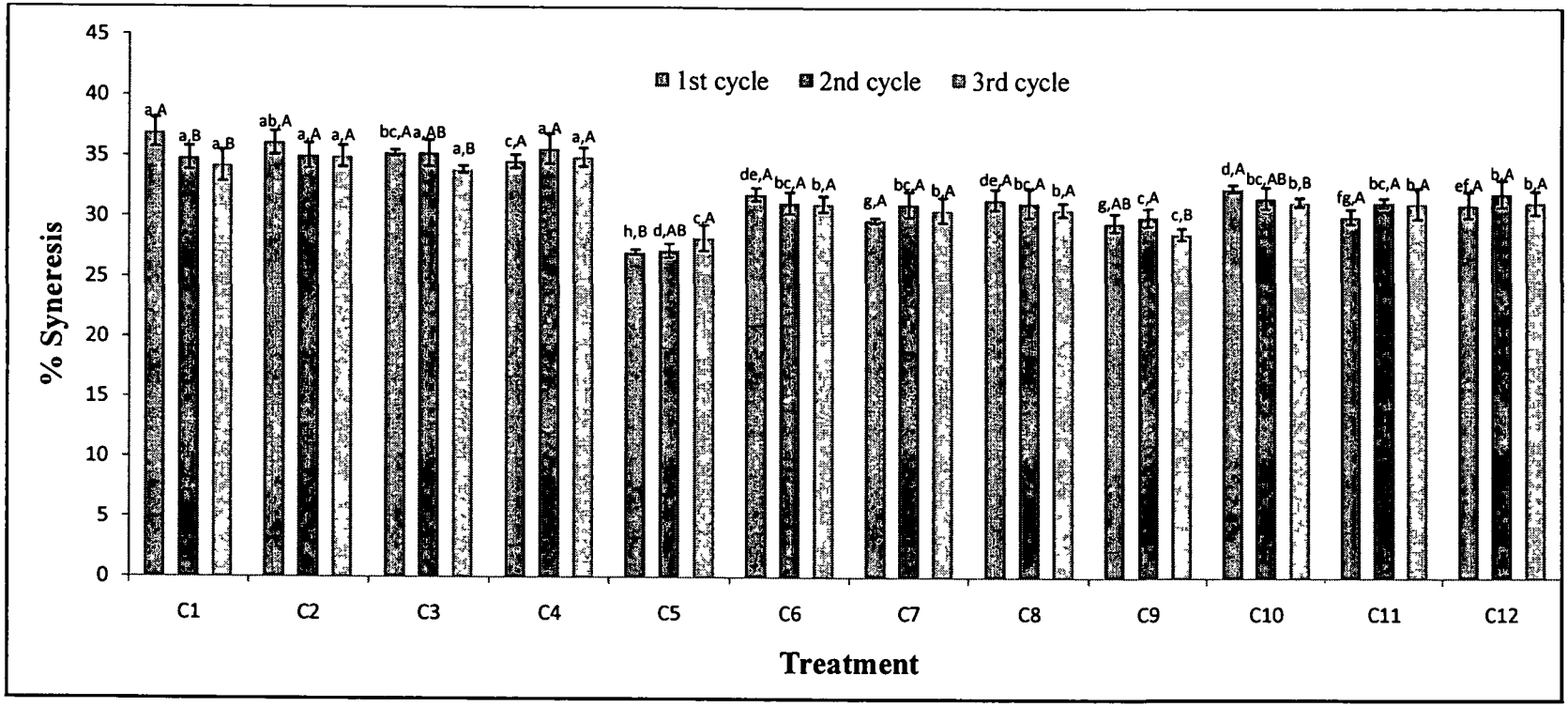

Fig. 6.1 Freeze-thaw behaviour of starch gels up to 3 cycles for different treatment conditions

C1 to C12 Treatment conditions as described in Table 6 1

Vertical error bars above the columns represent standard deviation (S D) of three replications

