STUDIES ON PHYSICO-CHEMICAL AND FUNCTIONAL PROPERTIES OF DIOSCOREA (SP) STARCHES

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CERTIFICATE

This is to certify that the Thesis entitled "**Studies on Physico-Chemical and Functional properties of Dioscorea (SP) Starches**" submitted herewith by Smt. Jancy K. John is an authentic scientific record of the research work carried out by her under our guidance and supervision, and no part thereof has been submitted for any degree or award.

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DECLARATION

The work incorporated in the thesis entitled **"Studies on Physico-Chemical and Functional properties of Dioscorea (SP) Starches"** was carried out by me in the Biochemical Processing and Waste Water Technology Division of the Regional Research Laboratory (CSIR), Trivandrum and Division of Crop Utilization and Biotechnology of Central Tuber Crops Research Research Institute (ICAR), Trivandrum, under the joint guidance of Dr. S.N. Moorthy, Senior Scientist, CUBT, CTCRI, Trivandrum and Dr. K.C.M. Raja, Scientist EII, BCPWWT, RRL, Trivandrum. I further declare that the work embodied in this thesis has not been submitted for any degree or award or diploma or any other similar title.

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Dated: .8.1999

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To my father-in-law

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INTRODUCTION

Starch is the major organic component present in a large class of agricultural crops such as roots, tubers, legumes and cereals. Since tubers are considered as an important staple food in the diet of tropical population, they are more widely cultivated and distributed than cereals and other crops. Tubers are generally consumed after boiling, but sometimes after elaborate processing either at domestic scale or different industrial levels[1,2]. Since the diet of the people in developing countries lacks in calorie, the tuber crops provide a cheap and readily available source of energy especially in the humid tropics[3].

Although cassava and sweet potato are the two economically important starchy food crops, minor tubers covering *Dioscorea, Colocasia, Xanthosoma* and *Amorphophallus* sp. also invariably function as important food sources[4,5]. While cassava is more popular in Asia and Latin America, yams predominate in the African continent.

Starches from different botanical sources exhibit distinct and characteristic properties depending on a number of integrated factors embracing polymer composition, molecular conformation and the nature of the non-starchy constituents present. Inherently, starch occurs in the form of semi-crystalline granules consisting of essentially linear amylose and highly branched amylopectin, as two basic subunits.

In addition to being a major constituent of human diet, starch also functions as an excellent raw material for modifying the texture and consistency of foods, owing to its ability to form viscoelastic gels when heated in water. Besides their food uses, starch-derived products have considerable applications in pharmaceuticals, textiles, alcohol-based fuels and adhesives[6]. Emerging uses of starch include as low-calorie fat substitutes, biodegradable packing materials, thin films and thermoplastic materials with improved

thermal and mechanical properties[7-14]. Starch modification is a common practice for development of processed foods, having both textural and nutritional advantages. Recent advances made in the knowledge of structure and physical chemistry of starch reveal many striking similarities between starch and synthetic semi-crystalline polymers[15-18].

Unlike in the case of starches of major tuber and root crops, studies on the structural and functional aspects of starches from minor tubers such as edible yams and aroids and on their utilization still remain inadequate. Although the major organic constituent of all the minor tubers is starch, accounting for 10-35% on fresh weight basis[19], recovery of starch from them is invariably low. This is mainly due to the fact that the above tubers, often contain extraneous constituents that interfere during starch extraction. For example, taro corm contains water soluble mucilage comprising of a wide variety of non-starch polysaccharides and arabinogalactan proteoglycans[20] which interfere during the extraction of starch. The present study was taken up to examine the efficiency of starch extraction by pretreating fresh tubers of *Dioscorea* genus with a few selected chemicals and to investigate in detail the changes occurring in the physico-chemical and biochemical characteristics of the starch samples thus obtained.

Modified starches have wider applications than unmodified ones as the latter often remain less suitable for many end-use specific applications. Of all the chemical modifications, acid modification has the single advantage that it alters the functional properties of starch without affecting its granular nature. Acid modified starches have wide applications in various starch-based industries[21]. Hence, studies were also taken up to assess the influence of acid modification on structural and granular properties of *Dioscorea* starches.

which are circular in cross section, but others, eg. of *D. alata* may be stellate, rectangular or polygonal[2] in shape. Shape, size and colour of yam leaves vary considerably and being dioecious, male and female flowers are produced on different plants. The yam fruit is a dry, dehiscent capsule, 1-3 cm long. The yam seed is small and possesses a flattened wing structure.

1.4. Tuber Characteristics

The structure of yam tuber varies considerably, depending on the species. The weight of the individual tuber may vary from a few grams to over 50 kg and tuber lengths of 2-3 m have been recorded[2]. Most of the tubers are cylindrical, covered with a thick corky layer.

1.5. Global Production

The global production of yams during 1989-1997 is summarized in Table 1[22]. By far, the largest acreage and the greatest amount of yam production is in West Africa.

Table 1. Global production of yams (in 1000 MT) during 1989-1997[22]

	Production of Yam			
	1989-91	1995	1996	1997
World	21,278	31,531	33,551	29,943
Africa	20,073	30,126	32,100	28,477
North America	396	483	497	459
South America	317	403	424	477
Asia	208	232	242	242

1.6. Composition of Fresh Tuber

The chemical composition of the tuber varies with species and cultivar. Even within the same cultivar, the composition may vary depending on the environmental conditions under which the crop is grown. The proximate composition of three Dioscorea yam species is given in Table 2[23]. By far, the largest component of the fresh tuber is water, which accounts for about two-third of the fresh weight. Carbohydrate, mainly represented by starch, is the major dry matter component of yams, approximately making up for one-quarter of the fresh weight of the tuber. The content of free sugars in yams is often less than 1% on fresh weight basis. But the percentage of sugar may go up to 2-4% as reported for *Dioscorea esculenta*. Protein content of yams is rather low and ranges from 1-2%. Mucilages which exude from the yam tuber consist of mainly glycoproteins. Vitamins and minerals also make up the minor components of a fresh yam tuber. Significant amounts of vitamin C are present in the range of 6-10 mg/100 g of tuber tissue[23].

Table 2. Composition of Fresh tuber[23]

	D. alata	*D. rotundata*	*D. esculenta*
Moisture (%)	65-75	58-73	67-81
Carbohydrate (%)	22-29	23	27-33
Fat (%)	0.03-0.27	0.12	0.04-0.29
Crude protein (%)	1.12-2.78	1.09-1.99	1.29-1.87
Ash (%)	0.67-2.06	0.68-2.56	0.050-1.24
Crude fibre	0.65-1.4	0.35-0.79	0.18-1.51

1.7. Isolation of Starch from Plant Tissues

Extraction of starch from plant tissues and its subsequent purification are relatively simple compared to that of other naturally occurring constituents. As starch exists in a relatively free form, disintegration and maceration of the concerned tissues in excess of water will quantitatively release the granules into the aqueous phase. Extraneous contaminants are normally removed by a simple process of repeated washing with water, followed by sedimentation or centrifugation. However in such cases, where starch granules coexist with non-starchy components such as proteins as in the case of wheat and rice, a pre-treatment with dilute alkali enables easy isolation of starch[24]. In the case of crops such as maize, the material is steeped in water containing sulphur dioxide so as to facilitate starch separation by softening the outer coating[25]. On the other hand, in the case of potato tubers, an initial pulping makes starch extraction considerably easy[24]. Use of mercuric chloride is very often practised to prevent the action of degradative endogenous enzyme on starch, which in turn also ensures better starch quality[26]. In the case of aroids and yams, the major problem encountered is due to the presence of mucilaginous substances. The latter keeps the starch as a suspension without settling. Pretreatment of mucilagenous tubers using 0.3 M ammonia is reported to improve the yield and quality of starch and this method was found to be quite effective during extraction of starch from *Colocasia* tubers[27]. Pretreatment of fresh sweet potato slices with chemical agents such as carbon tetrachloride and carbon disulphide was reported to improve extraction efficiency[24]. Advantages of using lime to solubilize pigments and flocculate certain undesirable contaminants during extraction of starch from sweet potato are exploited industrially[25]. Hoover and Hadziyev[28] used sodium bisulphite during homogenization and extraction of starch from potato tubers.

1.8. Properties and Structure of Starch

Carbohydrates constitute a major class of organic compounds that play a vital role in sustaining and supporting plant life. Starch is the reserve carbohydrate of the plant kingdom and is important to plants as much as glycogen to animals. Starch is generally deposited in the seeds, tubers or roots of plants in the form of minute granules or cells varying from 2 to 150 μm or more in diameter[25]. Starches from different botanical sources exhibit characteristic functional properties, depending on a number of integrated factors including polymer composition, molecular structure, interchain organization and the levels of minor constituents such as lipids, proteins and phosphate ester groups present in association with the polysaccharide.

1.8.1. Granule morphology

The size, distribution, shape and morphology of starch granules are markedly influenced by their botanical origins[29-33]. Thus, cereal starches are generally small and polyhedric, as against tuber starches which are often large and ellipsoidal or spherical in shape. Some of the cereal starches viz of amaranth and cowcockle exist uniformly as very small granules having diameters ranging from 0.5-2.0 μm, whereas cassava starch granules have diameters up to 175 μm. Granule characteristics of starches from different plant sources are given in Table 3[34].

Starch granule composition, morphology and their supermolecular organization are also partly under genetic control[31,35]. Plant biotechnology offers new approaches to modulate starch quality and quantity[36]. A major breakthrough with respect to starch composition could be made by allowing the inactivation of granule-bound starch synthase, in potato tubers using antisense RNA mediated inhibition. The results showed that starch of the transgenic plants had very low amylose content of <5%[36]. In addition to being able to

modify the biosynthesis routes by controlling the expression of endogenous genes in transgenic plants, genetic engineering may also offer answers to more fundamental questions concerning the *in vivo* role of starch synthesis in plants.

Table 3. Granule size and shape of different starches[34]

Starch	Granule size (μm)	Granular shape
Cassava	5-35	Round, flat at one end
Colocasia	1-10	Mostly round
Xanthosoma	10-50	Variable
Amorphophallus	3-30	Round, polygonal
D. alata	16-100	Round or variable
D. esculenta	2-15	Mostly round
D. rotundata	10-70	Oblong, oval, elliptical
Coleus	5-20	Round, oval
Potato	15-110	Oblong, oyster shape
Maize	1-10	Round, polygonal
Rice	2-15	Round, polygonal

1.8.2. Granular properties of starch

1.8.2.1. Gelatinization

Starch granules are insoluble in cold water, but when an aqueous starch suspension is heated above a critical temperature that varies with the type of starch, the hydrogen bonds responsible for the structural integrity of the granule weaken, allowing penetration of water and consequent hydration of the linear

segments of amylopectin. As a result, the molecules start to form helices or coils, creating a tangential pressure causing the granules to imbibe water and swell manyfold to their original volume. Within a specific range of temperature, the hitherto opaque aqueous starch suspension transforms to a translucent gel. The above process or transition taking place in the starch granules is termed gelatinization. The gelatinization results in loss of crystalline and birefringence properties, swelling of granules and leaching of amylose bringing about considerable changes in the viscoelastic properties.

Differential scanning calorimetry (DSC) is an important technique to study the thermodynamics of starch gelatinization, revealing several endothermic phenomena during heating of moistened starch, in terms of initial, peak and end point gelatinization temperatures and thermal energy changes. During gelatinization, DSC measures the extent of disruption primarily of the hydrogen bonds that stabilizes the double helices within the starch granules and quantifies the heat energy, i.e. the enthalpy involved in the transition of starch from a semi-crystalline granule to an amorphous gel[37]. During the study of starch-lipid interaction by DSC, it has been observed that surfactants like sodium dodecyl sulphate (SDS) lowers the gelatinization temperatures of starch, whereas substances like monoglycerides, either delay it or have negligible impact[38,39].

1.8.2.2. Viscosity

An important property of starch is its ability to form a viscous paste when heated in the form of an aqueous dispersion. Generally, root or tuber starches swell more rapidly within a narrower range of temperature than that generally observed in the case of common cereal starches. A Brabender Viscoamylograph is commonly used to measure and study the pasting

properties of starch covering pasting temperature, peak viscosity, hot paste viscosity, hot gel stability, cold paste viscosity and setback value. The starch gels formed from sources such as maize and potato are generally found to retain excellent hot-gel stability. Although starch gel formed from cassava starch has excellent clarity, it is extremely weak, and shows a sharp fall in hot paste viscosity when held at cooking temperature, ie. 95°C. The above difference in the hot gel property could be primarily attributed to the length of the amylose chain, and also to the degree of branching. Shorter the amylose chains, greater are the chances of their getting cleaved and leached into water. It has been observed that incorporation of surfactants affected the viscosity differently. Presence of glyceryl monostearate at higher concentrations was found to reduce the peak viscosity of cassava starch, whereas that of sodium lauryl sulphate increased the peak viscosity[40].

1.8.2.3. Viscoelasticity

An ideal starch for many food products is the one that at low concentration produces a smooth texture with a heavy-bodied paste, which remains soft and flexible at low temperature and retains its thickening power at high temperatures and high shear. In this respect, the study of rheological properties is of major importance. The viscoelastic behaviour of gelatinized starch dispersions is dependant on the shear conditions used during their preparation. Storage and loss moduli (G' and G") can be measured using the Oscillatory mode of a Bohlin rheometer. Generally during heating, there is an increase in the storage (G') and loss moduli (G"), and a decrease in the phase angle, indicating the phase change from sol to gel. A gelatinized starch system has been described as a suspension of swollen starch granules in a continuous phase[41,42]. The initial increase in G' and G" is due to a progressive swelling of starch granules, finally leading to formation of a close-packed system. When

the starch granules become very soft, deformable and compressible, a decrease in G' and G" is observed[43]. When the elastic nature of the material exceeds the viscous nature of the paste, G' predominates over G". Most of the reported values for G' and G" are of starch pastes which have been held at room temperature for several hours after heating. G' for 6% corn and potato starch solutions at 60°C have been reported to be 132 Pa and 124 Pa respectively[42], while G' values for 10% corn starch suspension at 70°C have been found to be 1000 Pa[44]. Reported values of G" for 5% corn starch suspension at 20°C and 4% cassava starch pastes at 25°C are 497 and 16.57 Pa respectively[45].

Presence of emulsifiers like glyceryl monostearate (GMS) and sodium stearoyl lactylate (SSL) at 1% concentration affected the viscoelastic behaviour of starch suspension of corn, wheat, potato and waxy barley by delaying the onset of G' and G". The above effect was, however, less pronounced in the case of the waxy barley starch[46]. The G" of the hot starch pastes except that of wheat starch increased in the presence of GMS, SSL and SDS which resulted in a higher tan δ (G"/G') value compared to that of free-lipid gels. In a subsequent study[47], a cationic surfactant like cetyltrimethyl ammonium bromide (CTAB) was found to increase the G' of native as well as modified corn starch pastes.

1.8.2.4. Retrogradation of starch

When starch granules swell in hot water and the temperature of the system exceeds the gelatinization temperature of the starch concerned, smaller amylose fragments leach into water. When the above starch-water system is cooled under controlled conditions, the solubilized fragments reassociate and tend to precipitate. The above reassociation and reprecipitation is jointly termed as retrogradation. Due to the difference in the intrinsic nature of starch granules, the extent of retrogradation is characteristic for a given starch.

Even though numerous studies have been made on the kinetics of starch retrogradation, the actual mechanism, in particular at the molecular level is still not clearly understood. It has been established that retrogradation consists of two simultaneous processes caused by two components of starch; the rapid gelation of solubilized amylose leading to inter helical reaggregation and the slow re-crystallisation of the short amylopectin chains within the gelatinized starch granules[48-50]. Although the process of retrogradation has been described as the return to the crystalline state[51], the crystal pattern is believed to be considerably different from that of the native starch granules. For fully gelatinized cereal starches, retrogradation induces a change from A to B type of X-ray pattern[52]. The above pattern originates from hexagonal packing of double helices and it is possible that, during retrogradation, both amylose and amylopectin contribute to the formation of double helices in clusters.

The process of retrogradation takes place even in the solid state. The staling of bread is such a process[53], and starches precipitated by alcohol when left moist also readily retrograde. It is, however, possible to increase the shelf-lives of many starch-based foods by adding certain polar monoacyl lipids[54-56]. The added lipids affect the crystallization properties of starch by forming inclusion complexes with the amylose fraction and the outer branches of amylopectin[57,58].

An important consequence of conformational ordering and aggregation of double helical chain segments of retrograded amylose is their resistance to acid and enzyme hydrolysis[59,60]. The amylase-resistant linear amylodextrin originating from retrograded amylose constitutes a fraction of the so called "resistant starch", ie. the starch resisting digestion in the small intestine and becoming available for fermentation in the large intestine of man[61,62]. This type of resistant starch is formed during heat-moisture treatments and also

during cooling of amylose-containing starchy foods, as a result of amylose retrogradation. There is presently considerable interest in the nutritional implications of resistant starch in foods, for they seem to have an influence on reduced plasma glucose and insulin levels[63], increased feacal bulking[64] and the potential for beneficial effects on the health of colonic epithelial cells[65].

1.8.2.5. Alkali absorption and swelling

Alkali absorption and swelling of starch granules bear importance from the point of view of industrial application, as many of the techniques adopted for modifying and derivatizing starch are catalysed by alkali. There is found to be a positive correlation between swelling of starch granules and alkali concentration. It is generally seen that at a constant temperature, starch granules in alkali suspension swell as a function of the equilibrium concentration of alkali. When starch granules are placed in a strong alkaline solution, protons of the -OH group are dissociated leaving behind negative charges on the starch molecules. It is the repulsion between negative charges that results in swelling of starch granules[66].

1.8.2.6. Amylolytic susceptibility

Native starch granules differ in their susceptibility to amylase hydrolysis, depending on both their botanical origin and enzyme sources. Differences in susceptibility are not only ascribed to the degree of hydrolysis but also to the mode of attack and the products of hydrolysis. These behavioural differences are mostly determined by the structure of the granules[67,68]. Due to the differences in their botanical origin, susceptibilities to amylolysis of tropical tuber starches are quite different from other starchy sources[69,70]. Cereal starches are generally more susceptible to enyzamatic hydrolysis than starches extracted from roots, tubers and fruits[71,72]. Potato

starch is found to be much more resistant to amylolysis than many other tuber starches[71]. Most of the starches from *Dioscorea* sp. are less hydrolysed than native potato tuber starch[69]. Unlike potato and *Dioscorea* tubers, cassava starch is as much susceptible as cereal starches such as rice and wheat.

Enzymes responsible for the breakdown of starch are widely distributed in nature. Amylases act on starch by hydrolysing the α-1,4 glycosidic linkages. The amylases may be divided into three groups, the α-amylases which split the bonds in the interior of the substrate (endoamylases); β-amylases which hydrolyse units from the non-reducing end of the substrate (exoamylases) and the glucoamylases which split off glucose units from the non-reducing terminal of the substrate molecule[73].

Different sources of α-amylases are barley malt, porcine pancrease, human saliva, *Aspergillus oryzae* and a few *Bascillus* species. The action of α-amylase on the amylose fraction of starch proceeds in two stages. Initially a complete, rapid degradation of amylose into maltose and maltotriose takes place. The second stage is much slower than the first step, involves a slow hydrolysis of the oligosaccharides with the formation of glucose and maltose as final products. α-Amylolysis of amylopectin yields glucose, maltose and a series of α-limit dextrins, oligosaccharides of four or more glucose residues, all containing α-1,6-glycosidic bonds. The cleavage of glycosidic bonds takes place with retention of configuration.

Jean-Claude et al[74] have studied the mode of action of porcine pancreatic and *Bascillus subtilis* α-amylases on native tuber starches of yam (*D. alata*), sweet potato (*I. batatas*) and tannia (*X. sagittifolium*) and compared with that of potato and cassava starches. It was found that enzyme action on yam starch was three times less efficient than in potato and tannia starches, while sweet potato starch was hydrolysed to half the extent of cassava starch.