Similar small letters above the bars denote values are not significantly different ($p>0$ 05) between treatments for same freeze-thaw cycle

Similar capital letters above the bars denote values are not significantly different ($p>0$ 05) between different freeze-thaw cycles for the same treatment

noticed in % syneresis between the freeze-thaw cycles. The study suggests that the change in freeze-thaw behaviour is due to ultrasonication only. Because of ultrasonic treatment breakage of starch chains in the amorphous region caused extensive reordering of the chain segments. Therefore, amount of water expelled from the starch gels during thawing was less for the starches where ultrasound was applied for isolation of compared to when ultrasound was not applied for isolation. It can be attributed to breakage and reordering of greater number of hydrophilic bonds exposed due to ultrasonication, which could hold more water during thawing and thereby reducing syneresis. A similar observation on freeze-thaw behaviour of ultrasonically treated maize starch was made by Luo et al..[7] Retrogradation of native starch makes them unacceptable in many food applications.[8] Better freeze-thaw stability of the ultrasonically extracted starches make taro starch more acceptable for application in food meant for refrigeration.

6.3.3 Pasting properties of starch

Table 6.3 shows the pasting profile of the starches extracted by different treatments combinations. The pasting temperature, hold viscosity and final viscosity of the starches extracted using ultrasound as a pretreatment were found to be higher compared to control and starches extracted using enzymatic pretreatment alone. This shows that the change in pasting properties of the starches is due to ultrasonication which might have partially disrupted the starch granules. The results obtained were found to be different from those obtained by Wang and Wang[1] for rice starch and Luo et al.[7] for maize starches where viscosities decreased due to ultrasonication, but were in accordance with those obtained by Zhang et al.[9] for ultrasonically separated corn starch. The increase in pasting temperature indicates that starch granules after ultrasound treatment continue to swell at higher temperatures compared to control sample before further distortion or disruption of the granular structure and leading to reduction of viscosity. Some amount of disruption might have already taken place during ultrasonication which allowed water to be absorbed at higher temperatures. The peak viscosities of the starches isolated by pretreating the slurry with ultrasound for 10 min were found to be lower or similar to that of control or starches isolated by enzymatic pretreatment alone, while ultrasound treatment for 20 min increased the peak viscosity significantly. This might be attributed to greater disruption of the granule structure for more sonication time, which allowed more water to be absorbed

and thereby increasing the peak viscosity. Increased values of peak viscosity, hold viscosity and final viscosity and lower values of breakdown viscosity suggest that the starch paste obtained after treatment with ultrasound could be more resistant to shearing and form a more rigid gel after cooling. The change in the viscosity profile of the starches were slight indicating that higher yield of starch can be achieved using combination of ultrasound and enzymatic pretreatment without much affecting the pasting properties.

Table 6.3 Pasting properties of starch pastes for different ultrasonic treatment conditions

Treatment[1,2]	PT, °C	PV, cP	HV, cP	FV, cP	BV, cP	SV, cP
C1	84.8±0.06^{d}	4323±23^{d}	2013±19^{f}	2939±52^{cd}	2310±25^{abc}	926±15^{a}
C2	84.7±0.00^{e}	4336±51^{cd}	2017±18^{f}	2921±26^{d}	2319±48^{ab}	904±14^{bcd}
C3	84.8±0.06^{d}	4295±25^{d}	2009±15^{f}	2946±27^{bcd}	2286±56^{bc}	937±11^{a}
C4	84.8±0.10^{d}	4327±41^{cd}	2014±29^{f}	2939±49^{cd}	2313±28^{abc}	925±22^{ab}
C5	84.9±0.00^{c}	4308±42^{d}	2179±32^{a}	3119±72^{a}	2129±34^{f}	940±19^{a}
C6	85.1±0.05^{b}	4297±48^{d}	2153±28^{ab}	3029±29^{b}	2144±29^{ef}	876±14^{def}
C7	84.9±0.00^{c}	4310±69^{d}	2103±14^{cd}	2973±48^{bcd}	2207±71de	870±13^{ef}
C8	85.2±0.00^{a}	4350±53^{cd}	2054±41^{ef}	2949±56^{bcd}	2296±28^{abc}	895±21^{cde}
C9	85.2±0.06^{a}	4395±37^{bc}	2104±29^{c}	2994±49^{bcd}	2291±62^{bc}	890±26de
C10	85.1±0.10^{b}	4469±29^{a}	2115±25^{bc}	2968±78^{bcd}	2354±64^{ab}	853±18^{f}
C11	84.9±0.06^{c}	4439±27^{ab}	2067±19de	3007±48^{bc}	2372±43^{a}	940±13^{a}
C12	85.1±0.00^{b}	4365±31^{cd}	2096±37^{cd}	3018±34^{bc}	2269±27^{cd}	922±24^{abc}