Levels of hydrolysis were higher with porcine pancreatic amylase than with *Bascillus subtilis* amylase except for yam starches. Manelius et al[75] while describing the mode of action of bacterial and pancreatic α-amylases on wheat starch have observed that the endosperm is made up of large and small starch granules (A and B). Further it was noticed that 'B' granules undergo faster solubilization during α-amylolysis.

The morphological changes in starch granules as a result of amylolysis have been largely explored[76-79]. It is possible that the action of enzymes causes surface alterations by corroding and degrading the external part of the granule. When endocorrosion occurs, the internal part of the granule is corroded forming small pores by which enzymes penetrate the granule[78,79]. Hydrolysed granules exhibit successive internal layers, which correspond to alternate region with strong and weak enzyme susceptibilities[72,74,76-79].

Different sources of β-amylase are wheat, barley, sweet potato and soybean. The enzyme acts in an exo-fashion from the nonreducing terminals of amylose, amylopectin etc and hydrolyses alternate (1→4)-α-D-glucosidic linkages to liberate one maltose unit at a time in which the reducing D-glucopyranose residue has the β-anomeric configuration. Since the enzyme is unable to bypass α1→6-linkages, the enzymic degradation of branched α1→4-linked D-glucans remains incomplete, whereas the degradation of amylose goes almost completely to maltose. The so called β-limit dextrins therefore contain all the branch points of the parent polysaccharide, but with the outer chains trimmed down close to the outermost branch points.

1.9. Structural aspects

It is now of common knowledge that starch granule is essentially composed of two major components - amylose and amylopectin, as evidenced by dispersion of the granular material and successful separation of the above

two polymeric sub-units[31]. As in the case of many other polysaccharides, amylose and amylopectin subunits in starch are heterogeneous and they widely vary in their size and arrangement of chains. Most of the starches contain between 20 and 35% amylose. Plant breeders have, however, developed mutants that contain waxy starches with practically little amylose, as well as high amylose starch accounting for 50-85% of amylose. Starch owes much of its functionality to its total fine structure as well as to the relative properties of its two distinct subunits. The physical organization of starch polymers into larger structural domains in the solid state is also another highly determinant factor. Further, the minor noncarbohydrate components present in the granular starch particularly lipids, proteins and phosphorous may also be of significance in determining the processing quality and functional properties of commercially important starches[80,81].

1.9.1. Amylose

Structurally, amylose often referred to as the linear starch fraction, mainly consists of α-(1→4)-linked D-gluco-pyranosyl residues. However, it is now evident that this polymer is slightly branched having occasional α-1→6 branch points. According to Hizukuri et al[82] a starch molecule may have 9-20 branching points and side chains comprising of 4 to 100 glucosyl units. The fact that extent of branching depends on the source of starch[83] and increases with the molecular size of amylose from a particular source is reflected from the varying levels of β-amylolysis ranging between 73-95%. The above variation reported depends on the botanical origin of amylose and the extraction procedure[31,84]. When subjected to concurrent action by pullulanase and β-amylase, conversion of amylose to maltose is about 97-100%[85]. For amyloses isolated in laboratory, the reported molecular weight varies between $2.0x10^5$ and $1.2x10^6$ Kd, with polydispersity indices (Mw/Mn) between 1.3

and 5.8[31,84,86,87]. Branching in amylose being inconsequential, it behaves essentially like a linear polymer in forming films and complexing with ligands.

Conformational pattern of amylose in solutions has been a subject of extensive research[31]. Early investigations indicated that the conformation of amylose depends on the type of the solvent employed. Amylose in neutral solutions as well as in solvents such as dimethyl sulfoxide (DMSO), formamide and aqueous alkali behaves like a random coil, whereas presence of a complexing agent enables it to assume a helical conformation in both neutral and alkaline media[31,88]. For high molecular weight amyloses (> 10^5), the conformation in water and DMSO are essentially similar. Small angle X-ray scattering data confirms the view that the average conformation of amylose in aqueous solution is in the form of a highly disordered coil involving many discernible sequences of short-range helical structure that are irregular and labile[89].

There are two features of amylose in solution that are of special relevance to its functionality. The first is the ability of amylose to form helical inclusion complexes with an appropriate ligand. Thus, iodine forms an inclusion complex with amylose that gives rise to the typical blue colour (λ_{max}= 640 nm) and is the basis for quantitative assessment of amylose. Besides iodine, a variety of polar and nonpolar compounds induce coil $\rightarrow$ helix transition of amylose in an aqueous solution. It is the ability of these materials to satisfy the solvation requirements of the hydrophobic helical cavity that enables the polysaccharide chain to adopt a regular conformation where the ligand molecule resides within the helix[31]. The interactions that stabilize the helix are intrachain hydrogen bonds between adjacent glycosyl residues, interturn hydrogen bonds and numerous intra- and intermolecular VanderWaals contacts[90,91]. The second important feature regarding the solution behaviour of

amylose is its tendency to form interchain double helix between chain segments comprising of less than 100 glucose units. The above two features contribute to the instability of amylose in solutions compared to that of amylopectin.

1.9.2. Amylopectin

Amylopectin with a molecular weight of the order of 10^7-10^9 [31,92] is one of the largest known naturally occurring macromolecules. Studies on methylation and periodate oxidation of starches have indicated that amylopectin molecule has 4-5% of interchain α-1→6 linkages leading to a highly branched, but a compact network structure.Because of its branched nature, amylopectin displays relatively low intrinsic viscosity in the range of 120-200 $mL.g^{-1}$, despite its high molecular weight[31].Although the average length of unit chains in an amylopectin molecule is of the order of 20-25[93], the chains differ considerably in their length and distribution[94,95]. A-chains in an amylopectin molecule are unbranched and linked to the molecule through their reducing end groups.On the other hand, B-chains (B_1 - B_4) are joined to the molecule in the same way as A-chains, but the former carries one or more A-chains. The third type called C-chain has the free reducing end group of the amylopectin molecule (Fig.1). The shortest chains A and B_1 have 14-18 and the longer ones B_2-B_4 have 45-55 glucose units.The molar ratio of short to long chains varies between 3:1 and 12:1, depending on the botanical origin of the starch[93]. Compared to tuber starches, cereal starches generally have larger amounts of short chain fractions[93,94]. Further, both short and large chain fractions are made up of shorter unit chains[93,94]. Various enzymic methods have been devised for structural analysis of amylopectin[31,96-100]. The polymodal chain distribution in amylopectin, as revealed from gel filtration chromatography of their enzymic digests obtained using debranching enzymes, coupled with other relevant structural analysis of acid-resistant

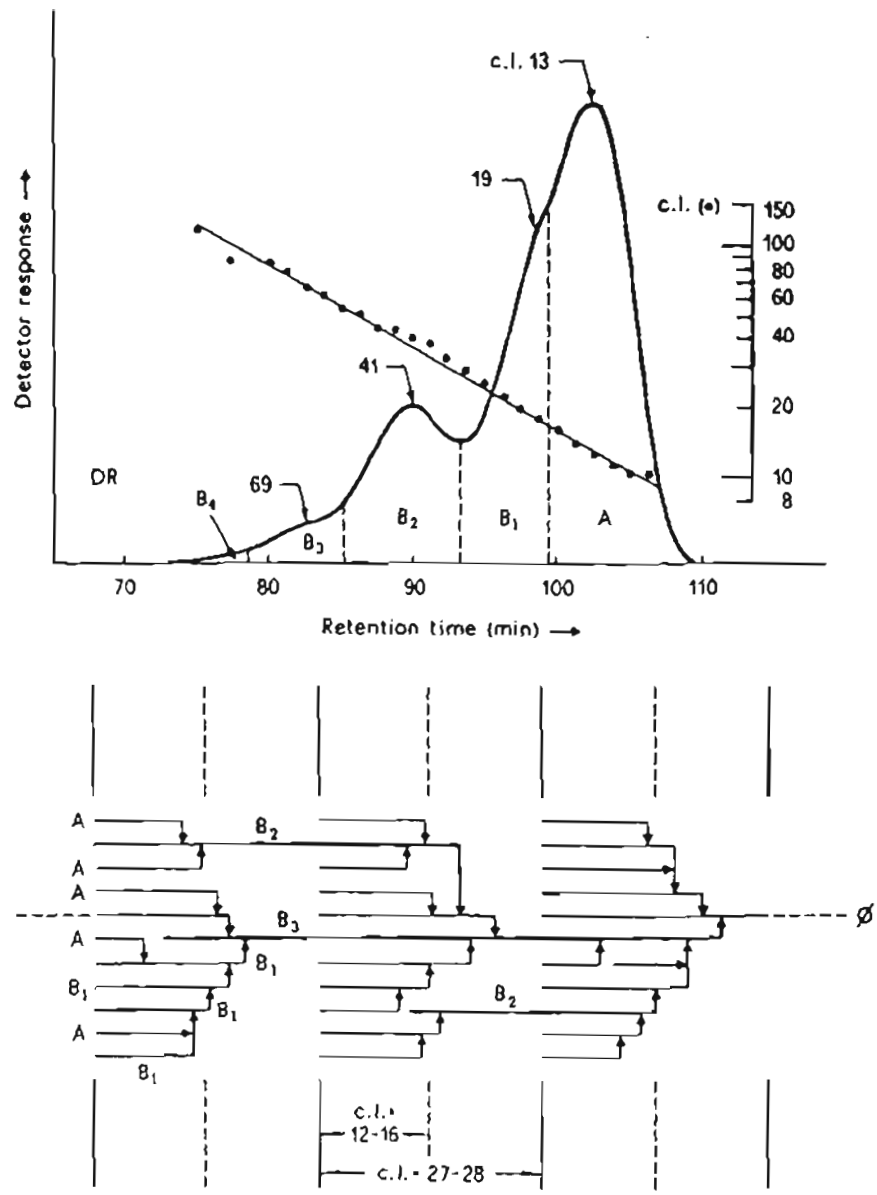


Fig 1: Chain distribution profile (waxy rice starch) and model for amylopectin structure according to Hizukuri[94].

amylodextrins[101-105] confirms the cluster-type model proposed by French[101] and Robin et al[102]. Two general features of the above model are that amylopectin is composed of compact parts of oriented chains existing as crystalline clusters and that the branching points are distributed in an orderly manner throughout the macromolecule. Conformational analysis and molecular modelling of the branching points have revealed that side chains can remain parallel to the main backbone strand, allowing formation of double helices[106]. As amylopectins, particularly those from root and tuber starches, contain phosphate (ester) groups, mainly at C_3 and C_6 positions, they impart properties of a polyelectrolyte to the macromolecule[31]. Physico-chemical and biochemical studies hitherto carried out on potato starch indicate that the C_3 phosphorylation level is almost constant, independent of the cultivars, while the extent of phosphorylation at C_6 shows variation[107,108].

The extensive branching in the amylopectin molecule maintains a lower level of hydrolysis by β-amylase when compared with that of amylose. Also, unlike with amylose, iodine is unable to form stable and strong complexes with amylopectin because of short length of the unit chains. Small amounts of iodine (< 0.6%) however can bind with amylopectin forming a red-brown complex having an adsorption maxima between 530-540 nm. The ratio of Mw/Mn for amylopectin has been reported to be about 300, indicating a wide distribution of molecular sizes imparting more stability to amylopectin solutions.

Starch granules from crops such as amylomaize and wrinkled pea contain substantial amount of an intermediate subunit which is heterogeneous with respect to structure and molecular size. According to Banks and Greenwood[31], the above fraction consists of linear chains with DP of 50-200 and lightly branched low-molecular weight molecules with greater chain length

than normal amylopectin. Amyloses having branches upto 20 or more may also be a part of the intermediate material[100]. Because of its uniqueness in structure, heterogeneity and high instability in solution leading to extensive retrogradation, the intermediate material has not been easily fractionated and fully characterized.

1.9.3. Fine structure of native starch

Transmission and scanning electron microscopy (TEM and SEM) have largely contributed to the elucidation of the fine structure and morphological features of granular starch. A general picture that has emerged from the ultrastructural studies of granular starch is that the growth rings having thickness in the range of 120-400 nm, consist of alternating crystalline and amorphous lamellae[35]. According to current models of amylopectin[35,94,102,109] parallel helices assemble to form radially oriented clusters. Oostergetel and Van Bruggen[110] have however proposed a superhelical lamellar structure for amylopectin in potato starch based on TEM and electron diffraction data of nondisrupted granule fragments. The crystalline regions of 5 nm thickness consist of double helices comprising short chains of amylopectin that interpenetrate forming a continuous superhelical network. The above arrangement of crystalline domains in a helical fashion leaves behind voids of ~8 nm in diameter.

Gallant et al[111] based on their review of the old and new microscopic studies have proposed a novel "blocklet concept" to elucidate the structural organization of starch granule. By analogy with the 'superhelical' amylopectin structure[110] the above authors claimed that the crystalline and amorphous lamellae of amylopectin are organized into larger and more or less spherical 'blocklets' having diameters ranging between 20 and 500 nm. The size and diameter of the blocklet are found to be dependent on botanical source of starch

as well as its location in the starch granule and each blocklet accommodates clusters of 5-50 amylopectin short - DP chains. With the above type of granule organization, amorphous material could exist in different regions (1) in each lamella, (2) between clusters of side chains within each lamella, (3) around each blocklet of side-chain clusters and (4) in radially arranged channels in the granules, through which amylose can leach out during gelatinization. The blocklets may be viewed as the structural elements of alternating crystalline and partially crystalline shells of the starch granules as often seen in SEM micrographs of granular starches corroded by α-amylases. The size and arrangement of blocklets in a native starch could be important determinants of the granule resistance to enzymic attack.

Crystallinity of native starch granules is characterized by their distinct wide-angle X-ray powder diffraction patterns, that could be classified into A, B and C types. 'A' form is typical of cereal starches, while the 'B' pattern is attributed to tuber starches as well as high-amylose and some waxy starches[112]. The C-pattern is believed to be a superimposition of A and B types. Starches with short chain amylopectin comprising of less than 20 glucose residues exhibit A-type crystallinity whereas those of longer average chain length display 'B' type pattern[94,113]. Another crystallographically distinct structure of starch, typically observed when amylose forms complexes with various ligands is the V-pattern[57,114,115]. In V-amylose, the chain conformation is a left-handed single helix with six residues per turn for complexes with aliphatic alcohols and monoacyl lipids[90,91].

In the case of normal starches only a small portion of the starch granule appears to be crystalline. Kainuma and French[116] have visualized the hydrated starch granule as an amorphous gel matrix in which the crystalline regions are embedded. Crystallinity values for granular starches range from 15-45% with

the A-type showing values between 33-45%[32]. Waxy starches give good X-ray diffraction patterns, while high amylose starch shows very low crystallinity ranging between 15-22%[32,117].

Zobel[32] and Blanshard[15] suggested that amylose remains separated from amylopectin in corn and wheat starch granules while it remains associated with amylopectin in potato starch. Later works[118,119] have confirmed the cross-linking of amylose with amylopectin and also reported that there is no cross-linking among amylose molecules in potato starch. It should be therefore inferred that in native potato granular starch, amylose molecules do not exist in the form of bundles at amorphous region, but they are dispersed among the amylopectin molecules. It is also possible that some of the amylose molecules participate in the formation of double helices with amylopectin, thereby becoming less prone to aqueous leaching or complexation with iodine, particularly in potato starch.

1.10. Modifications of Starches

Apart from the direct use of cereals and tubers as a staple food, the starch extracted from the above crops also finds immense use in various food and non-food industries (Table 4[120]). As no single starch in its natural form is uniformly suitable for performing all its functions, starches are often modified and tailor-made so as to meet with specific requirements. Modified food starches provide improved viscosity control over a broad range of processing variables like pH, temperature and shear. Modifications of the properties of native starches are mainly directed to improve their functions and expand their usefulness in industrial applications. Starches are modified by means of physical, chemical or biochemical methods. The distinct objectives of modifications of starches are presented in Table 5[121].

Table 4. Industrial uses of starch[120]

Industry	Use of starch/modified starch
Adhesive	Adhesive production
Agrochemical	Mulches, pesticide delivery, seed coatings
Cosmetics	Face and talcum powders
Detergent	Surfactants, builders, co-builders, bleaching agents and bleaching activators
Food	Viscosity modifier, glazing agent
Medical	Plasma extender/replacers, transplant organ preservation, absorbent sanitary products
Oil drilling	Viscosity modifier
Paper and board	Binding, sizing, coating
Pharmaceuticals	Diluent, binder, drug deliver
Plastics	Biodegradable filler
Purification	Flocculant
Textile	Sizing, finishing and printing, fire resistance

Table 5. Distinct objectives of starch modification[121]

To modify cooking characteristics
To decrease retrogradation
To decrease gelling tendencies of pastes
To decrease freeze-thaw stability of pastes
To decrease paste and/or gel synerisis
To improve paste and/or gel clarity and shear
To improve paste and/or gel texture
To improve film formation
To improve adhesion
To add hydrophobic groups (for emulsion stabilization)

1.10.1. Physical modifications of starches

During the early periods, starches were physically modified essentially to improve viscosity, solubility and to impart cold water swelling power. The early methods hence stressed mainly on particle size reduction by a simple process of grinding. The extent of reduction in viscosity achieved was found to

be directly related to the duration of grinding[24]. A technique for making soluble starch was developed by subjecting the starch to a shearing action of two closely fitted rollers moving in opposite direction[24]. Use of atomized jet spraying under pressure or atomizing starch milk in hot air-stream yielded powder that disintegrated faster and having better gelling property[24]. The overall advantage of the above process was that it reduced the swelling of starch granules in hot water and hence lowered the viscosity of the solution. Cooking and drying of starches on hot drums or spray dryers were found to impart cold water swelling property to starches[122].

1.10.2. Modifications of functional properties - later approaches

Subsequent to the earlier simple mechanical devices to modify starch properties, consistent efforts have been successfully made to improve or modify the functional properties of starches by various physico-chemical methods. Cooked-up starches were prepared by means of pregelatinization followed by spray-drying. Lorenz and coworkers[123] initiated studies on impact of heat-moisture treatment on physico-chemical properties of starches from various plant origins. The above studies disclosed that a root starch such as cassava starch when heat modified in presence of restricted amount of moisture undergoes changes in the molecular orientation to such an extent that its pasting characteristics closely resemble with that of cereal starches. Further, heat- moisture treatment was found to retard swelling power of starch granules and impart higher amylolytic susceptibility than untreated samples. Later work proved that steam-pressure treatment of cassava starch is very effective for producing low but stable viscosity starch[124]. The effect of steam pressure treatment on the physico-chemical properties of *Dioscorea* starches has been reported recently and the results suggest that starch granules are compressed by the steam pressure treatment, leading to vast changes in physico-chemical

properties[125]. Parboiling and subsequent roasting is the most effective method for upgrading the cooking characteristics of cassava flour[126]. The functional properties of fresh cassava for use as chips and flour can be modified by steam-hydrothermal treatment[127]. Paste stability and setback properties of cassava flour can be improved also by the presence of salts like sodium sulphate[128].

Properties of cassava starch could be modified by subjecting it to alkali treatment under controlled experimental conditions such that treatment causes a reduction in amylose content and higher alkali number and susceptibility to α-amylase[129]. These properties could be advantageously made use of for preparing maltodextrins having DE 20-23.

Granular cold-water-soluble starches were prepared from maize starch by treating it with mixtures of ethanol and sodium hydroxide solution at controlled temperature[66]. These starches swelled instantaneously when rehydrated in cold water and most of the pastes had better viscosity and freez-thaw stability.

1.10.3. Chemical modification of starches

General techniques adopted for chemical modification involve derivatization of starch by means of oxidation, esterification or etherification[130] using appropriate chemical agents. Starches are derivatized essentially to modify their gelatinization and cooking characteristics. As a result of derivatization, high amylose starch granules show lower retrogradation and gelling tendencies. Correspondingly, there is an increase in water-binding capacity of starch dispersions at low temperature, thereby minimizing syneresis. Depending upon the nature of the chemical used, derivatized starchcould display increased hydrophilic or hydrophobic properties. In general, derivatization involves introduction of a suitable substituent group through interaction with free hydroxyl groups of the glucose moiety.

An alternate approach to derivatization is to prepare cross-linked starches by chemical agents such as sodium trimetaphosphate[130]. The latter randomly spotwelds the starch molecule. The reinforced starch gel becomes a heavier bodied and more viscous material during heat processing. The inter-molecular phosphate bonding within the starch granule causes a reduction in the chain length and prevents further breakdown during heat processing. Starches cross-linked as above are more suitable for application in textile and paper industry. Other common cross-linking agents are epichlorohydrin, vinyl sulfone, diepoxides, phosphorous oxychloride etc.

Chemical modification by esterification and etherification[131-135] has been extensively studied. Acetylation of starch can be carried out using acetic acid, acetic anhydride, vinyl acetate etc. A rapid method for preparation of starch succinates by means of reactive extrusion has been recently reported[136]. Acetylation[130] and succinylation[136] impart a number of desirable properties which could be advantageously made use of in food, paper and pharmaceutical industries.

Complexation of cassava starch with dicarboxylic acids like oxalic, malonic and succinic acids increases the solubility in water and DMSO[137]. The most important cationic derivatives of starch contain tertiary amino or quarternary ammonium groups. The gelatinization temperature of cationic starches decreases as the number of cationic substituent group increases and the starch dispersions show improved stability and clarity[130]. In addition to the above chemical modifications, starch xanthate, hydroxyalkyl starches and dialdehyde starches have been successfully prepared and used for a variety of purposes[130].

Some types of starches in their natural form exhibit 'syneresis', during cooling, due to reassociation of linear fragments chopped off from the granule

during heat processing. The above tendency can be prevented by introduction of anionic groups. Such anionic starches are used as ingredients in canned foods.

Grafting of unmodified starches with synthetic polymers such as acrylonitrile and methyl methacrylate yields a material having higher water absorbency, and hence find greater applications in agriculture and plant tissue cultures.

Treatment of starch with acid, without substantially changing the granular form results in modified starch with properties that have commercial value. Studies on the effect of treating potato starch with mineral acids were pioneered by Kirchoff and later pursued by researchers such as Lintner, Nageli, Bellmas and Duryea[138]. Later, a lot of work has been carried out on the effect of acid modification on starches from various sources such as rice[139], waxy maize[140], maize and cassava[141], wheat[142], *Cannavalia ensiformis*[143] and arrowroot[144]. According to reaction kinetics, there exists two phases, i.e. a faster hydrolysis of the amorphous fraction and a slower degradation of crystalline starch grain fraction[140]. Acid modification plays an important role in the manufacture of other types of modified starches and may be used as a pre-modification step in some cases or as postmodification step in others. Industrial uses of acid modified starches include textile, building products, starch gum candy, paper and paperboard manufacture.

1.10.4. Biochemical modifications

Biochemical modifications or more precisely enzyme modifications of starch produce a number of industrially important compounds. Cyclodextrins are cyclic, nonreducing maltooligosaccharides produced from starch by the enzyme cyclodextrin glucosyl transferases isolated from certain bacteria. The enzymes are capable of cleaving starch to dextrins containing six, seven and

eight glucopyroanosyl residues and rearrange them to form cyclic compounds having a torus shape. They are called cyclo hexa, hepta and octaamyloses represented as α, β and γ-cyclodextrins having very wide applications in biotechnology[145]. Cyclodextrins and their derivatives enhance the solubility of complexed substrates in aqueous media, but do not damage the microbial cells or the enzymes. Cyclodextrins or their fatty acid complexes can substitute mammalian serum in tissue cultures. A highly soluble γ-cyclodextrin-nystatin complex can protect tissue cultures from fungal infections[145]. The tolerance level to toxic compounds during biological detoxification of organic chemical industries sewage can be elevated by admixing small amounts of β-cyclodextrin to the system. Cyclodextrins form inclusion complexes with numerous different guest molecules, hence they can stabilize labile compounds, emulsify oils, mask tastes, flavours or odours, increase solubility and convert viscous or oily compounds into powders[146]. They are also used for catalysis of chemical reactions or as models for enzyme systems.

Enzyme catalysed starch hydrolysis yield products which include maltodextrins, corn syrups, glucose syrups, high maltose and high fructose syrups and are widely used for their functional properties in food, textile and brewing industry. Enzymatic hydrolysis possesses some distinct advantages over acid hydrolysis. Griffin et al[147] prepared maltodextrins from milled rice flour using heat-stable α-amylase at a processing temperature of 80°C.

Possibilities for biological modification of starch also include plant breeding, mutant generation and crossing and genetic engineering of plants[121]. Molecular biology has accomplished more to date in increasing starch content in potato tubers[148] than in modifying cereal grain starches.

1.11. Applications of Starch

Apart from the application of native and modified starches in various industries like paper, textile, food, pharmaceuticals, adhesives, foundries, fertilisers, detergents, mining engineering and metallurgical industries which are rather traditional[6], applications of chemically or biochemically modified starches have opened new avenues for utilizing this naturally abundant polymer in many industries either as a substitute or in combination with synthetic polymers. Starch has received considerable attention as one of the most favourable materials from which biodegradable plastics can be developed[149]. An extrusion processing of starch with synthetic polymers was developed for the preparation of starch-based biodegradable plastics[150] in the early 1980's. Granular starches have been used as fillers in biodegradable polymers, in an attempt to make these new polymers price-competitive with petrochemical plastics[151]. Biodegradable plastic production is still in its infancy when compared to petrochemical plastic production. Many different approaches are being pursued in order to produce materials with the desired properties. Starch can play and is playing an important role in these developments. Versatile biodegradable materials have been produced by microbial fermentations that use starch as a carbon source[152]. Koch et al[153] have remarked that it is possible to produce a new generation of detergents in which the surfactants, builders and co-builders and bleaching activators could all be derived from starch. The interest in starch has recently been renovated because of its inherent biodegradability and it is envisaged to have great potential for food packing edible films[7-14]. A good deal of work has been carried out by Arvanitoyannis et al[9,10] on edible films made from starches of corn and wheat blended with microcrystalline cellulose or methyl cellulose and these films proved to have properties for food packaging applications. Studies on biodegradable films

made from low density polyethylene, ethyleneacrylic acid, polycaprolactone and wheat starch have shown that with higher starch content the composite materials exhibited lower tensile strength and modulus, higher gas permeation and water vapour transmission rate and higher biodegradability[7].

In addition to the present day use of starches, researches in the area of starch conducted world over unambiguously suggest that starch could be an alternate material of choice to meet the exceedingly high demand for petroleum based chemicals in the non-food industries like polymer synthesis. Polyamides are one of the most important synthetic polymers and methods have been developed to synthesize carbohydrate derived or starch derived polyamides[154]. Increased application of renewable resources in view of the continuous depletion of fossil raw materials may render the development of novel carbohydrate or starch-derived materials more technologically and industrially relevant during the coming decades.

CHAPTER II

MATERIALS AND METHODS

2.1. Materials

2.1.1. Raw materials

Fresh and matured tubers of *Dioscorea alata, Dioscorea rotundata* and *Dioscorea esculenta* were procured from the local farm-holdings and brought to the laboratory and made use of in the present study.

2.1.2. Chemicals

Sodium hexametaphosphate (SHMP), glycerylmonostearate (GMS), potassium metabisulphite (KMS), sodium chloride (NaCl), ammonia (NH_4OH), citric acid, disodium hydrogen phosphate, sodium acetate, acetic acid, and dextrose of Analytical/Guarantied reagent (AR/GR) grade were supplied by S.D. Fine Chemicals Ltd., Ranbaxy and Merck (India). Dinitrosalicylic acid (DNS) and Folin's reagent were procured from Spectrochem Ltd., Mumbai. All common chemicals including mineral acids and alkalies used for the present study were either AR or quality reagents (QR) grade.

2.1.3. Bovine Serum Albumin (BSA)

BSA, used as protein standard was procured from Sigma (USA).

2.1.4. α-amylase

Bacterial (*Bacillus* sp.) α-amylase having an activity of 22.5 units/mg solid was purchased from Sigma (USA).

2.2. Methods

2.2.1. Pretreatment of fresh tubers and preparation of control and experimental starch samples

Starch samples were prepared in the laboratory from fresh roots of *Dioscorea* (sp.) as described below.

250 g fresh roots were peeled and washed with tap water and sliced to samples of ~3 cm thickness. The samples were ground with 150 ml tap water in a homogenizer, and then mixed with 1 litre of water and settled for one hour. The homogenate was filtered through a sieve of 180 mesh size and washed with 1 litre of water and the residue was discarded. The filtrate containing starch was left overnight at room temperature (28-30°C) under a layer of toluene in order to settle the starch. The settled starch was separated by decanting the supernatant and again washed with 1 litre water and kept for 3 hours. The supernatant was decanted and the wet starch was initially air-dried and subsequently dried in a mechanical drier at a temperature $\leq 60^{\circ}C$ to a moisture content of $\leq$ 10%. The above starch sample was used as the control. For preparing experimental samples, the above procedure was followed and instead of water, aqueous solutions containing 1-5% w/v of NaCl, KMS, NH_4OH, SHMP and 0.025-0.125% w/v of GMS were used for the extraction. Each of the starch samples obtained after extraction was dried to the same moisture range as that of control sample and stored in a desiccator pending detailed analytical studies.

2.2.2. Acid modification of starch samples

Acid modified samples of *Dioscorea alata*, *Dioscorea rotundata* and *Dioscorea esculenta* were prepared by slightly modifying the technique of Jenkins et al[142]. 1.5 g each of the starch samples was weighed and transferred to a 250 ml conical flask, into which 100 ml 2.2 M HCl was added. The flask was held at room temperature of about 30°C for durations of 8, 24, 32, 48, 56 and 72 hours. The zero hour sample was prepared by reducing the contact time of starch with acid as minimum as possible, but maintaining other experimental conditions similar to that for preparation of rest of the samples. The conical flasks containing the sample solutions were gently agitated periodically to re-

suspend the starch sample uniformly in the acid. After the respective reaction time, each sample solution was filtered using a vacuum filter pump and the starch residue was washed repeatedly with distilled water and dried at room temperature of 28-30°C under vacuum. After vacuum drying, the samples were left exposed to room temperature of 28-30°C so as to enable the sample to condition the moisture content.

2.3. Analysis

2.3.1. Moisture content

The moisture content in the sample was determined by air-oven method as per AOAC[155]. For the above, the samples were dried for 18 hours at 100°C in an air-oven provided with thermostatic control.

2.3.2. Starch content

Starch content in the samples was determined as follows: 500 mg starch was accurately weighed and transferred into a 100 ml conical flask followed by the addition of 20 ml of 80% ethyl alcohol. Samples were kept overnight, filtered and washed with water. The residue was transferred to the conical flask with 20 ml 2N HCl followed by washing with water and the solution was heated till a pale yellow colour was obtained. After cooling to room temperature (28-30°C), the volume of the solution was made up to 100 ml. 1 ml aliquot from the above solution was pipetted into a 100 ml volumetric flask and the volume was made up to the mark using distilled water. The total carbohydrate was estimated using 1 ml of the above diluted solution by phenol-sulphuric acid method[156] and the values were converted to starch content by multiplying with a factor of 0.91.

2.3.3. Ash Content

Ash content of the starch was determined as per the method of Smith[157].

2.3.4. Protein estimation

The protein content in the starch samples was determined essentially by Lowry's method[158], with the following modifications. 250 mg starch sample was dissolved in 10 ml of 1N sodium hydroxide and made up to 25 ml in a volumetric flask using distilled water. After centrifugation at 10,000 rpm, aliquots of 2 ml were transferred to test tubes, followed by addition of 5 ml mixed copper reagent. Samples were allowed to stand for 10 minutes and 0.5 ml phenol reagent (Folin-Ciocalteu reagent) was added and kept for further 30 minutes. The absorbance was measured at 660 nm using a spectrophotometer (Spectronic-20) against reagent solution as blank.

A standard curve was calibrated using Bovine serum albumin (BSA) as the protein standard.

2.3.5. Lipid content

The total lipids in the samples were extracted by stirring 1 g sample in 50 ml of 85% aqueous methanol, for 2 hours at room temperature. The solution was filtered through Whatman filter paper No.1 and the filtrate was transferred to a pre-weighed petridish and dried at 100°C for 18 hours in an air-oven. The dish along with residue was cooled, weighed and the percentage of lipid was calculated from the value obtained.

2.3.6. Amylose Blue value

Apparent, true and hot water soluble amylose contents were determined essentially by the reported procedures[159,160] as described below and expressed as blue value[161].

2.3.6.1. Apparent amylose

100 mg of starch was accurately weighed and quantitatively transferred to a 100 ml standard volumetric flask and the sample was dispersed in 1.0 ml of 95% ethyl alcohol and 9.0 ml of 1N sodium hydroxide, by keeping overnight at room temperature of 28-30°C. The volume of the starch solution was made up to the mark with distilled water. 2.0 ml aliquots of the solution were pipetted out into 50 ml volumetric flask, and the solution was neutralized by dropwise addition of 1N hydrochloric acid using phenolphthalein as indicator. Subsequently, 1.0 ml of iodine solution (0.02% iodine in 0.2% potassium iodide) was added and volume of the solution was made up to the mark. After 20 minutes, the intensity of the blue colour was read at 630 nm in a spectrophotometer (Spectronic 20) against a reagent blank. The amylose blue value was calculated using the equation:

$$\text{Blue value} = \frac{\text{absorbance} \times 4}{100}$$

2.3.6.2. True amylose

100 mg of starch sample, defatted using 85% methanol as described in 2.3.5 was taken and the amylose blue value was determined as already mentioned (2.3.6.1).

2.3.6.3. Hot water soluble amylose

100 mg of starch was accurately weighed and transferred into a 100 ml loosely stoppered conical flask followed by addition of 40 ml distilled water. Starch was gelatinized by immersing and shaking in a boiling water bath for 10 minutes after which the flask was taken out from the water bath and was allowed to cool to room temperature (28-30°C). After making up the volume to 100 ml, the solution was filtered. 5.0 ml aliquot from the filtrate was pipetted

into a 50 ml volumetric flask and 1 ml iodine reagent was added. After adjusting the volume of the solution to the mark, the intensity of the blue colour was read at 630 nm in a spectrophotometer (Spectronic 20) after 20 minutes against a reagent blank. The amylose blue value was calculated using the equation:

$$\text{Blue value} = \frac{\text{absorbance} \times 4}{250}$$

2.3.7. Free reducing Sugar

Free reducing sugar content of the starch samples were determined as follows. 1 gm starch sample was dispersed in 100 ml of distilled water taken in a 200 ml volumetric flask by stirring for one hour at room temperature (28-30°C). The solution was filtered and 2.0 ml aliquot from the filtrate was analysed for reducing sugar content by DNS method[162].

2.3.8. Cold water solubles

The cold water solubles in the starch samples were determined as per the experimental procedure given below. 1 gm starch sample was dispersed in 50 ml of distilled water taken in a 100 ml volumetric flask by stirring for 2 hours at room temperature (28-30°C). The solution was filtered and 10 ml of the filtrate was transferred to a pre-weighed petri dish and dried at 70°C by placing in an air-oven. The dish containing the residue was cooled by keeping in a desiccator and the weight of dish with residue was determined. From the weight of the residue thus obtained, percentage cold water solubles was calculated using the equation:

$$\frac{W2-W1}{W} \times 100$$

2.3.9. Swelling volume and solubility

Swelling volume (ml/g of starch) and solubility (%) of starch samples were determined by adopting Schoch's procedure[163] as follows.

100 mg each of the samples was accurately weighed and transferred to 50 ml conical flask. After adding 10 ml of distilled water, the flask was placed in a boiling water bath for 10 min to get a translucent suspension of the sample. Subsequently, the solution was centrifuged in a Remi R8C model Centrifuge at 2,000 rpm for 10 minutes and the volume of the residue was noted. 2.0 ml portion of the aliquot from the filtrate was pipetted and transferred to a pre-weighed petridish and dried at 100°C by keeping in an air-oven for 18 hours. The dish was cooled and weighed, and from the value obtained, the percentage solubility was calculated using the equation:

$$\%\,\text{Solubility} = \frac{\text{Difference in weight} \times (10 - \text{volume of the residue})}{01. \times 2} \times 100$$

2.3.10. Intrinsic viscosity[164]

To determine the intrinsic viscosity (η_i) of the starch sample, the relative viscosity (η_r) of 0.5% alkaline(NaOH) solution of starch sample was determined at 30°C using Ostwald viscometer. From the η_r value thus obtained, the intrinsic viscosity, η_i was calculated using the equation

$$\eta_i = \frac{2.303 \log \eta_r}{\text{concentration}}$$

2.3.11. Differential Scanning Calorimetry (DSC)

The gelatinization characteristics of the samples viz. onset and completion of gelatinization denoted by T_i and T_f and gelatinization enthalpy

(ΔH) were examined using a differential scanning calorimeter, Seiko S II 6200 model (Japan) equipped with a standard soft-ware. The instrument was calibrated using Indium, H (fusion) = 6.80 cal. g^{-1}. Starches of known moisture content were weighed (1 to 3 mg of dry starch) in aluminium pans and moistened by adding deionized water maintaining a material to water ratio 1:3 and the pans were hermetically sealed. Sealed pans were allowed to equilibrate for 1 hour before heating. An empty pan was used as the reference material and the sample was heated from 15°C to 100°C at a rate of 5°C/min and cooled from 100°C to 30°C at a rate of 30°C/min. The experiment was performed under inert atmosphere by allowing a stream of nitrogen to flush through at 30 ml/min. Each experiment was done in replicates of 2-3 and the mean value was calculated. DSC analyses of the retrograded starches were done after keeping the starches in a refrigerator at 4°C for 7 days.

2.3.12. Rheology

2.3.12.1. Brabender Viscoamylograph

5-7% aqueous starch suspension was prepared by dispersing 20-28 g of starch (on dry weight basis) in 400 ml distilled water, and the suspension was quantitatively transferred to the stainless steel bowl fitted on a Brabender viscoamylograph. The experiment was conducted by allowing the bowl to rotate at 70 rpm while heating the suspension from ambient to 95°C at a rate of 1.5°C/minute. The starch slurry was held at 95°C for 15 minutes and subsequently cooled to ambient conditions for 30 minutes. From the viscogram, values for pasting temperature (°C), peak viscosity (P) and hot paste viscosity (H) values were recorded.

2.3.12.2. Bohlin rheometer

1.25g starch was slurried with 25 ml distilled water so as to get a final concentration of 5% (by weight) starch and 95% (by weight) water. To avoid

settling of the starch during the test in the rheometer, each sample was heated in a water bath to 75°C or until all the starch became a paste, before placing the sample in the rheometer. After heating in the water bath, the slurries were poured into the cup of a C-25 measuring system of a VOR Bohlin rheometer operating in the oscillation mode. The experimental conditions used were -

torque element	1.542 g/cm
amplitude	3%
sensitivity	1%
thermal equilibrium time	10 sec.
heating rate	1.5°C/minute.

The samples were heated from 75-95°C, held at the above temperature for 10 minutes and cooled at the same rate as in heating, from 95 to 35°C. It was allowed to remain at 35°C for 60 minutes. Storage and loss moduli (G' and G") were measured using the oscillatory mode of the Bohlin rheometer. G' is associated with the periodic storage and complete release of energy in a sinusoidal deformation process. G" reflects the non-recoverable use of applied mechanical energy to cause flow in the specimen.

2.3.13. X-ray Diffraction

X-ray diffraction pattern of starch samples were studied on a Philipp's X-ray Diffractometer (model PW 1070) using Cuk_{α} radiation under the following conditions.