C1 to C12: Treatment conditions as described in Table 6.1

[1]Values reported as Mean ± S. D. of three replications

[2]Means followed by same small letter superscripts within a column are not significantly different (p>0.05)

6.3.4 Solubility and swelling of starch

The solubility and swelling of the starches isolated by different treatment combinations is presented in Table 6.4. The solubility and swelling of the starches isolated by ultrasonication, and ultrasonication followed by enzymatic treatment were found to be higher compared to starches isolated by conventional method and those extracted by enzymatic method alone, although the differences were very small. The

results indicate that the increase in solubility and swelling is due to the effect of ultrasound only and not due to enzymatic treatment. The swelling of the starch granules were more when the ultrasonication time was more. This may be attributed to higher breakdown of the starch structure with time thereby exposing more hydrophilic groups to water and leading to higher water uptake and retention.[10, 11] Similar increase in starch swelling and solubility due to ultrasonication has been reported by Chan et al.[12] for mung bean and sago starch, and Jambrak et al.[11] for corn starch.

Table 6.4 Solubility, swelling and clarity of starch pastes for different treatment conditions

Treatment[1, 2]	Solubility, %	Swelling, g/g	Clarity, %T
C1	20.22 ± 0.84^{bc}	14.23 ± 0.36^{de}	26.36 ± 1.02^{bcde}
C2	20.46 ± 0.12^{abc}	14.19 ± 0.21^{e}	27.58 ± 0.96^{ab}
C3	20.13 ± 0.39^{c}	14.34 ± 0.51^{cde}	27.85 ± 0.99^{a}
C4	20.17 ± 0.47^{bc}	14.28 ± 0.25^{de}	27.43 ± 0.84^{abc}
C5	20.86 ± 0.85^{abc}	14.69 ± 0.68^{abcde}	25.47 ± 1.15^{de}
C6	20.67 ± 0.29^{abc}	14.52 ± 0.48^{bcde}	27.51 ± 0.69^{abc}
C7	20.49 ± 0.49^{abc}	14.37 ± 0.11^{cde}	26.94 ± 0.47^{abc}
C8	21.37 ± 0.57^{a}	14.85 ± 0.48^{abcd}	27.12 ± 0.59^{abc}
C9	20.14 ± 0.56^{c}	15.16 ± 0.28^{ab}	25.15 ± 0.87^{e}
C10	20.48 ± 0.29^{abc}	14.87 ± 0.37^{abcd}	26.14 ± 1.02^{cde}
C11	21.09 ± 0.53^{ab}	14.96 ± 0.47^{abc}	26.59 ± 0.29^{abcd}
C12	20.94 ± 0.64^{abc}	15.24 ± 0.12^{a}	26.37 ± 0.75^{bcde}

C1 to C12: Treatment conditions as described in Table 6.1
[1]Values reported as Mean ± S. D. of three replications
[2]Means followed by same small letter superscripts within a column are not significantly different ($p>0.05$)