Voltage	40 KV
Current flow	20 mA
Scanning speed	2.4" (2θ) per minute
Chart scale	1 cm = 1° (2θ)
Intensity	1ε3

2.3.14. Enzyme Susceptibility

The enzyme susceptibility of the samples with respect to α- amylase was studied by the method already reported by Raja et al[141] after appropriate modifications as given below.

2.3.14.1. Preparation of citrate-phosphate buffer[165]

Citrate-phosphate buffer (pH 5.6) was prepared by mixing 21 ml of 0.1M solution of citric acid and 29 ml of 0.2 M solution of dibasic sodium phosphate and diluted to 100 ml using distilled water.

2.3.14.2. *In vitro* α-amylase susceptibility

50 mg starch in 50 ml of citrate-phosphate buffer (pH 5.6) was gelatinized by keeping in a hot water bath at 70-80°C. After cooling the solution to ambient conditions 1.0 ml aliquot was transferred to test tubes preincubated at 70°C by keeping in a constant temperature water bath. Equal volume (1.0 ml) of the α-amylase, prepared by dissolving 25 mg enzyme in 25 ml citrate-phosphate buffer and further diluting the solution by 1:9 using the same buffer, was added to each of the tubes. Samples in triplicate were subsequently incubated at the temperature mentioned above for a total period not exceeding 45 minutes. After successive intervals of 0, 15, 30,45 and 60 minutes, corresponding set of three samples was removed from the water bath and the enzyme reaction was terminated by adding 2.0 ml of dinitrosalicylic acid (DNS) reagent. The reducing sugar content in each sample was estimated using dextrose as the standard[162].

2.3.15. Moisture Sorption Study

The moisture uptake pattern of starch samples was examined at different RH(%), but at a temperature of 25-27°C. The experimental procedure adopted

was as follows. Sulphuric acid of four different normalities, 8.3, 7.4, 6.3 and 5.2 N to give corresponding relative humidity, RH(%) of 70, 75, 80 and 85 were taken in four desiccators and desiccators were initially equilibrated for the respective RH conditions of known water content. 2-3 g of starch samples were accurately (upto four decimal) weighed in preweighed petri dishes and were placed in the desiccators and exposed to the respective RH conditions. The gain or loss in weight of the samples were determined on 2nd, 4th, 8th, 12th day etc till a constant weight was obtained. The experiment was terminated once the sample attained the constant weight. From the change in the weight and the initial moisture content, the moisture uptake of the samples at different RH were calculated and the pattern was compared.

2.3.16. Statistical Analysis

The results of the experiments were analysed statistically and the standard deviation was determined wherever possible.

CHAPTER III

EFFECT OF CHEMICAL PRETREATMENT OF DIOSCOREA TUBERS ON PHYSICO-CHEMICAL PROPERTIES OF DIOSCOREA STARCHES

INTRODUCTION

Although the major commercial sources of starch are cereals such as maize, wheat and rice and tubers covering cassava, arrowroot, potato and sweet potato, several other edible crops have been identified and reported as potential sources of starch[166-172]. One of them for the future could be *Dioscorea* tubers. As mentioned earlier, the major problem encountered with the extraction of starch from the above class of tubers is the presence of mucilagenous substances which keep the starch granules in suspension without allowing it to settle. This chapter discusses the results of the study carried out for developing an effective method for extraction of starch from tubers of *Dioscorea alata*, *Dioscorea rotundata* and *Dioscorea esculenta* by pretreating the fresh tubers with a few selected chemicals ie. sodium hexametaphosphate (SHMP), sodium chloride (NaCl), potassium metabisulphite (KMS) and ammonium hydroxide (NH_4OH) in the concentration range of 1-5% w/v and glyceryl monostearate (GMS) in the concentration range of 0.025-0.125% w/v as already mentioned in Chapter III. The starch samples prepared were analysed and compared with control for their yield, purity (% starch concent) and some of the essential physico-chemical characteristics such as intrinsic viscosity, amylose content, swelling and solubility and pasting behavior.

3.0. Results and Discussion

3.1. Yield and Purity of Starch

The average yields of starch obtained from samples of *Dioscorea alata*, *Dioscorea rotundata* and *Dioscorea esculenta* by direct aqueous extraction without subjecting the tuber samples to any chemical pretreatment were 46, 58

and 18 g per 250 g of fresh tuber respectively (Table 6). Pretreatment of fresh tubers was found to improve the yield of starch although to varying levels, except in the case of samples of *D. alata* and *D. rotundata* pretreated with GMS.

Table 6: Yield (g/wt of tuber) and purity (%) of *Dioscorea* Starches

Sample	*D.alata*		*D.rotundata*		*D.esculenta*	
	Yield (g/250g)	Starch Content* (%)	Yield (g/250g)	Starch Content* (%)	Yield (g/250g)	Starch Content* (%)
Control	43.04	79.08	64.35	75.22	18.16	74.23
SHMP 1%	52.55	81.77	60.22	74.61	32.32	88.05
SHMP 2%	47.19	81.19	65.57	74.37	40.25	86.28
SHMP 3%	42.98	81.29	62.80	71.25	37.01	86.81
SHMP 4%	40.96	78.98	61.47	70.79	31.10	88.93
SHMP 5%	44.76	79.46	64.68	76.77	38.78	89.81
Control	43.35	78.82	53.82	77.61	18.16	74.23
KMS 1%	43.43	79.56	57.05	74.88	18.39	88.93
KMS 2%	43.87	77.45	57.20	79.49	19.92	88.93
KMS 3%	45.42	79.72	56.73	71.81	19.01	88.93
KMS 4%	53.32	78.89	60.18	71.47	13.72	89.81
KMS 5%	49.27	80.10	61.31	74.20	18.47	89.81
Control	40.93	74.21	58.00	72.83	18.16	74.23
NaCl 1%	40.57	71.91	59.06	72.43	23.42	86.28
NaCl 2%	41.76	75.82	52.85	72.56	23.22	86.31
NaCl 3%	41.18	74.86	60.82	77.59	20.06	89.10
NaCl 4%	43.82	76.27	50.16	76.14	22.11	88.93
NaCl 5%	43.21	75.22	55.34	76.42	28.05	88.99
Control	40.93	74.21	55.79	81.01	18.16	74.23
NH_4OH 1%	43 53	90.79	61.95	81.62	40.78	88.04
NH_4OH 2%	46.06	90 73	65.01	81.19	37.91	86.28
NH_4OH 3%	49.80	88.04	59.43	82.32	38.42	86.81
NH_4OH 4%	49.23	89.06	61.13	81.43	42.32	88.93
NH_4OH 5%	47 63	90.42	62.61	80.55	42.96	89.81
Control	55 74	78.41	53 82	77.61	18.16	74.23
GMS 0.025%	53.91	68.17	53 27	82.05	22.21	86.30
GMS 0.050%	52 34	71.66	47.04	83.16	20.32	89.81
GMS 0.075%	57.10	71.80	48.54	83.16	20.17	88.93
GMS 0.100%	51 92	70 38	56 07	83.83	25.48	83.09
GMS 0.125%	58.28	70.38	49.27	82.75	29.85	86.95

* Starch content on fresh weight basis
Moisture content of starch samples 9-13%

As shown in Table 6, pretreatment of fresh tubers of *D.alata* with aqueous solutions containing lower concentrations of SHMP, especially at 1% concentration of the salt, increased the yield of starch from 43 to 53 g

compared to that of control. However, increasing the concentration of SHMP beyond 2% did not show any additional advantage with respect to starch yield. A similar trend was also observed regarding the purity of starch obtained wherein the concentration of SHMP in the range of 1-3% had a beneficial effect. Increasing the salt concentration beyond 3%, however, maintained only the same level of purity as that of control. Unlike in the case of SHMP pretreatment, use of higher concentrations (3-5%) of KMS had generally a positive impact on starch extractability. Further, it could be noticed that pretreatment with KMS showed an unique and distinct pattern, wherein the starch yield steadily increased on treatment with 1 to 4% concentration of the salt and later showed a tendency to decline. KMS treatment also yielded starch with more or less same or marginally higher purity as compared to control. From the data as given in Table 6, it could be inferred that NaCl pretreatment did not show any distinct benefit on the extractability of starch especially when tubers were pretreated using low levels of salt. Even at the highest concentration of 5% NaCl, there was only a marginal change in the starch yield compared to that of control. It was observed that pretreatment of fresh tubers of *D. alata* with NH_4OH at all concentrations brought about higher yield of starch. The maximum yield of starch in the above case was in the sample that pretreated with 3% NH_4OH, showing an increase from 41 to 50 g of starch per 250 g fresh tuber. A noticeable impact of NH_4OH pretreatment was that it improved the purity of starch to an extent of about 91% compared to 74% for the control sample. Pretreatment of *D. alata* tubers with GMS generally resulted in suppressing the yield and purity of starch, although 0.125% level of the GMS treatment indicated a tendency to enhance the starch yield from 56 to 58 g per 250 g of fresh tuber.

The impact of chemical pretreatment of *D. rotundata* tubers was less pronounced compared to that of *D. alata* tubers. Pretreatment with SHMP did not bring much improvement on the starch extractability compared to that of

control. Further, it was noticed that the percent of starch content also slightly lowered as a result of treatment of tubers with 3 and 4% SHMP solution, while rest of the concentrations showed more or less similar purity as the control sample. Similar to *D. alata* tubers, *D. rotundata* tubers also showed improved starch yield as a result of pretreatment with KMS. The highest yield was obtained with 5% KMS treatment where an increase in the yield from 54 to 61 g per 250 g fresh tuber could be observed. But starch purity was lowered by an extent of 4.4%; the values declining from 77.61 to 74.2%. Pretreatment with NaCl in general had an effect of suppressing the yield of starch extracted, but purity of starch extracted was found to be better. However, treatment of tubers with 3% NaCl resulted in increasing the starch yield as well as purity. Similar to *D. alata* tubers, *D. rotundata* also showed a positive response towards starch extractability as well as starch purity as a result of pretreatment with NH_4OH. The highest yield was obtained by pretreatment with 2% NH_4OH, showing an increase in yield from 56 to 65 g per 250 g fresh tuber. As observed in the case of *D. alata* tubers, pretreatment of fresh *D. rotundata* tubers with GMS also showed an overall tendency to lower the starch yield, although at 0.100% concentration level, the yield of starch got slightly increased. The above reduction in starch yield however did not affect the purity of starch. It was found that the starch prepared from tubers pretreated with GMS improved the starch purity accounting for a starch content of 83% which was considerably higher than 78% purity observed in the case of sample prepared by conventional water extraction.

Unlike in the case of *D. alata* and *D.rotundata* tubers, all pretreatments in general were found to favour both starch extractability and starch purity of *D. esculenta* tubers. A pronounced increase in the yield of starch was noticed as a result of both SHMP and NH_4OH treatments of tubers. Among different concentrations of SHMP tried, the maximum yield of starch was found to be in the case of tuber samples pretreated with 2% SHMP accounting for an increase

from 18 to 40 g per 250 g fresh tuber. All the concentrations of SHMP improved the starch content and an increase in starch content by about 16% compared to control was observed in the case of starch samples prepared from 5% SHMP pretreated tubers. Unlike in the case of *D.alata* and *D.rotundata* tubers, the effect of pretreatment with KMS was less pronounced in the case of *D.esculenta* tubers although the starch purity got considerably improved touching a level of 90% compared to control sample. Pretreatment of *D. esculenta* tubers with NaCl also increased the yield, although to a less extent. Among the different concentrations of NaCl, the highest yield was obtained with 5% concentration where the yield increased from 18 to 28 g per 250 g of fresh tuber. It was noticed that purity of starch (%) increased with increase in concentration of NaCl used for pretreatment. Among the chemicals tried, treatment with NH_4OH gave the most favourable effect on the yield of starch and pretreatment with 5% NH_4OH raised the starch yield almost 2.4 times from that of control. In conjunction with improving the starch yield, pretreatment with NH_4OH also enhanced the starch purity (Table 6). Unlike that of *D. alata* and *D. rotundata*, improvement in the yield and more specifically in the starch purity were more conspicuous in the case of starch samples prepared from *D. esculenta* tubers pretreated with GMS.

It could be generally inferred that the nature of impact of chemical pretreatment was generally similar in the case of *D. alata* and *D. rotundata* tubers, while the response of *D. esculenta* tubers to chemical pretreatment was markedly different. A common observation was that among the chemicals used for pretreatment, NH_4OH was found to be beneficial for the extraction of starch from the tubers in terms of both starch yield and purity. A similar observation has been reported earlier[27] where the pretreatment with 0.3 M ammonia was found to enhance the yield and quality of starch in the case of *Colocasia esculenta* tubers. This may be due to the interaction of NH_4OH with the mucilaginous substances and rendering starch to settle more easily.

3.2. Lipid and Protein contents

As shown in Table 7, starches extracted from fresh as well as chemically pretreated tubers of *D. alata*, *D. rotundata* and *D. esculenta* were analysed for their lipid and protein contents. *D. alata* and *D. rotundata* control starch samples contain about 0.021% protein whereas *D. esculenta* sample contain about 0.013% protein. No significant impact of chemical pretreatment on protein content of starch samples could be observed.

Table 7: Lipid* and Protein* contents of *Dioscorea* Starches

Sample	*D.alata*		*D.rotundata*		*D.esculenta*	
	Lipid (%)	Protein (%)	Lipid (%)	Protein (%)	Lipid (%)	Protein (%)
Control	0.20	0.021	0.29	0.021	0.22	0.013
SHMP 1%	0.39	0.017	0.19	0.024	0.19	0.012
SHMP 2%	0.39	0.016	0.19	0.016	0.49	0.010
SHMP 3%	0.30	0.021	0.39	0.017	0.49	0.010
SHMP 4%	0.20	0.023	0.19	0.014	0.39	0.010
SHMP 5%	0.20	0.020	0.19	0.016	0.49	0.012
Control	0.39	0.021	0.49	0.024	0.22	0.013
KMS 1%	0.39	0.016	0.77	0.024	0.59	0.016
KMS 2%	0.59	0.022	0.88	0.030	0.39	0.010
KMS 3%	0.50	0.023	0.47	0.026	0.39	0.010
KMS 4%	0.69	0.025	0.49	0.020	0.49	0.010
KMS 5%	0.69	0.024	0.58	0.024	0.49	0.010
Control	0.29	0.021	0.19	0.018	0.22	0.013
NaCl 1%	0.59	0.021	0.19	0.014	0.20	0.021
NaCl 2%	0.70	0.019	0.39	0.014	0.19	0.032
NaCl 3%	0.59	0.019	0.48	0.010	0.39	0.028
NaCl 4%	0.59	0.018	0.76	0.011	0.49	0.028
NaCl 5%	0.29	0.017	0.79	0.013	0.50	0.029
Control	0.29	0.021	0.39	0.021	0.22	0.013
NH_4OH 1%	0.29	0.016	0.39	0.012	0.20	0.010
NH_4OH 2%	0.39	0.017	0.39	0.017	0.39	0.010
NH_4OH 3%	0.69	0.017	0.29	0.017	0.19	0.010
NH_4OH 4%	0.59	0.018	0.49	0.015	0.19	0.011
NH_4OH 5%	0.69	0.017	0.40	0.017	0.39	0.010
Control	0.39	0.026	0.49	0.024	0.22	0.013
GMS 0.025%	0.51	0.026	0.58	0.026	0.40	0.023
GMS 0.050%	0.89	0.025	0.97	0.015	0.39	0.023
GMS 0.075%	0.89	0.026	0.98	0.015	0.30	0.025
GMS 0.100%	0.91	0.024	0.68	0.023	0.29	0.023
GMS 0.125%	0.99	0.026	0.68	0.015	0.38	0.023

* Mean of three determinants.

It has been noticed that pretreatment of fresh tubers with the chemicals generally enhanced the lipid extractability (Table 7). A major observation was that pretreatment of fresh tubers with GMS had a pronounced effect on enhancing the lipid extractability of the samples which is considered to be due to entrapment of GMS in the starch matrix. A similar observation has been reported earlier with *Xanthosoma* tubers pretreated with GMS[173].

3.3. Intrinsic Viscosity

As presented in Table 8, the average values of the intrinsic viscosity (η_i) values of starch samples prepared from *D. alata* and *D. rotundata* tubers without subjecting to any chemical pretreatment were more or less similar viz., 2.08 and 2.02 respectively. Compared to the above two species, *D. esculenta* starch showed perceptibly lower intrinsic viscosity, the value being 1.74.

The alkaline (NaOH) solutions of starch samples, extracted from *D. alata* tubers pretreated with higher concentrations of SHMP (5%) showed a narrow increase in viscosity from 2.08 to 2.14. Pretreatment of tubers with KMS adversely affected the intrinsic viscosity of starch samples and the highest reduction up to an extent of 6% was noticed in the case of samples prepared from tubers pretreated with 5% KMS. Pretreatment of *D. alata* tubers with NaCl showed a common tendency to enhance the intrinstic viscosity of the starch samples. Thus, 3% NaCl treated sample showed an increase in viscosity value from 2.09 to 2.19. Pretreatment of tubers with NH_4OH also resulted in lowering the intrinsic viscosity of the starch samples, similar to KMS pretreatment. But the extent of reduction in the intrinsic viscosity as a result of pretreatment with NH_4OH was found to be greater. Thus, 5% NH_4OH treated sample showed about 11% reduction in viscosity. Pretreatment of tubers with GMS did not considerably affect the viscosity of the starch samples.

Similar to *D. alata*, in the case of *D. rotundata* tubers also, pretreatment with SHMP resulted in an increase in the intrinsic viscosity. But the extent of increase in intrinsic viscosity as a result of SHMP pretreatment of the *D. rotundata* tubers was more pronounced showing an increase by 14% for 4% SHMP treated sample compared to the corresponding control sample.

Table 8: Instrinsic viscosity data of *Dioscorea* starches

Sample	*D.alata**	*D.rotundata**	*D.esculenta**
Control	2.08±0.010	2.00±0.010	1.74±0.000
SHMP 1%	2.03±0.010	2.13±0.030	1.72±0.000
SHMP 2%	2.05±0.040	2.25±0.030	1.72±0.010
SHMP 3%	2.13±0.010	2.25±0.040	1.68±0.010
SHMP 4%	2.14±0.010	2.28±0.020	1.66±0.020
SHMP 5%	2.14±0.005	2.25±0.010	1.68±0.005
Control	2.07±0.005	2.03±0.005	1.74±0.000
KMS 1%	1.96±0.010	1.90±0.005	1.59±0.000
KMS 2%	1.94±0.020	1.78±0.020	1.57±0.010
KMS 3%	1.95±0.010	1.73±0.010	1.59±0.010
KMS 4%	1.94±0.010	1.73±0.010	1.54±0.005
KMS 5%	1.94±0.030	1.70±0.000	1.58±0.010
Control	2.09±0.000	2.00±0.010	1.74±0.00
NaCl 1%	2.16±0.010	2.19±0.030	1.71±0.01
NaCl 2%	2.14±0.010	2.07±0.010	1.76±0.00
NaCl 3%	2.19±0.020	2.08±0.005	1.77±0.01
NaCl 4%	2.07±0.010	2.05±0.005	1.76±0.02
NaCl 5%	2.05±0.003	2.09±0.003	1.77±0.01
Control	2.09±0.010	2.00±0.010	1.74±0.000
NH_4OH 1%	2.07±0.010	2.06±0.010	1.74±0.005
NH_4OH 2%	2.06±0.020	1.90±0.020	1.62±0.010
NH_4OH 3%	1.88±0.010	1.93±0.010	1.59±0.010
NH_4OH 4%	1.85±0.020	1.85±0.010	1.56±0.005
NH_4OH 5%	1.86±0.005	1.76±0.005	1.40±0.020
Control	2.08±0.010	2.03±0.005	1.74±0.00
GMS 0.025%	2.10±0.010	2.04±0.010	1.75±0.02
GMS 0.050%	2.09±0.010	2.06±0.010	1.74±0.03
GMS 0.075%	2.03±0.010	2.01±0.020	1.76±0.01
GMS 0.100%	2.04±0.010	2.07±0.010	1.76±0.02
GMS 0.125%	2.04±0.005	2.03±0.004	1.73±0.01

* Mean of three determinants were taken and the dispersion is indicated by the Standard Deviation

Similar to *D. alata* tubers, KMS treatment of *D. rotundata* tubers also caused a lowering in the intrinsic viscosity and the effect was more pronounced in the

latter case which showed a very distinct pattern. A general observation was that intrinsic viscosity lowered along with increase in the concentration of KMS and 5% KMS pretreated sample exhibited nearly 16% reduction in intrinsic viscosity, compared to that of control. A reduction in intrinsic viscosity was noticed also for NH_4OH treated samples of *D. rotundata* at concentrations above 1% and the extent of reduction for 5% NH_4OH treated sample was about 12%. As a result of NaCl pretreatment of *D. rotundata* tubers, a positive impact on intrinsic viscosity was noticed especially at lower concentrations such that the sample pretreated with 1% NaCl showed an increase in viscosity by 9.5%. Similar to *D. alata*, pretreatment of *D. rotundata* tubers with GMS also did not have any significant impact on intrinsic viscosity.

Similar to *D. alata* and *D. rotundata* tubers, KMS treatment of *D. esculenta* tubers also resulted in lowering the intrinsic viscosity. The extent of reduction shown by 4% KMS treated sample was about 11.5%. The pattern of reduction in intrinsic viscosity of *D. esculenta* samples pretreated with NH_4OH was in a distinct manner that the intrinsic viscosity was lowered progressively with increase in the concentration of NH_4OH. Pretreatment of tubers with 5% NH_4OH evinced a reduction by 20% in the intrinsic viscosity as compared to the control starch sample. The pretreatment *of D. esculenta* tubers with either SHMP or GMS did not show any significant impact and NaCl treatment showed.only marginal improvement (Table 8).

As could be observed from the results (Table 8), the nature and extent of impact of chemical pretreatment of fresh tubers on the intrinsic viscosity of *D. alata* and *D. rotundata* starches were more or less of a similar pattern, whereas the response of *D. esculenta* starch differed noticeably. It could be noticed that samples prepared from tubers pretreated with KMS made the alkaline (NaOH) starch suspension less viscous although to different extents (Table 8). The

above reduction could be attributed to the degradative action of sulphite on starch polysaccharides[174]. Similarly, in the case of samples pretreated with NH_4OH, some degradation of starch molecules caused by breakage of inter-helical H-bonds by virtue of the alkalinity would be expected resulting in lowering the viscosity. It has been already reported that the addition of salt like NaCl to a starch solution increased the viscosity[175]. Osman has reported that the addition of salts to corn starch solutions, caused an increase in viscosity which is dependent on the position of anions in the lyotropic series[176]. An increase in viscosity has been reported[177] also in the case of starches extracted from fresh tubers of *Amorphophallus* pretreated with NaCl.

3.4. Swelling Volume and Solubility

Results of the experiments conducted on swelling and solubility of starch samples prepared from fresh as well as chemically pretreated roots are presented in Table 9. The swelling volumes (ml/g of starch) of native starches of *D. alata*, *D. rotundata* and *D. esculenta* were in the range of 25-28, 18-25 and 34 respectively. Pretreatment of tubers with SHMP showed a favourable impact on swelling property of *D. alata* samples, of which 5% SHMP treated sample showed the highest increase in value from 28.0 to 35.5. Pretreatment of *D. alata* tubers with KMS increased the swelling volume more or less to a similar extent as that by SHMP (Table 9). Pretreatment with NaCl also showed a positive effect on swelling volume and an increase in swelling volume from 25 to 33 ml was observed in the case of 5% NaCl treated sample. Although pretreatment with NH_4OH at lower concentrations marginally improved the swelling volume, at higher concentrations (4 and 5%) no change in swelling volume could be observed. It was noticed that, of the chemicals used for pretreatment, GMS alone suppressed the swelling volume of starch samples and the extent of reduction in swelling volume changed reciprocally with increase in concentration of GMS. A decrease in swelling volume from 28 to

21 ml/g of starch was observed for starch samples prepared from *D. alata* tubers pretreated with 0.125% GMS (Table 9).

Table 9: Swelling volume* (S.V) and solubility* data of Dioscorea starches

Sample	*D.alata*		*D.rotundata*		*D.esculenta*	
	S.V (ml/g of starch)	Solubility (%)	S.V (ml/g of starch)	Solubility (%)	S.V (ml/g of starch)	Solubility (%)
Control	28 0	19.80	25.0	15.20	34.0	6.60
SHMP 1%	35.0	16.25	30.0	15.28	32.0	6.60
SHMP 2%	33.0	18.40	27.5	14.50	32.0	6.80
SHMP 3%	35 0	14.50	27.5	12.68	33.0	6.80
SHMP 4%	35.0	11.35	28.0	21.60	32.0	6.85
SHMP 5%	35.5	10.87	25.5	20.45	32.0	6.70
Control	28 0	12.60	22.0	9 77	34.0	6.60
KMS 1%	30 0	12.00	18 5	8.20	27.0	7.25
KMS 2%	32 0	11.90	18.0	8.20	27.5	7.30
KMS 3%	33.0	10.05	20.0	8.00	27.0	7.30
KMS 4%	35.0	13.00	22.0	11 70	28.0	7.13
KMS 5%	34.0	13.20	23.0	11.55	28.0	7.05
Control	25.0	15 68	18.0	12.30	34.0	6.60
NaCl 1%	31.0	10.65	20 0	14.00	34.5	6.60
NaCl 2%	31 5	10.05	20 0	14.40	34.0	6.25
NaCl 3%	30 5	15.68	21.0	17.88	35.0	6.30
NaCl 4%	29.0	10.65	18.0	16.40	35.5	6.30
NaCl 5%	33.0	10.06	19.0	12.15	35.0	6.38
Control	25.0	15.68	25.0	18.75	34.0	6.60
NH_4OH 1%	26.0	22.20	25.0	19.00	35.0	9.40
NH_4OH 2%	28.0	21 60	27.5	18.88	34.5	9.60
NH_4OH 3%	25.5	18 63	27.5	18.13	34.0	9.60
NH_4OH 4%	25.0	15 20	28	18.00	34.0	9.50
NH_4OH 5%	25.0	12.95	26.5	18.38	34.5	9.48
Control	28.0	19.80	22.0	9.77	34.0	6.60
GMS 0.025%	28.0	16.20	18.0	8.10	28.0	3.60
GMS 0.050%	26.0	12.95	17.5	8.25	28.0	3.70
GMS 0.075%	23.0	11 55	18.0	8.20	29.5	3.60
GMS 0.100%	23.0	11 70	17.3	8.30	27.5	3.50
GMS 0.125%	21.0	9.95	17.5	8.25	27.0	3.70

*Average of two determinants.

Similar to *D. alata* tubers, SHMP pretreatment of *D. rotundata* tubers also increased the swelling volume of starch. The maximum effect was observed in the case of the sample treated with 1% SHMP where the swelling volume increased from 25 to 30 ml/g of starch. Unlike in the case of *D.alata*, pretreatment of *D.rotundata* tubers with NaCl and NH_4OH did not show any noticeable impact in swelling volume, while pretreatment with GMS resulted in

lowering the swelling volume of starch from 22 to 17.3ml/g of starch. KMS treatment of *D. rotundata* tubers at lower concentrations showed a decline in swelling volume while at higher concentrations, swelling volume remained unchanged compared to control sample.

In the case of *D. esculenta* tubers, pretreatment with KMS and GMS alone showed any significant impact on swelling volume. In both the above cases, the swelling volume was lowered to the same extent. A reduction in volume from 34 to 27 ml/g of starch was observed in the case of starch samples prepared from GMS as well as KMS treated samples. Pretreatment with NaCl and NH_4OH did not bring any change in the swelling volume of the *D. esculenta* starch, whereas SHMP treatment marginally lowered the swelling volume.

It could be noticed that the effect of KMS pretreatment differed noticeably among the three tuber starches as mentioned above. It has been reported that addition of 0.01% sulphite brings reduction in the swelling volume of cassava, potato and sago starches at 95°C, as against rice and wheat starches which show a similar effect only at a higher temperature of 121°C while maize was little affected even at the above temperature[178]. The above reduction in swelling volume of starch observed as a result of KMS pretreatment could be due to the oxidative-reductive depolymerization (ORD) effected by KMS on the starch molecule. Pretreatment of *D. alata* tubers with NaCl brought a positive effect by increasing the swelling volume, while *D. rotundata* and *D. esculenta* did not show any considerable effect. It has been reported by the earlier workers[178] that addition of 0.01% sodium chloride does not have any impact on the swelling volume of sago, cassava, rice, wheat and maize starches while the swelling volume of potato starch gets reduced noticeably. A second major observation regarding swelling volume was that starches extracted from tubers pretreated with GMS displayed noticeable reduction in swelling volume at all concentrations studied. It is now known that

swelling of starch granules is affected by the presence of lipids and surfactants. GMS and sodium stearoyl lactylate (SSL) are reported to give a restricted swelling of the starch granules[46], whereas the presence of sodium dodecyl sulphate (SDS) has improved the potential of swelling for wheat and potato starch granules[179]. The restricted swelling of starch granules in presence of GMS is attributable to the formation of a complex between amylose and GMS. It has been reported that when amylose leaches out of the granules during gelatinization, the lipids either intrinsically present or externally added, could form complexes with the exuded amylose probably on the surface of the granules and retard their swelling[180,181].

Among the control samples of *Dioscorea* starches, *D. esculenta* starch showed the highest swelling volume while that of *D. rotundata* showed the lowest volume. When starch is suspended in hot water, the individual granules swell and a portion of the starch dissolves in the aqueous medium. The degree of swelling and dissolution of starch granules will depend on the type of starch[163]. As the bonding forces within the starch molecules largely determine the swelling power, highly associated starch granules having an extensive and strongly bonded micellar structure generally display relatively greater resistance towards swelling[182]. Accordingly, since the granules of *D. esculenta* starch exhibit greater swelling, it should be inferred that the *D. esculenta* starch granules contain less associative forces than both *D. alata* and *D. rotundata* starches.

Among the three samples, the highest solubility was displayed by *D. alata* while *D. esculenta* starch was found to be the least soluble. A comparison of the results on the impact of chemical pretreatment on starch solubility of *D. alata* starch samples showed that, all the chemicals tried, excepting (1-3%) NH_4OH and 4 and 5% KMS adversely affected the solubility of starch. Among the chemically pretreated samples, maximum solubility was observed in the case of starch sample prepared from roots pretreated with 1%

NH_4OH. It was also found that the solubility decreased as the concentration of NH_4OH increased. Among the chemically pretreated samples, maximum reduction in the solubility was noticed for those extracted from tubers pretreated with GMS. Starch prepared from tubers pretreated with 0.125% GMS showed 50% reduction in solubility from that of the control. Pretreatment with SHMP also resulted in lowering the solubility of starch in a consistent manner that solubility decreased with increase in the concentration of SHMP.

Unlike *D. alata* starch, pretreatment of *D. rotundata* tubers with 4 and 5% SHMP and (1-4%) NaCl increased the solubility of starch samples, whereas NH_4OH treatment did not have any noticeable impact. Similar to that observed in the case of *D. alata* tubers, pretreatment of *D. rotundata* tubers with GMS suppressed the solubility. Pretreatment of tubers with KMS at lower concentrations of 1-3% decreased the starch solubility, while at higher concentrations a marginal increase in solubility could be observed.

The impact of pretreatment on solubility of *D. esculenta* starch differed noticeably from starch samples of both *D. alata* and *D. rotundata*. Pretreatment with SHMP and NaCl did not show any perceptible impact. But pretreatment with GMS was found to lower the solubility almost by 47 percent when compared to the control. Unlike the above, pretreatment with KMS and NH_4OH improved the solubility of starches. The above effect was more pronounced in the case of starch samples prepared from tubers pretreated with NH_4OH where an increase in solubility by about 45% was observed compared to the control sample.

A major observation was that among the chemicals tried, GMS reduced the solubility of starch samples presumably as a result of insoluble amylose-lipid complex formation, while pretreatment with KMS at higher concentrations facilitated the release of higher amount of polysaccharide into the supernatant. The latter phenomenon could be attributed to the degradative

action of KMS on starch structure. It has been reported that addition of 0.01% sulphite increased the solubility of potato, sago, cassava, rice, wheat and maize starches[178]. The increase in solubility caused by NH_4OH treatment of tubers could be due to partial disruption of starch granule structure thereby facilitating the release of higher amount of polysaccharides into the supernatant.

3.5. Apparent, True and Water soluble amylose content

Results of a comparative study on amylose contents in the control and experimental starch samples measured in terms of blue value are presented in Table 10. Defatting with methanol effectively increased the amylose blue

Table 10: Apparent amylose content of Dioscorea starches

Sample	*D.alata**	*D.rotundata**	*D.esculenta**
Control	0.01647±0.0004	0.01538±0.0003	0.0106±0.0000
SHMP 1%	0.01579±0.0002	0.01489±0.0002	0.0106±0.0007
SHMP 2%	0.01543±0.0006	0.01496±0.0006	0.0104±0.0002
SHMP 3%	0.01522±0.0008	0.01443±0.0003	0.0104±0.0004
SHMP 4%	0.01594±0.0010	0.01532±0.0010	0.0100±0.0000
SHMP 5%	0.01532±0.0007	0.01532±0.0010	0.0104±0.0006
Control	0.01668±0.0001	0.01576±0.0000	0.0106±0.0000
KMS 1%	0.01648±0.0004	0.01555±0.0007	0.0104±0.0006
KMS 2%	0.0164±0.0000	0.01561±0.0007	0.0104±0.0008
KMS 3%	0.01640±0.0070	0.01528±0.0010	0.0106±0.0001
KMS 4%	0.01692±0.0002	0.01583±0.0005	0.0106±0.0007
KMS 5%	0.01697±0.0004	0.01602±0.0010	0.0112±0.0009
Control	0.01584±0.0001	0.01613±0.0006	0.0106±0.0000
NaCl 1%	0.01568±0.0003	0.01495±0.0009	0.0104±0.0002
NaCl 2%	0.01431±0.0009	0.01577±0.0002	0.0104±0.0005
NaCl 3%	0.01450±0.0009	0.01528±0.0005	0.0102±0.0002
NaCl 4%	0.01396±0.0007	0.01496±0.0009	0.0102±0.0040
NaCl 5%	0.01312±0.0010	0.01483±0.0010	0.0103±0.0007
Control	0.01584±0.0001	0.01645±0.0007	0.0106±0.0000
NH_4OH 1%	0.01540±0.0007	0.01613±0.0001	0.0106±0.0009
NH_4OH 2%	0.01416±0.0006	0.01615±0.0001	0.0092±0.0001
NH_4OH 3%	0.01376±0.0003	0.01527±0.0009	0.0108±0.0009
NH_4OH 4%	0.01366±0.0005	0.01543±0.0008	0.01068±0.0007
NH_4OH 5%	0.01360±0.0009	0.01505±0.0001	0.0104±0.0004
Control	0.01647±0.0004	0.01576±0.0000	0.0106±0.0000
GMS 0.025%	0.01536±0.0001	0.01499±0.0009	0.0098±0.0000
GMS 0.050%	0.01543±0.0004	0.01409±0.0002	0.0094±0.0009
GMS 0.075%	0.01345±0.0007	0.01455±0.0007	0.0096±0.0000
GMS 0.100%	0.01412±0.0000	0.01399±0.0009	0.0090±0.0006
GMS 0.125%	0.01226±0.0010	0.01373±0.0000	0.0096±0.0002

* Mean of four determinants were taken and the dispersion is indicated by the Standard Deviation

value accounting for true amylose in all the starch samples (Table 11). A comparison of true, apparent and water soluble amylose contents of control samples from *D. alata*, *D. rotundata* and *D. esculenta* samples indicates that in general, 93-99% of the total amylose in each of the starch samples is accounted by apparent amylose and 42-47% of the latter by water soluble amylose (Table 12).

Table 11: Total amylose content of Dioscorea starches

Sample	*D.alata**	*D.rotundata**	*D.esculenta**
Control	0 01665±0.0002	0.01629±0.0006	0.01140±0.0001
SHMP 1%	0.01669±0.0007	0 01519±0.0003	0.01120±0.0002
SHMP 2%	0.01600±0 0009	0.01507±0.0009	0.01120±0.0004
SHMP 3%	0.01626±0.0002	0.01621±0.0002	0.01096±0.0001
SHMP 4%	0.01636±0.0001	0.01600±0.0003	0.01060±0.0003
SHMP 5%	0.01635±0.0001	0.01617±0.0009	0.01160±0.0007
Control	0.01737±0.0006	0.01640±0.0000	0.01140±0.0001
KMS 1%	0.01668±0.0002	0.01620±0.0005	0.01100±0.0006
KMS 2%	0.01652±0.0009	0.01619±0.0005	0.01140±0.0002
KMS 3%	0.01703±0.0003	0.01583±0.0008	0.01160±0.0009
KMS 4%	0 1793±0 0005	0.01691±0.0001	0.11000±0.0002
KMS 5%	0.01804±0.0006	0.01617±0.0002	0.01200±0.0007
Control	0.01613±0.0003	0.01631±0.0001	0.01140±0.0001
NaCl 1%	0 01609±0.0002	0.01504±0 0008	0.01100±0.0006
NaCl 2%	0.01655±0 0009	0 01601±0.0007	0 01100±0.0004
NaCl 3%	0.01605±0.0004	0 01536±0.0001	0.01140±0.0002
NaCl 4%	0.01629±0.0007	0.01513±0.0002	0.01120±0.0002
NaCl 5%	0.01638±0.0002	0.01526±0.0000	0.01100±0.0001
Control	0.01613±0 0003	0.01684±0.0003	0.01140±0.0001
NH_4OH 1%	0.01676±0 0004	0.01723±0.0008	0.01260±0.0007
NH_4OH 2%	0.01681±0 0005	0.01665±0.0002	0.01080±0.0006
NH_4OH 3%	0.01662±0.0006	0 01651±0.0000	0.01140±0.0001
NH_4OH 4%	0.01685±0.0003	0.01637±0.0007	0.01140±0.0003
NH_4OH 5%	0.01658±0.0007	0.01684±0.0009	0.01108±0.0004
Control	0 01665±0 0002	0.01640±0.0000	0.01140±0.0001
GMS 0.025%	0.01664±0.0002	0.01516±0.0006	0.01080±0.0006
GMS 0.050%	0 01583±0.0001	0.01463±0.0001	0.01040±0.0004
GMS 0.075%	0 01484±0.0005	0.01515±0.0009	0.01060±0.0004
GMS 0.100%	0.01395±0.0009	0.01480±0.0000	0.01040±0.0002
GMS 0.125%	0.01400±0 0001	0.01396±0.0006	0.01080±0.0009

* Mean of four determinants were taken and the dispersion is indicated by the Standard Deviation

In general, chemical pretreatment of *Dioscorea* tubers brought down the amylose content except in the case of samples prepared from tubers pretreated

Table 12: Water soluble amylose content of Dioscorea starches

Sample	*D.alata**	*D.rotundata**	*D.esculenta**
Control	0.00698±0.00005	0.006436±0.00003	0.004983±0.00005
SHMP 1%	0.007308±0.00002	0.006032±0.00009	0.004928±0.00000
SHMP 2%	0.007340±0.00001	0.006360±0.00005	0.004955±0.00008
SHMP 3%	0.007700±0.00004	0.006480±0.00004	0.005072±0.00002
SHMP 4%	0.007380±0.00004	0.006420±0.00004	0.004960±0.00006
SHMP 5%	0.007552±0.00006	0.006780±0.00002	0.005344±0.00003
Control	0.007580±0.00003	0.006620±0.00000	0.004983±0.00005
KMS 1%	0.007420±0.00000	0.006404±0.00000	0.004720±0.00003
KMS 2%	0.007536±0.00008	0.006572±0.00005	0.004613±0.00006
KMS 3%	0.007664±0.00006	0.006660±0.00003	0.004608±0.00002
KMS 4%	0.008020±0.00003	0.007016±0.00009	0.004747±0.00001
KMS 5%	0.008304±0.00001	0.006780±0.00005	0.005077±0.00009
Control	0.007256±0.00008	0.006748±0.00002	0.004983±0.00005
NaCl 1%	0.007408±0.00002	0.006510±0.00007	0.004445±0.00000
NaCl 2%	0.007364±0.00005	0.006860±0.00004	0.004608±0.00003
NaCl 3%	0.007352±0.00005	0.006720±0.00002	0.004404±0.00002
NaCl 4%	0.007616±0.00001	0.006780±0.00004	0.004400±0.00002
NaCl 5%	0.007700±0.00000	0.007000±0.00001	0.004464±0.00005
Control	0.007256±0.00008	0.007460±0.00001	0.004983±0.00005
NH_4OH 1%	0.007708±0.00002	0.007268±0.00003	0.004429±0.00007
NH_4OH 2%	0.007436±0 00001	0.007568±0.00008	0.004395±0.00009
NH_4OH 3%	0 007486±0.00001	0.007664±0.00003	0.004352±0.00006
NH_4OH 4%	0.007536±0.00009	0.007636±0.00005	0.004423±0.00001
NH_4OH 5%	0.007692±0.00003	0.007744±0.00006	0.004872±0.00008
Control	0.00698±0.00005	0.006620±0.00000	0.004983±0.00005
GMS 0.025%	0.006920±0.00003	0.005396±0.00004	0.004912±0.00000
GMS 0.050%	0.006656±0.00002	0.005244±0.00003	0.004712±0.00002
GMS 0.075%	0.006432±0.00001	0.005004±0.00001	0.004592±0.00004
GMS 0.100%	0.005848±0.00008	0.004796±0.00007	0.003880±0.00009
GMS 0.125%	0.005484±0.00010	0.004596±0.00009	0.003712±0.00007

* Mean of four determinants were taken and the dispersion is indicated by the Standard Deviation

with 4 and 5% KMS (Table 10). All the starch samples extracted from tubers pretreated with GMS displayed noticeable reduction in amylose blue value. The above reduction in the value as a result of pretreatment with GMS was to an extent as high as 26% in the case of *D. alata* starch samples and to a relatively less extent in the case of *D. rotundata* and *D. esculenta* samples, reaching a level of 13 and 15% respectively. The effect of SHMP pretreatment

on amylose content of the starch samples was however less pronounced. Among the three *Dioscorea* starches, percent reduction in amylose induced by NaCl and NH_4OH was more pronounced in the case of *D. alata* starch samples, the respective percentage reduction in the above two cases being 17 and 14%.