6.3.5 Clarity of starch pastes

The clarity of the starch pastes extracted by enzymatic method were found to be more compared to conventional method and starch extracted by ultrasonication alone (Table 6.4). The increase in clarity of the starch pastes due to enzymatic treatment may be attributed to the breakdown of the cell wall components like cellulosic fibres into soluble fragments which got removed during centrifugation

which would otherwise contributed to the impurities of the isolated starch. There was significant decrease in the clarity of the starch pastes isolated by ultrasonication only. The results were not in agreement with the results obtained by Jambrak et al.,[11] Sujka and Jamroz[13] and Zheng et al.[14] for various starches treated with ultrasound where the clarity of the starch pastes increased after ultrasound treatment. They also obtained lower viscosity values in pasting property which may explain for increase in clarity. In the present study the decrease in clarity was found to be consistent with increased viscosity values in pasting profile.

6.4 Conclusions

Both ultrasound and enzymatic methods using cellulase and xylanase increased the yield of starch from taro compared to conventional method. Combination of ultrasound along with the enzymes can increase the yield of starch significantly as sonication partially broke the cell walls making the substrate accessible to the enzymes. Combination of ultrasound and enzymatic pretreatment can be effectively used to increase the recovery of starch from taro tuber without much affecting the physicochemical properties of taro starch.

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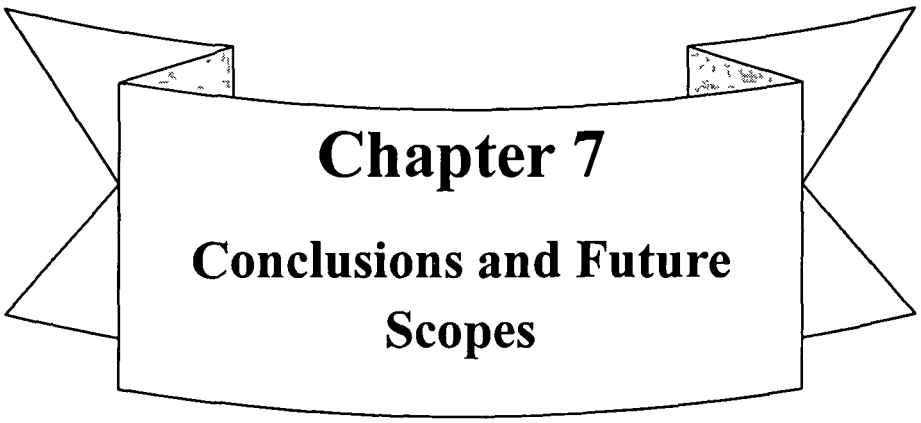

Chapter 7

Conclusions and Future Scopes

7.1 Conclusions

In the present investigation characterization of starches from various taro cultivars of Assam was carried out. Properties of the taro starches were compared with that of rice, maize and potato starches. The cultivar whose starch had comparatively better functional properties among the taro cultivars was selected for further studies. The starch from the selected cultivar was incorporated in preparation of tomato ketchup. The effect of incorporation of starch on quality of tomato ketchup was examined and compared with tomato ketchup prepared using maize starch and ketchup prepared without using any starch. The starch extraction process using enzymes from the tuber of the selected taro cultivar was optimized. The properties of the starch extracted by the optimized enzymatic process were compared with the starch extracted by conventional method. Further, the effect of various ultrasound parameters on yield and properties of taro starch was investigated. The effect of combination of ultrasound and enzymatic pretreatment on yield and properties of starch was examined in the present study.

The salient findings of the thesis are summarized below:

I. **Physicochemical, functional, textural and colour characteristics of starches isolated from different cultivars of taro of Assam and their comparison to starches from rice, maize and potato**

- The amylose content of taro starches varied from 11.87 to 18.14% which were lower compared to normal rice, maize or potato starches with amylose content of 21.57, 24.20 and 25.75% respectively.
- The amylose content of the starches of the "eddoe" type taros were comparatively higher than that of "dasheen " type taros.
- The average size of the starch granules varied from 2.22 to 3.29 μm for the various taro cultivars and were smaller compared to that of rice, maize or potato starches, but were closer to that of rice starches.
- The shape of the taro starch granules were polygonal and irregular similar to that of rice starch.
- Taro starches presented A-type XRD pattern similar to that of rice and maize starch.
- The swelling and solubility of all the starches increased with temperature.

- The swelling of taro starches were comparable to that of rice and maize starches but lower than potato starch.
- The solubility of *Kani* and *Panchamukhi* taro starches were much higher than the other starches at 90°C.
- The clarity and stability of the taro starches were much lower than potato and maize starch and comparable to rice starch.
- Among the taro starches, clarity of starch from *Panchamukhi* taro was most stable.
- The pasting and gelatinization temperatures of the taro starches were much higher than rice, maize and potato starches.
- Starches from *Kani* and *Muktakashi* taro were least viscous among the taro starches.
- The viscosities of the rest of the taro starches were closer to rice and maize starch and were significantly lower than potato starch.
- The freeze-thaw stability of *Panchamukhi* taro starch was better than the starches from other taro cultivars and rice and maize.
- The firmness and cohesiveness of the taro starch pastes were not significantly different from the starch pastes of rice and maize.
- The firmness of potato starch paste was considerably higher than the other starch pastes.
- *Panchamukhi* and *Garu* starch pastes were significantly lighter than starch pastes of other taro cultivars and potato starch paste exhibited highest lightness.
- The colours of the taro starch pastes were found to be comparable to the colour of rice and maize starch pastes.
- The L* values of all the starches were more than 90.
- The L* value of *Panchamukhi* starch (95.88) was the highest and *Ahina* starch (92.29) recorded the lowest among the taro starches.

II. **Effect of incorporation of taro and maize starch on various quality parameters of tomato ketchup**

- Ketchups prepared by addition of starch from *Panchamukhi* taro and maize at 1 and 2% concentrations significantly lowered the serum loss as compared to the control sample throughout the storage period.
- The firmness and consistency of the ketchups significantly increased due to incorporation of starch which acts as a thickening agent.
- The firmness also increased as the concentration of the starch in the ketchup samples were increased from 1 to 2%.
- Cohesiveness values of the ketchup samples prepared by incorporation of the starches were significantly higher compared to the control sample for all storage periods.
- *Panchamukhi* starch could be used in preparation of tomato ketchup for improving the texture and mouthfeel without affecting the acceptability.

III. **Optimization of starch isolation from taro using enzymes and comparison of properties of starches isolated by enzymatic and conventional methods**

- For enzymatic extraction process starch yield increased with incubation period but decreased as the temperature was increased.
- Starch yield increased with increase in concentration of cellulase/xylanase for all incubation periods.
- Starch isolated by enzymatic method swelled more significantly as compared to conventional method.
- Starch isolated by conventional method had higher solubility compared to enzymatic method.
- The clarity of the starch pastes extracted by enzymatic method was found to be more as compared to conventional method.
- The pasting properties of the starches isolated by the two methods evinced no significant differences.
- The freeze-thaw stability of the starch pastes isolated by enzymatic method was more stable.

IV. **Effect of ultrasound on yield and functional properties of taro starch**

- Starch yield significantly increased from control for all ultrasonic treatment combinations.
- Starch yield increased with increase in treatment time when both cycle and amplitude were constant.
- The solubility and swelling of the extracted starches increased significantly from control with ultrasound pretreatment
- The clarity of the pastes prepared using starch extracted by ultrasonic pretreatment was observed to be lower compared to that extracted by conventional method.
- The pasting temperature, peak viscosity, hold viscosity, final viscosity and setback viscosity of the starches extracted by ultrasound pretreatment were significantly higher compared to control, while the breakdown viscosity was found to be lower.
- The firmness and consistency of the ultrasonically treated starch pastes were significantly higher compared to the untreated starch paste.
- The per cent syneresis of the starch gels recovered by sonication treatments were found to be substantially lower than the control starch gel during all the three freeze-thaw cycles.
- The whiteness of the starches extracted by ultrasonication was lower compared to starch extracted by conventional method although the differences were not significant.
- Ultrasonically extracted starches also exhibited more redness and yellowness compared to conventionally extracted starch.