Compared to *D. alata* samples, the effect of chemical pretreatment on amylose content was less pronounced for *D. rotundata* samples. The extent of reduction in amylose content as a result of SHMP pretreatment was about 6% whereas the percentage reduction of amylose content in samples from tubers subjected to NH_4OH and NaCl treatment was about 8%. Present studies also revealed that chemical pretreatment of the *D. esculenta* tubers does not bring about any noticeable change in the amylose contents except when the fresh tubers are pretreated with GMS. In the latter case, percent reduction in amylose was around 15% as already mentioned. Treatment of *D. esculenta* tubers with rest of the chemicals did not bring any conspicuous difference in amylose between control and experimental samples.

The reduction in amylose blue values observed as a result of pretreatment of tubers with different chemicals could be explained as follows. The present study leads to a possible inference that the decline in amylose values as a result of GMS treatment could be due to complexation of the surfactant with the amylose making it less prone to cleavage. In the case of cassava starch it has been already reported that anionic and cationic surfactants reduce the amylose content considerably[40]. It is possible that helical structure of amylose gets stabilized by hydrocarbon part of the surfactant[183]. A similar observation has been reported in the case of *Xanthosoma* and *Amorphophallus* tubers as a result of pretreatment with GMS [173,177]. Reduction of amylose in the samples prepared from tubers pretreated with SHMP could be viewed as a result of interaction of phosphate groups with the free OH groups, thus making amylose unable to react with iodine to form the blue complex. Effect of NaCl could be due to ionic interaction of starch with easily ionizable salt such as

NaCl especially in the linear region. The reduction of amylose observed in starches extracted from tubers pretreated with NH_4OH could be attributed to the partial hydrolytic degradation in an alkaline environment. The above explanations are indeed empirical and warrant confirmation from further detailed structural studies.

Regarding water-soluble amylose contents, a common observation was that pretreatment with GMS lowered the values in the case of all the three types of *Dioscorea* tuber starches (Table 12). The above reduction of soluble amylose in *D. alata* starch was almost by 21%. Pretreatment of *D. alata* tubers with rest of the chemicals showed an increase in water soluble amylose contents. In the case of starches prepared from tubers of *D. alata* pretreated with SHMP, an increase in water soluble amylose contents to an extent of 10% was noticed while the corresponding increase caused by NH_4OH and NaCl treatments was only about 6%. For starch samples extracted from tubers pretreated with KMS, it was found that amylose showed a progressive increase and 5% KMS treated sample showed an increase by 9.5% compared to the control. Among the three tubers, viz., *D. alata*, *D. rotundata* and *D. esculenta*, reduction in soluble amylose by GMS pretreatment was highest for *D. rotundata* starch sample showing a decline in the value by about 31%. No major change could be observed owing to the pretreatment of *D. rotundata* tubers with rest of the chemicals. The reduction in soluble amylose in *D. esculenta* starch as a result of GMS pretreatment was very much consistent, that the values progressively decreased with increase in concentration of GMS and the sample pretreated with 0.125% GMS showed maximum reduction of 26% in the amylose blue value. For pretreatment with chemicals such as NaCl, KMS and NH_4OH, although a reduction in soluble amylose, the magnitude of reduction was noticeably less compared to that of GMS treatment.

From a comparative evaluation of the results of the study conducted, it could be noticed that the effect of chemical pretreatment on the pattern of

change in soluble amylose content considerably differed among the three tuber starches depending on the nature of the chemicals as well as the source of starch. However, in the case of GMS treatment, all the Dioscorea starch samples showed a reduction in soluble amylose (Table 12). The above perceptible reduction in soluble amylose presumably suggests that GMS could penetrate the starch matrix and complex inside the helical region, thereby restricting the leaching of amylose fragments in to the aqueous phase. The above explanation is supported by the observation that starches prepared from GMS-treated tuber samples showed a corresponding reduction in solubility at 90°C. A similar pattern of result has been reported in the case of cassava starch as a result of treatment with GMS[34].

3.6. Paste Rheology

As presented in Table 13, the control samples of *D. alata* showed a pasting temperature in the range of 79-82°C. As there were considerable variations in the pasting behaviour among the individual samples, results of each set of experimental samples were compared and evaluated with the corresponding control sample of the respective set. The pasting temperatures of the treated samples were more or less same or slightly lower than that of the control except in the case of GMS treated sample. In the above case, it was noticed that swelling of the starch granules got delayed and pasting temperature of the control sample got raised from 79°C to 85°C. Starch samples pretreated with SHMP and KMS showed an increase in peak viscosity indicating strong cohesive forces within the granules. Unlike the above, a decrease in the peak viscosity was observed for starch samples prepared from tubers pretreated with GMS as well as NH_4OH. No perceptible decrease in peak viscosity was noticed as a result of pretreatment of tubers with NaCl within the concentration range of 1-3%. However, at higher concentrations of 4 and 5% NaCl, a noticeable reduction in peak viscosity could be observed. The breakdown ratio (H/P) which is the ratio of hot paste viscosity (H) to peak viscosity (P) calculated

from the Brabender viscogram was in the range of 0.5818-0.8600 for the control samples of *D. alata*. Pretreatment with low levels of KMS, especially at 2% concentration, imparted more stability to the hot gel as reflected from the

Table 13: Paste Rheology of *D.alata* starch samples.

Sample	Starch Slurry Conc^n (%)	Paste Properties			
		Pasting Temp. (°C)	Peak viscosity (P) BU	Hot Paste Viscosity (H) BU	Breakdown Ratio (H/ P)
Control	6	82	500	430	0.8600
SHMP 1%	6	80	710	460	0.6479
SHMP 2%	6	76	740	450	0.6081
SHMP 3%	6	79	680	440	0.6471
SHMP 4%	6	80	680	400	0.5882
SHMP 5%	6	80	580	410	0.7069
Control	6	80	550	320	0.5818
KMS 1%	6	80	500	320	0.6400
KMS 2%	6	82	560	400	0.7143
KMS 3%	6	82	620	380	0.6129
KMS 4%	6	80	620	310	0.5000
KMS 5%	6	80	780	330	0.4231
Control	7	80	1000	700	0.7000
NaCl 1%	7	79	960	800	0.8333
NaCl 2%	6	79	920	660	0.7174
NaCl 3%	7	80	1000	680	0.6800
NaCl 4%	6	79	650	440	0.6769
NaCl 5%	6	79	620	480	0.7742
Control	6	82	500	430	0.8600
NH_4OH 1%	6	82	500	240	0.4800
NH_4OH 2%	6	85	410	230	0.5610
NH_4OH 3%	6	80	440	200	0.4545
NH_4OH 4%	6	80	410	200	0.4878
NH_4OH 5%	6	80	510	210	0.4118
Control	6	79	310	210	0.6774
GMS 0.025%	6	85	220	110	0.5000
GMS 0.050%	6	82	210	70	0.3333
GMS 0.075%	6	82	150	40	0.2666
GMS 0.100%	6	85	200	80	0.4000

breakdown ratio of 0.7143 compared to 0.5818 of the corresponding control sample. A similar effect was observed as a result of pretreatment with low concentrations of NaCl, that 1% NaCl treated sample showed a breakdown ratio of 0.8333 against 0.7000 of the control sample. From the results mentioned above,it is to be inferred that pretreatment of *D. alata* tubers with

low levels of KMS (2%) and NaCl (1%) helped to improve the hot gel stability, unlike that observed in the case of rest of the treatments (Table 13).

Table 14: Paste Rheology of *D.rotundata* starch samples.

Sample	Starch Slurry Conc^n (%)	Paste Properties			
		Pasting Temp. (°C)	Peak viscosity (P) BU	Hot Paste Viscosity (H) BU	Breakdown Ratio (H/ P)
Control	7	85	880	490	0.5568
SHMP 1%	7	85	940	570	0.6064
SHMP 2%	7	83	750	390	0.5200
SHMP 3%	7	85	860	490	0.5698
SHMP 4%	7	84	940	560	0.5957
SHMP 5%	7	84	690	340	0.4928
Control	6	85	370	180	0.4865
KMS 1%	6	89	130	10	0.0769
KMS 2%	6	89	130	20	0.1538
KMS 3%	6	85	190	30	0.1579
KMS 4%	6	88	320	160	0.5000
KMS 5%	7	86	520	360	0.6923
Control					
NaCl 1%	7	86	660	430	0.6515
NaCl 2%	7	86	480	170	0.3542
NaCl 3%	7	86	710	390	0.5493
NaCl 4%	6	89	240	50	0.2083
NaCl 5%	7	87	550	280	0.5091
Control	7	85	780	530	0.6795
NH_4OH 1%	7	85	780	590	0.7564
NH_4OH 2%	7	85	680	430	0.6324
NH_4OH 3%	7	86	660	490	0.7424
NH_4OH 4%	7	85	680	460	0.6765
NH_4OH 5%	7	85	660	466	0.7061
Control					
GMS 0.025%	7	88	330	40	0.1212
GMS 0.050%	7	89	330	50	0.1515
GMS 0.075%	7	88	260	30	0.1154
GMS 0.100%	7	89	380	60	0.1579
GMS 0.125%	7	89	240	20	0.0833

As presented in Table 14, the control starch sample of *D. rotundata* had a pasting temperature of 85°C and the starch sol showed a peak viscosity of 880 BU. Pretreatment of the tubers with GMS as well as KMS increase the range of pasting temperature up to 89°C as indicated from the higher range of pasting temperature of 88-89°C and 85-89°C respectively for GMS and KMS treated samples. No major change in pasting temperature could be observed in starch samples prepared from the tubers pretreated with SHMP and NH_4OH,

although a marginal increase in pasting temperature was noticed due to NaCl treatment. The partial resistance to swelling and gelatinization as observed in the case of GMS and KMS treated samples is reflected from the lower peak viscosity of starch solutions from that of the corresponding control starch sample. Further, between the above two types of chemically pretreated samples, those prepared from GMS pretreated roots showed considerably low breakdown ratio (H/P) in the range of 0.0833-0.1579, indicating the very weak nature of the hot gel. In the case of starch samples prepared from tubers pretreated with low concentrations of KMS (1-3%) also, a low breakdown ratio in the range 0.0769-0.1579 could be observed. However, pretreatment of tubers with higher concentrations (4 and 5%) resulted in getting starch samples with considerably higher H/P value of 0.5000 and 0.6923 respectively. From the above pattern of results it is to be concluded that pretreatment of tubers with 4 and 5% KMS impart better stability to the hot gel. Pretreatment with NH_4OH also yielded starch samples which produced apparently a stable hot gel as indicated by relatively higher breakdown ratio in the range of 0.6765-0.7564. From the overall results obtained in the case of *D. rotundata* samples it should be reasonably presumed that pretreatment with NH_4OH more favorably influenced the starch paste rheology compared to the rest of the salts tried. It is to be added that pretreatment with SHMP or NaCl failed to show any noticeable impact on paste rheology.

The control starch sample of *D.esculenta* had a pasting temperature of 67°C and the starch sol showed a peak viscosity of 530BU (Table 15). In general, pretreatment of tubers with chemicals did not showed any significant impact on pasting temperature of starches (Table 15). There was a general reduction in the peak viscosity of all the treated samples compared to control. However in the case of SHMP treated samples, the above reduction was only marginal. The breakdown ratio was found to be 1.0 in the case of control as

well as all the treated samples indicating that the chemical pretreatment did not alter the paste stability of the starch samples.

Table 15: Paste Rheology of *D.esculenta* starch samples.

Sample	Starch Slurry Conc^n (%)	Paste Properties			
		Pasting Temp. (°C)	Peak viscosity P (BU)	Hot Paste Viscosity H (BU)	Breakdown Ratio (H/ P)
Control	6	67	530	530	1
SHMP 2%	6	68	500	500	1
SHMP 4%	5	68	280	280	1
SHMP 4%	6	67	520	520	1
SHMP 5%	6	67	500	500	1
NaCl 1%	5	68	210	210	1
NaCl 2%	5	65	250	250	1
NaCl 3%	4.5	70	240	240	1
NaCl 4%	4.5	70	180	180	1
NaCl 5%	6	64	520	520	1
NH_4OH 1%	6	66	440	440	1
NH_4OH 2%	5	70	290	290	1
NH_4OH 3%	6	68	450	450	1
NH_4OH 4%	6	68	420	420	1
NH_4OH 5%	6	67	390	390	1
GMS 0.025%	5	64	360	360	1
GMS 0.100%	5	67	330	330	1
GMS 0.125%	6	67	510	510	1

An overall comparative evaluation of viscogram data of starch samples of *D. alata, D. rotundata* and *D. esculenta* have unambiguously indicated that, *D.esculenta* starch samples have got the highest paste stability as displayed by a breakdown ratio of 1.0 in the case of control as well as treated samples, suggesting a better utility as starch paste or gel after cooking or in those applications in which a prolonged period of high consistency is required. Similarly among the experimental samples, KMS (2%) and NaCl (1%) treated samples of *D.alata* and NH_4OH as well as KMS (4 and 5%) treated samples of *D. rotundata* could be more useful for the above purposes.

CHAPTER IV

EFFECT OF CHEMICAL PRETREATMENT OF DIOSCOREA TUBERS ON STRUCTURAL, RHEOLOGICAL AND DIGESTIBILITY PROPERTIES OF STARCHES

INTRODUCTION

Based on the results obtained with respect to yield and purity of starch samples as effected by pretreatment of tubers with 1-5% w/v SHMP, NaCl, KMS, NH_4OH and 0.025-0.125% w/v of GMS, experiments were repeated using those concentrations which resulted in the highest yield or purity of starch. For the purpose of study, 500 g of *Dioscorea* tubers were treated with the chemicals at the concentrations mentioned in Table 16. The extraction procedure and experimental details are same as mentioned in Materials and Methods (Chapter II). A detailed study on the changes occurring in structural, gelatinization and rheological characteristics of starches, as a result of pretreatment was conducted using control and experimental starch samples and the results have been compared.

Table 16: Concentration range of chemicals used for the pretreatment of *Dioscorea* tubers

Chemicals used for extraction	Concentrations of chemicals		
	D.alata	*D.rotundata*	*D.esculenta*
SHMP	1%	5%	2%
KMS	5%	5%	2%
NaCl	4%	3%	5%
NH_4OH	4%	2%	5%
GMS	0.075%	0.100%	0.125%

4.0. Results and Discussion

4.1. Moisture Sorption Study

Moisture sorption studies of *Dioscorea* starches were carried out at different RH levels of 70,75, 80 and 85%. Figs.2, 3 and 4 represent the moisture sorption profile of *D. alata*, *D. rotundata* and *D. esculenta* starch