V. **Effect of combination of ultrasound and enzymatic pretreatment on yield and functional properties of taro starch**

- It was observed that significant increase in yield of starch was obtained when ultrasound was applied as pretreatment.
- Significant difference was not observed when ultrasound time was increased.
- Starch yield also increased from control with the application of cellulase or xylanase alone and in combination.

- Treatment with single enzyme or their combination after ultrasonication of the starch slurry yielded significantly higher starch compared to ultrasonication or enzymatic pretreatment alone.
- It was observed that % syneresis of the freeze-thawed gels ultrasonically extracted starches were less compared to control and starch isolated by enzymatic alone .
- The pasting temperature, hold viscosity and final viscosity of the starches extracted using ultrasound as a pretreatment were found to be higher compared to control and starches extracted using enzymatic pretreatment alone.
- The solubility and swelling of the starches isolated by ultrasonication, and ultrasonication followed by enzymatic treatment were found to be higher compared to starches isolated by conventional method and those extracted by enzymatic method alone.
- The clarity of the starch pastes extracted by enzymatic method were found to be more compared to conventional method and starch extracted by ultrasonication alone.

Therefore, from the present investigation it is worth to conclude that taro starch could be used as a substitute of cereal starches in many food and non-food applications. Significantly higher yield of starch could be achieved using ultrasound or enzymatic pretreatment compared to conventional method. The yield could be further increased if ultrasound and enzymatic pretreatments were used in combination. Ultrasound resulted in better functional properties of the extracted starch.

7.2 Future scopes of the present investigation

- Other enzymes may be used for extraction of starch.
- Ultrasound or enzymatic treatments might be combined with other treatments like microwave.
- Structural changes in the amylose or amylopectin molecules due to ultrasonication or enzymatic treatment might be investigated.

- The effect of incorporation of starches extracted by different methods might be examined on the quality parameters of tomato ketchup.
- Other properties of starches like crystallinity, thermal properties etc., might be examined for the starches isolated by ultrasound and enzymatic pretreatment.

List of Publications

1. **Sit, N., Misra, S. and Deka, S. C.,** Physicochemical, functional, textural and colour characteristics of starches isolated from four taro cultivars of North-East India, *Starch/Stärke*, DOI: 10.1002/star.201300033 **(Available online)**
2. **Sit, N., Misra, S., Baruah, D., Badwaik, L. S. and Deka, S. C.,** Physicochemical properties of taro and maize starch and their effect on texture, colour and sensory quality of tomato ketchup, *Starch/Stärke*, DOI: 10.1002/star.201300120 **(Available online)**
3. **Sit, N., Misra, S. and Deka, S. C.,** Yield and functional properties of taro starch as affected by ultrasound, *Food Bioprocess Technol.* DOI: 10.1007/s11947-013-1192-7 **(Available online)**
4. **Sit, N., Misra, S. and Deka, S. C.,** Characterization of physicochemical, functional, textural and colour properties of starches from two different varieties of taro and their comparison to potato and rice starches, *Food Sci. Technol. Res.* **(Accepted)**
5. **Sit, N., Misra, S. and Deka, S. C.,** Optimization of starch isolation from taro using enzymes and comparison of properties of starches isolated by enzymatic and conventional methods, *J. Food Sci. Technol.* **(Under review)**
6. **Sit, N., Misra, S. and Deka, S. C.,** Effect of combination of ultrasound and enzymatic pretreatment on yield and properties of taro starch, *Innovative Food Sci. Emer. Technol.* **(Under review).**