Fig.2. Moisture sorption profile of *D. alata* starch samples at 80% RH

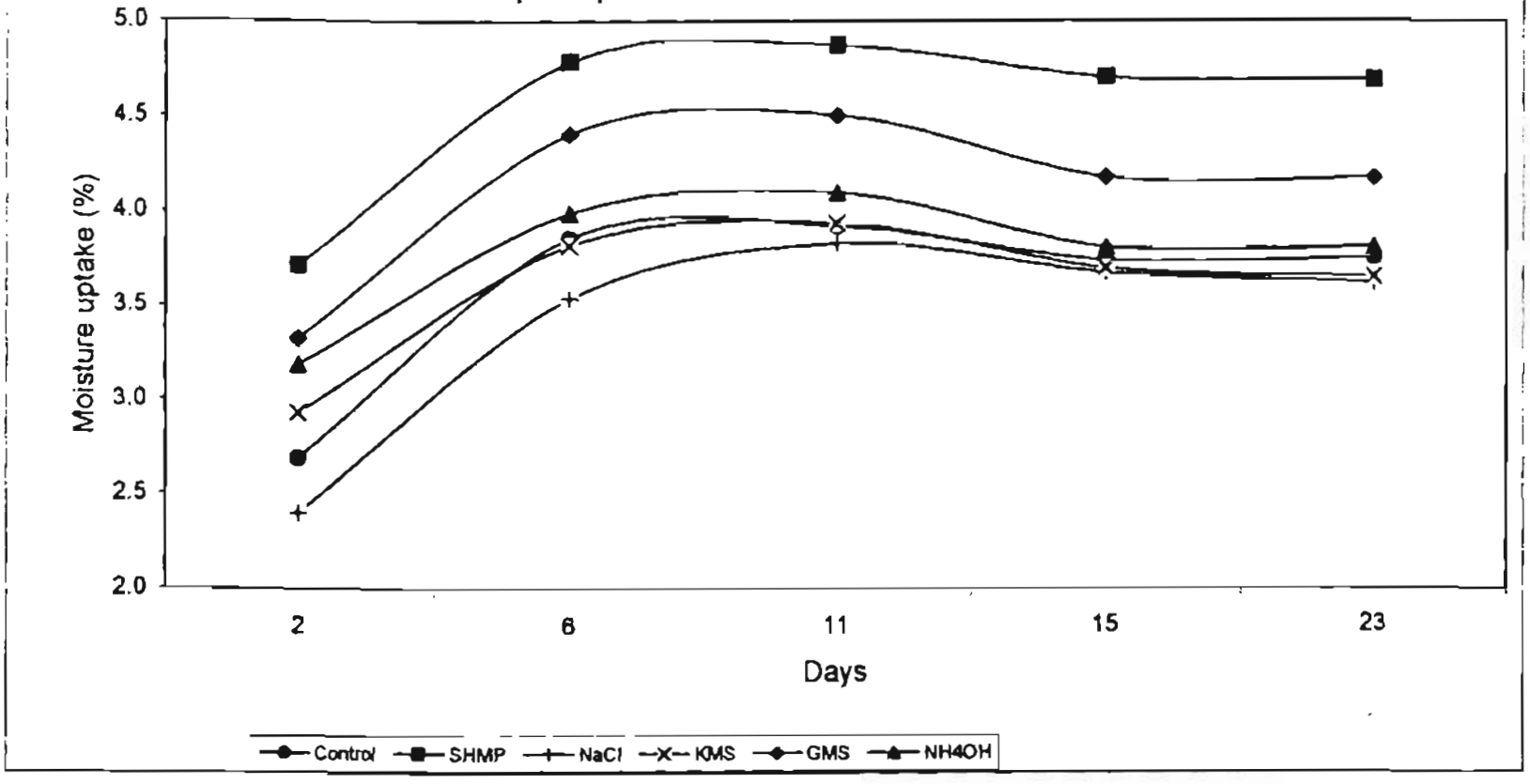


Fig.3. Moisture sorption profile of *D. rotundata* starch samples at 80% RH

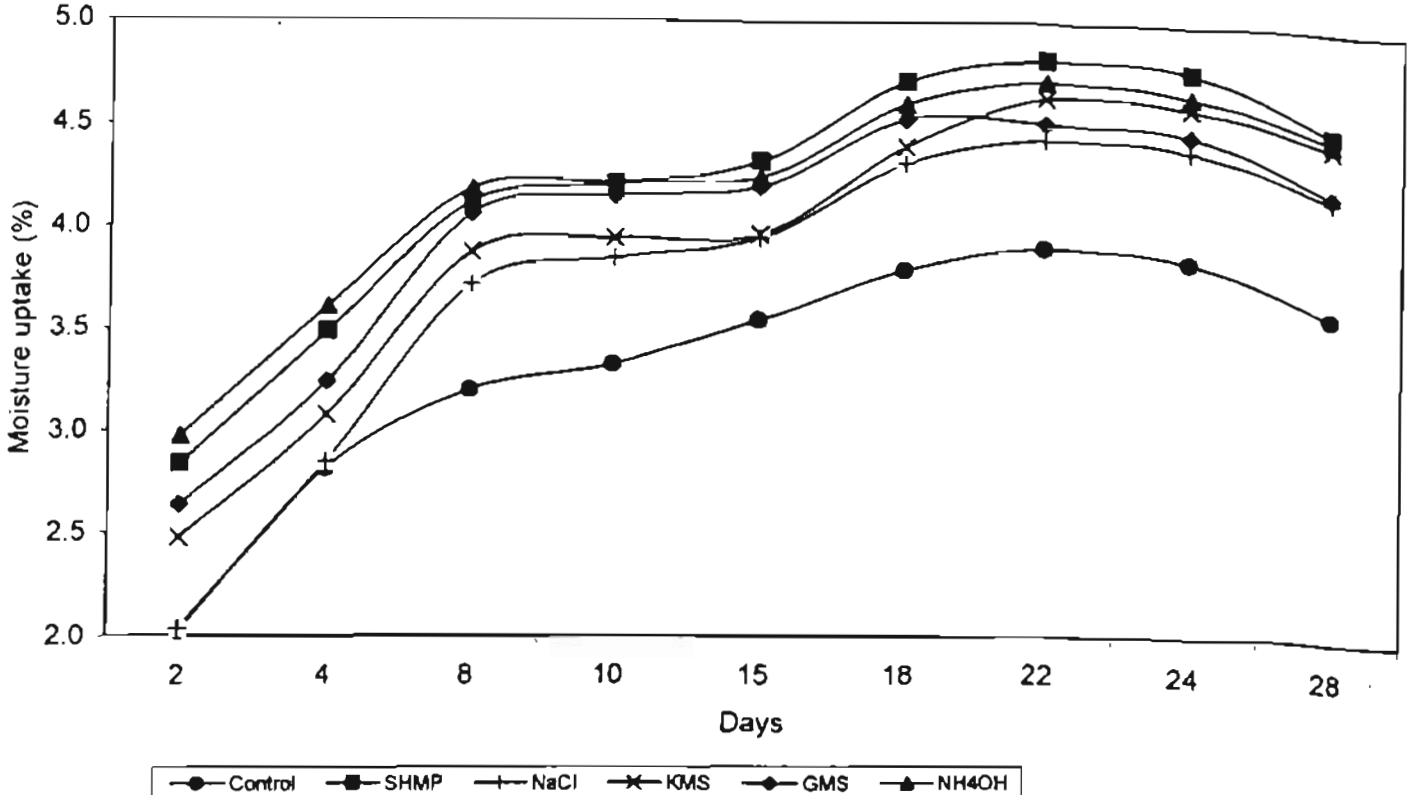
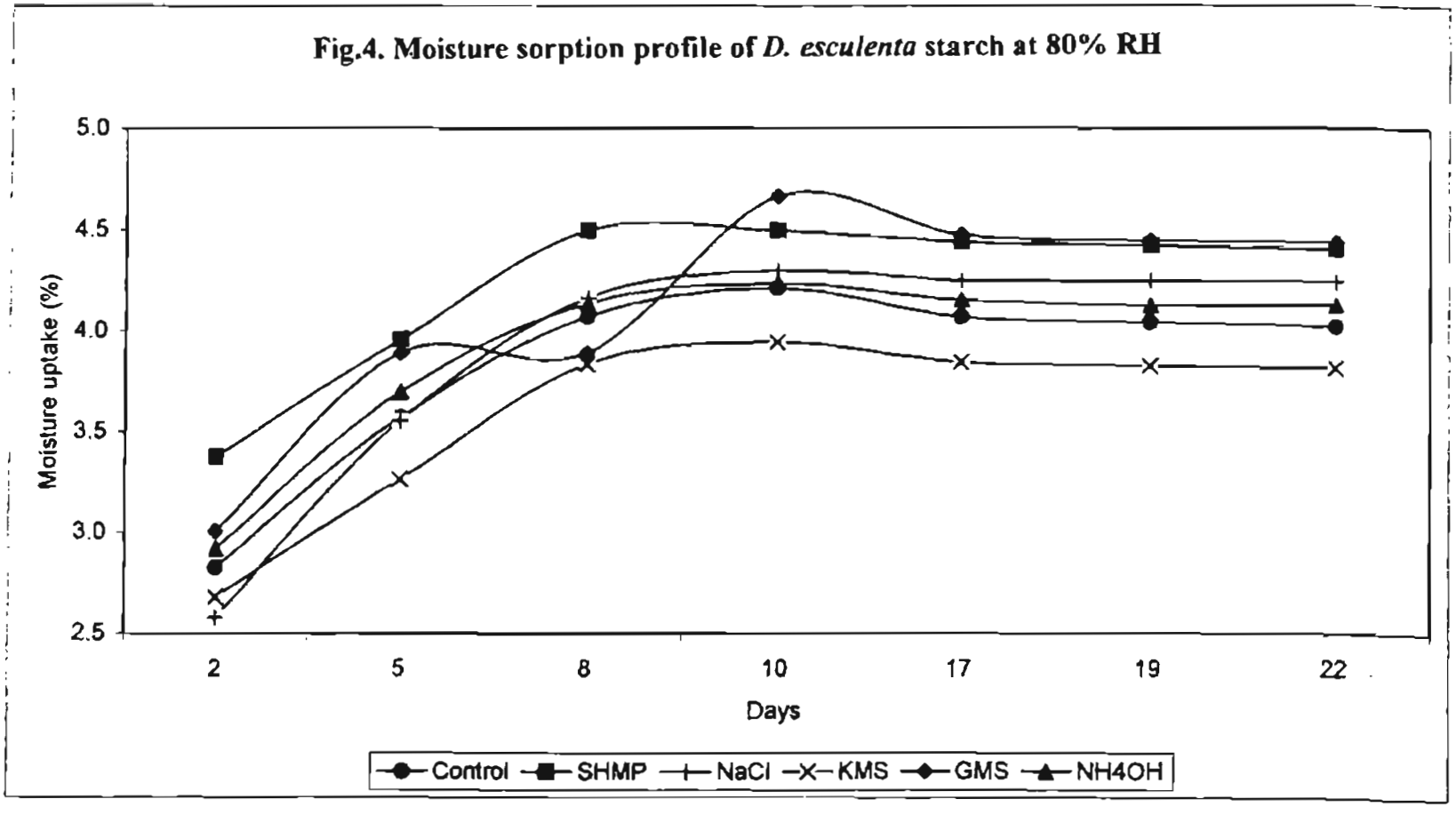


Fig.4. Moisture sorption profile of *D. esculenta* starch at 80% RH



samples at a relatively high RH of 80%. It was observed that when the starch samples were kept at different RH(%) conditions, after a few days, the moisture uptake remained almost constant. The time taken by each starch sample to attain the equilibrium moisture content (EMC) was found to differ among the starch samples. However, the pattern of moisture sorption did not show much variation in all the RH conditions tried in the present study. The overall pattern of moisture uptake by *D. alata*, *D. rotundata* and *D. esculenta* starch samples at all the four RH ranging from 70 – 85% are presented in Figs.5, 6 and 7. There was found to be an increase in the rate of moisture uptake corresponding to an increase in the relative humidity of the environment, independent of the source and type of the starch sample.

The moisture absorption pattern at 80% RH revealed that starch samples prepared from *D. alata* tubers pretreated with SHMP and GMS displayed higher moisture uptake compared to the corresponding control sample throughout the experiment. Although the initial moisture uptake (upto 11 days) was higher in the case of starch sample from NH_4OH pretreated roots, there was a subsequent desorption and the EMC value was almost the same as that of the control sample. The EMCs of starch samples prepared from KMS and NaCl treated tubers were found to be slightly lower than the control sample. Although all the experimental starch samples from *D.alata* tubers showed more or less identical sorption when kept at 70 and 75% RH (Fig.5), there was found to be a perceptible increase in the EMC value in the case of both NH_4OH and NaCl treated samples at 85% RH. Thus, an increase in the environmental RH to a level of 85% made the sample to imbibe more moisture prior to attaining the equilibrium moisture values. The above property was more prominent in the case of starch samples prepared from NaCl and NH_4OH treated tubers. The above pattern of higher moisture uptake at elevated RH has been observed and reported by earlier researchers. Gebre-Mariam et al[184] have reported that at

Fig.5. Moisture sorption (EMC) profile of *D. alata* starch at different RH(%)

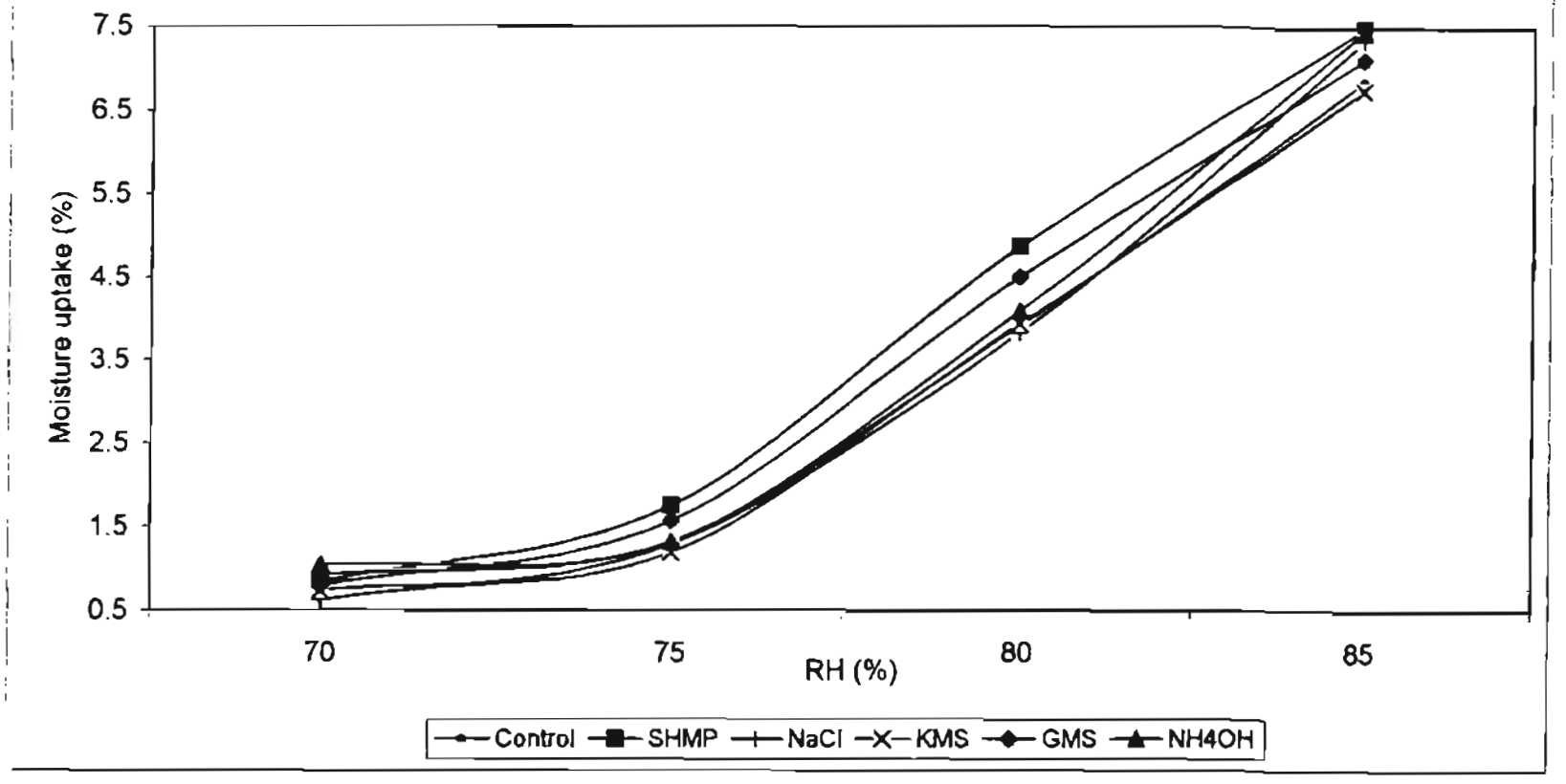


Fig.6. Moisture sorption (EMC) profile of *D. rotundata* starch at different RH(%)

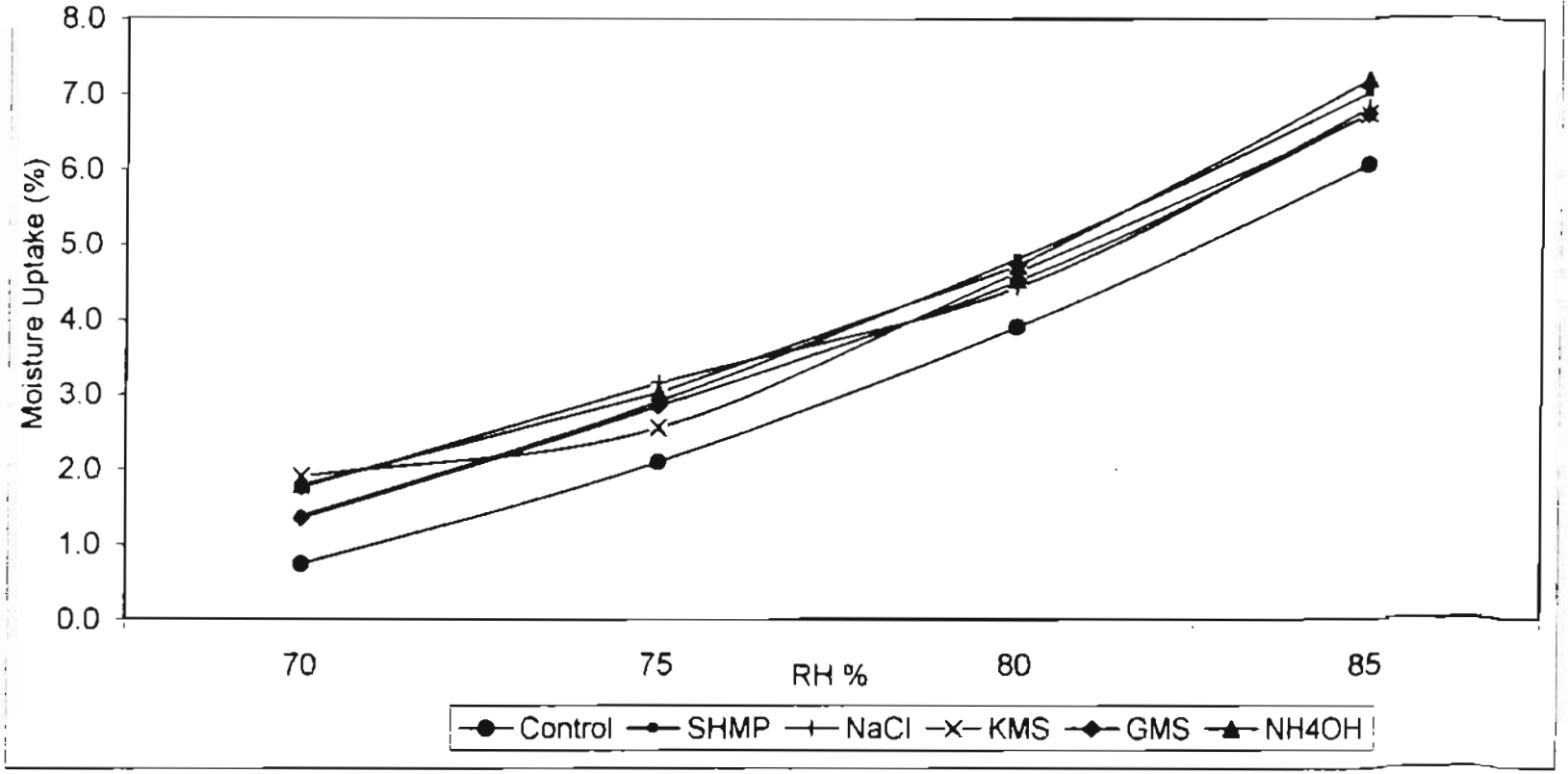
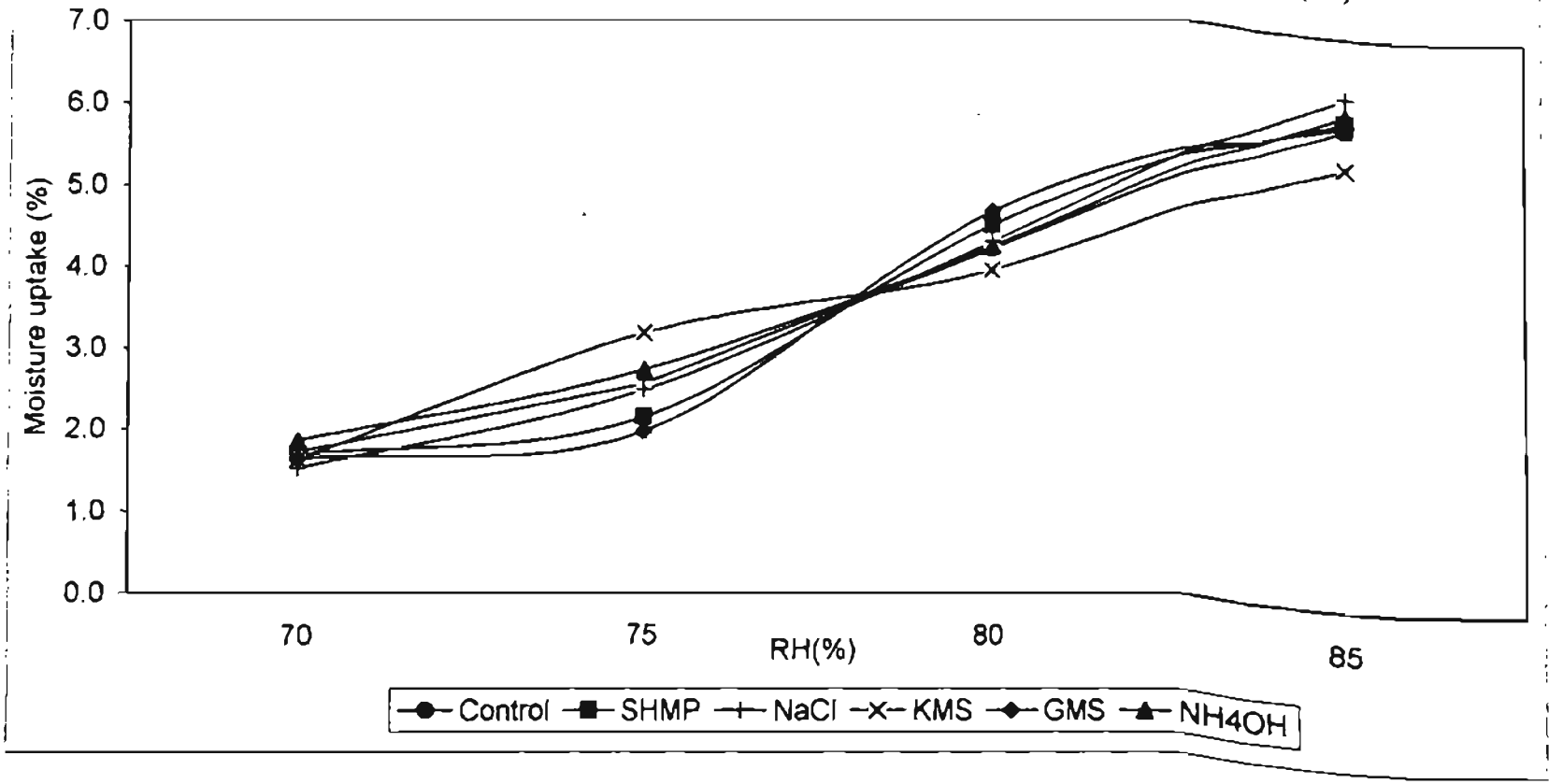


Fig.7. Moisture sorption (EMC) profile of *D. esculenta* starch at different RH(%)



lower humidities (10-30% RH), *Dioscorea abyssinica* and maize starches showed similar moisture sorption profiles, which are slightly lower than that of potato starch. However, when the humidity of the system was increased to a range of 40-100% *Dioscorea* and potato starches absorbed higher amount of moisture than maize starch, while studies on moisture sorption profiles of potato and enset[172] starch samples showed an identical pattern. This also speaks about the possible differences in moisture uptake pattern, depending on the starch source.

In the case of *D. rotundata* samples, the moisture uptake by all the treated samples was higher than that of the control starch sample at all RH(%) studied (Fig.6). The moisture uptake by the treated samples was in a similar pattern at 70, 75, 80and 85% RH. Thus the retention of moisture by the starch samples prepared by pretreatment appeared to be greater than the control starch sample.

In the case of *D. esculenta* starch samples, the pattern of moisture sorption was generally different at different RH conditions (Fig.7). At 70% RH, the EMCs of the control as well as treated samples were of the same order. When the environmental RH was raised to 75%, starch samples from KMS and NH_4OH pretreated roots retained higher moisture contents, whereas samples from SHMP and GMS treated roots showed lower moisture contents, compared to the control. The EMC of NaCl treated sample and the control sample was almost same. As the relative humidity of the system was increased to 80%, moisture uptake by KMS treated starch sample alone got lowered than the control sample (Fig.4). Samples from SHMP and GMS treated roots showed almost identical uptake of moisture which was higher than that of the control sample.

There is a growing awareness of the importance of water as a plasticizer in food processing operations[185]. The hydrophilic nature of many food biopolymers combined with their amorphous or semi-crystalline state means

that they are capable of sorbing continuously variable amounts of water[186]. This has a marked effect on the material properties. Moisture is known to modify the flow and mechanical properties of many powders including starches [187,188]. Therefore, a knowledge of moisture sorption profiles of starches is necessary where controlled powder flow or compaction is critical such as in the case of pharmaceutical tableting. A general inference that could be drawn from the present study is that pretreatment of *Dioscorea* tubers with SHMP and GMS results in enhancing the moisture uptake by the starch samples, while pretreatment with KMS causes a decline in the moisture uptake. It is possible that the amount of moisture imbibed by starch granules may be dependent on several factors such as chemical structure, particle size, temperature, the degree of molecular association by means of H-bonds, etc. The above increase in the moisture uptake by SHMP and GMS treated samples suggests that softening of starch matrix might have occurred as a result of pretreatment with SHMP and GMS, unlike KMS treatment of the tubers which may contribute to slightly higher rigidity or retention of the original rigidity of the starch matrix.

4.2. Gelatinization Properties

The gelatinization properties of the starch samples were studied by means of Differential Scanning Calorimetry (DSC) and it could be seen that chemical pretreatment of fresh tubers of *Dioscorea* did not effect any noticeable change in the above properties (Table 17). The pattern of DSC thermogram of *D.alata* starch samples is shown in Fig 8.

Control sample of *D.alata* showed a gelatinization range between 73.2° and 78.6°C representing the onset (T_i) and completion of gelatinization (T_f) respectively and the enthalpy change (ΔH) involved in the above process was 14.98 mj/mg. However, the above temperature range and enthalpy value are slightly different from the values already reported by earlier workers[189]. In the latter case the onset and final temperatures of gelatinization have been indicated to be 70.2 and 80.9°C respectively, with an enthalpy value of 21.3

mj/mg. The above differences in values may be attributed to both the innate differences in the starch quality, as influenced by agroclimatic conditions as well as to the minor changes in the conditions adopted in the experiment. Chemical pre-treatment of *D.alata*, resulted in an increase in the onset of gelatinization (T_i), gelatinization temperature range (T_i-T_f) and gelatinization enthalpy (ΔH). For Control starch sample of *D.rotundata*, the values for T_i, T_f and ΔH were 71.88°C, 80.44°C and 14.689 mj/mg respectively. Similar to *D.alata*, the pretreated samples of *D.rotundata* also showed only a marginal increase in the T_i, T_f, T_i-T_f and ΔH values. Compared to *D.alata* and *D.rotundata* starches, *D.esculenta* starch showed an early gelatinization as reflected from lower T_i and T_f values of 68.4° and 73.8°C respectively. There was also a corresponding decrease in ΔH value showing 11.963 mj/mg. Similar to *D.alata* and *D.rotundata*, pretreated samples of *D.esculenta* also showed only a marginal increase in T_i and T_i-T_f values compared to the

Table 17: DSC data of gelatinization of *Dioscorea* starch samples

Starch Sample	T_i* (°C)	T_f* (°C)	ΔH* (mj/mg)
D.alata			
Native	73.16±0.59	78.60±0.98	14.9882±0.37
SHMP	73.87±0.15	80.60±0.58	15.3667±0.06
KMS	74.20±0.10	80.80±0.15	15.2200±0.95
NaCl	74.06±0.05	80.60±0.45	15.9923±0.64
NH_4OH	74.23±0.23	81.23±0.41	15.8559±0.45
GMS	74.36±0.20	81.10±0.03	16.0233±0.57
D.rotundata			
Native	71.88±0.47	80.44±1.10	14.6893±0.52
SHMP	70.95±0.63	83.66±0.25	16.4260±0.23
KMS	72.35±0.21	82.35±0.49	15.4452±0.45
NaCl	72.53±0.30	82.30±0.50	16.2762±0.30
NH_4OH	72.33±0.11	83.00±0.15	16.4223±0.40
GMS	73.65±0.07	82.15±1.48	15.8250±0.23
D.esculenta			
Native	68.40±0.20	73.80±0.00	11.9626±0.17
SHMP	68.35±0.21	74.76±0.35	15.0730±0.39
KMS	69.00±0.30	75.33±0.30	15.5519±0.15
NaCl	69.26±0.11	76.03±0.11	16.1044±0.23
NH_4OH	69.00±0.00	74.76±0.32	15.5492±0.43
GMS	69.90±0.30	76.30±0.17	16.0970±0.74

*Mean of three determinants were taken and the dispersion is indicated by Standard Deviation

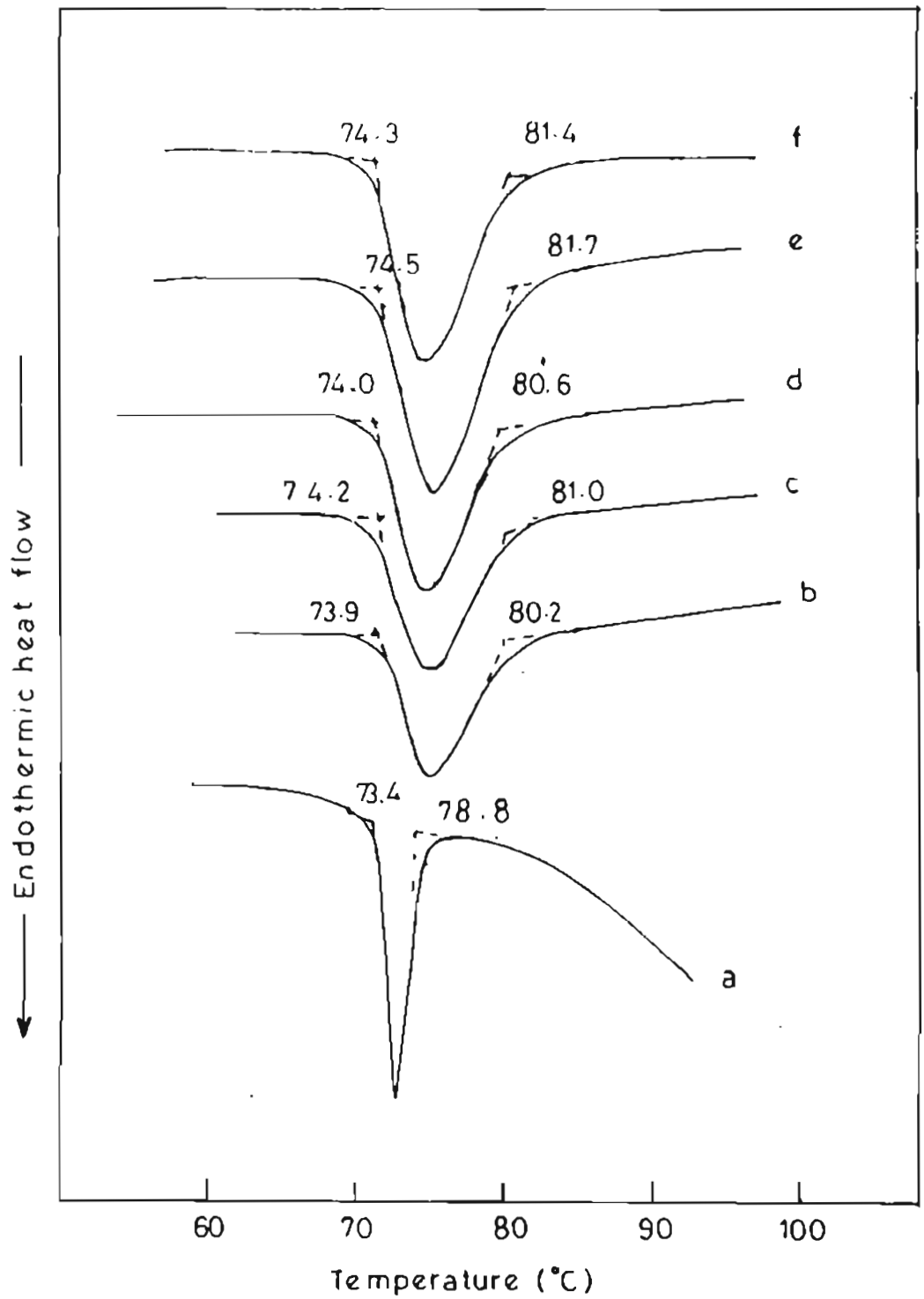


Fig 8: DSC thermogram of *D.alata* starch samples.
(a) Native (Control) (b) SHMP (c) KMS (d) NaCl (e) NH_4OH (f) GMS.

corresponding control. The ΔH values of pretreated samples ranged between 15-16 mj/mg while the corresponding value for the control sample was about 12 mj/mg.

Thus, the studies carried out on gelatinization properties of native as well as the treated samples of *Dioscorea* starches revealed that, pretreatment of fresh tubers with chemicals does not affect the granule strength or orientation. This was also noticed from the Brabender visco-amylograph data as discussed in Chapter III (Tables 13, 14 and 15), where only a marginal change in pasting temperature was observed as a result of pretreatment. Among the native *Dioscorea* starches, *D.esculenta* showed an early gelatinization lowering the T_i value and lower ΔH value compared to *D.alata* and *D.rotundata*. As the gelatinization temperature reflects the degree of orderly arrangement of the molecules in the starch granules, it should be concluded that the granular network in *D.alata* and *D.rotundata* are less fragile than *D.esculenta* starch.

A comparative study by means of DSC of the starch samples prepared from chemically pretreated *Dioscorea* tubers suggest that chemical pretreatment considerably lowered the onset (T_i) and final (T_f) melting temperature and also enthalpy of retrogradation (ΔH) (Table 18). Among the treated samples of *D.alata*, KMS treated samples showed lowest value (47.3°C) for the onset temperature of melting while other samples showed values in the range of 50.4 - 53.1°C. The highest value (53.1°C) for the onset of melting was shown by NH_4OH treated sample. T_i-T_f range as well as the enthalpy of melting was also highest for KMS treated sample. In the case of *D.rotundata* samples, KMS as well as GMS treated samples showed lower T_i values (48.5 and 49.4° C respectively) while SHMP treated samples showed the highest value of T_i (54.9°C). Widening of the melting endotherm as well as lowest ΔH value (5.5358 mj/mg) was observed with GMS treated sample. Contrary to the above case, GMS treated sample of *D.esculenta* showed highest value for T_i

(52.6°C) and a corresponding narrow melting endotherm was also observed. The enthalpy of retrogradation for all the treated samples are found to be in the range of 8.6861-9.8698 mj/mg. In general, retrogradation of the starch samples leads to lowering of T_i, T_f and ΔH values. This may be attributed to the weakening of starch matrix during retrogradation.

Table 18: DSC data of retrograded *Dioscorea* starch samples

Starch Sample	T_i* (°C)	T_f* (°C)	ΔH* mj/mg
D.alata			
SHMP Treated	50.4	79.7	9.65913
KMS	47.3	79.8	11.6539
NaCl	52.2	78.4	10.1723
GMS	51.8	71.5	9.3262
NH_4OH	53.1	74.0	9.2352
D.rotundata			
SHMP Treated	54.9	74.0	8.7344
KMS	48.5	74.3	9.1651
NaCl	51.2	73.8	7.6799
GMS	49.4	75.6	5.5358
NH_4OH	53.8	77.7	9.9369
D esculenta			
SHMP Treated	48.4	70.9	9.5772
KMS	48.5	72.5	9.6718
NaCl	50.5	72.8	8.6861
GMS	52.8	70.9	9.1823
NH_4OH	47.2	74.7	9.8698

*Average of two determinants

4.3.∝-amylase susceptibility

Pattern of ∝-amylase susceptibility of starch samples from untreated and chemically pre-treated root samples of *D. alata, D. rotundata* and *D. esculenta* is presented in Figs.9,10 and 11 respectively. In the case of *D. alata* samples, both the control and the starch samples prepared from fresh roots pretreated with SHMP and NH_4OH showed highest enzyme susceptibility at 45 minutes of incubation, beyond which there was a decline. For GMS, NaCl and KMS pretreated samples, the highest digestibility was observed at 30 minutes of incubation, but the extension of the incubation period showed a tendency to

Fig.9. α-Amylase digestibility pattern of *D. alata* starch samples

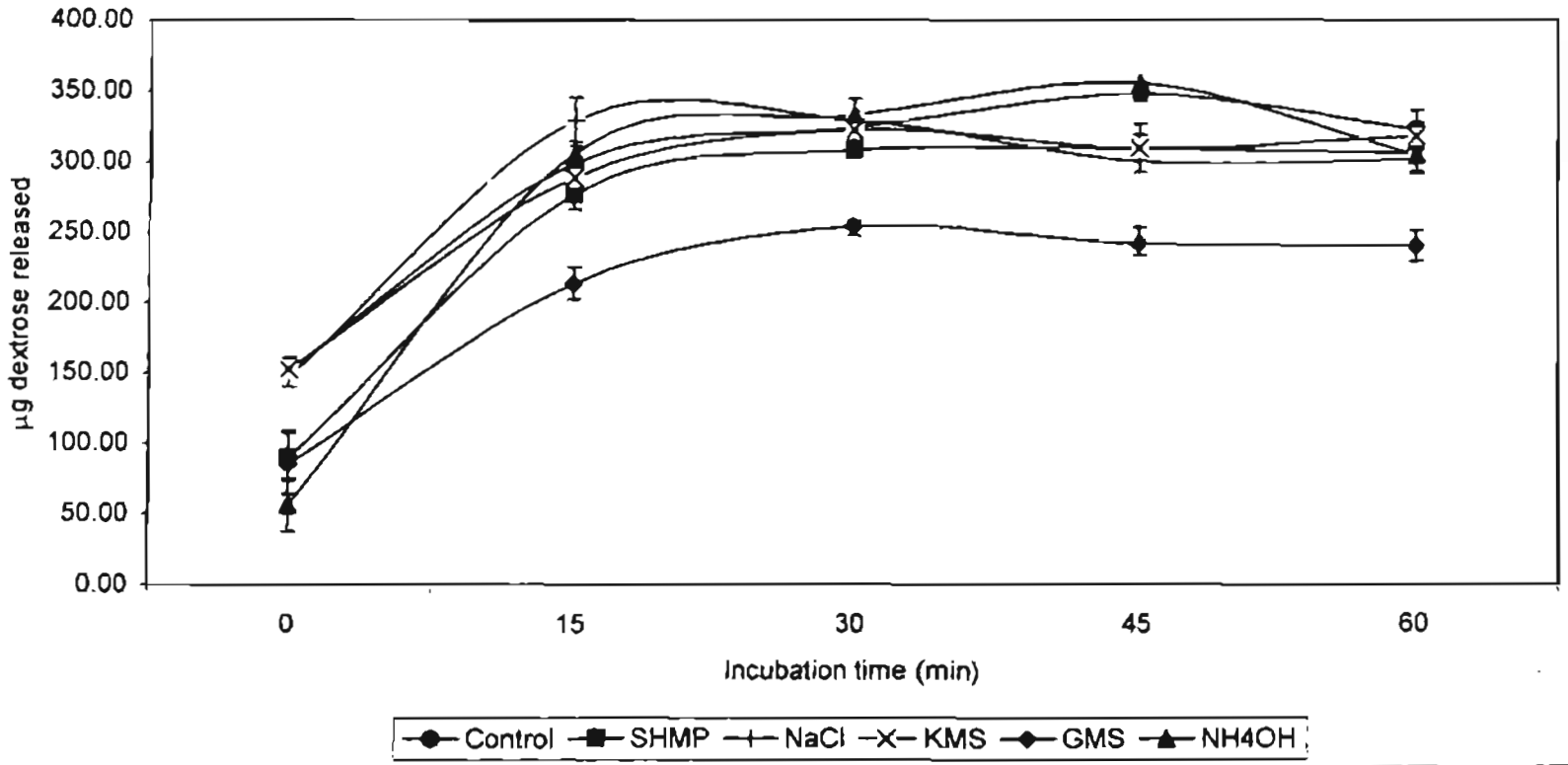


Fig.10. α-Amylase digestibility pattern of *D. rotundata* starch samples

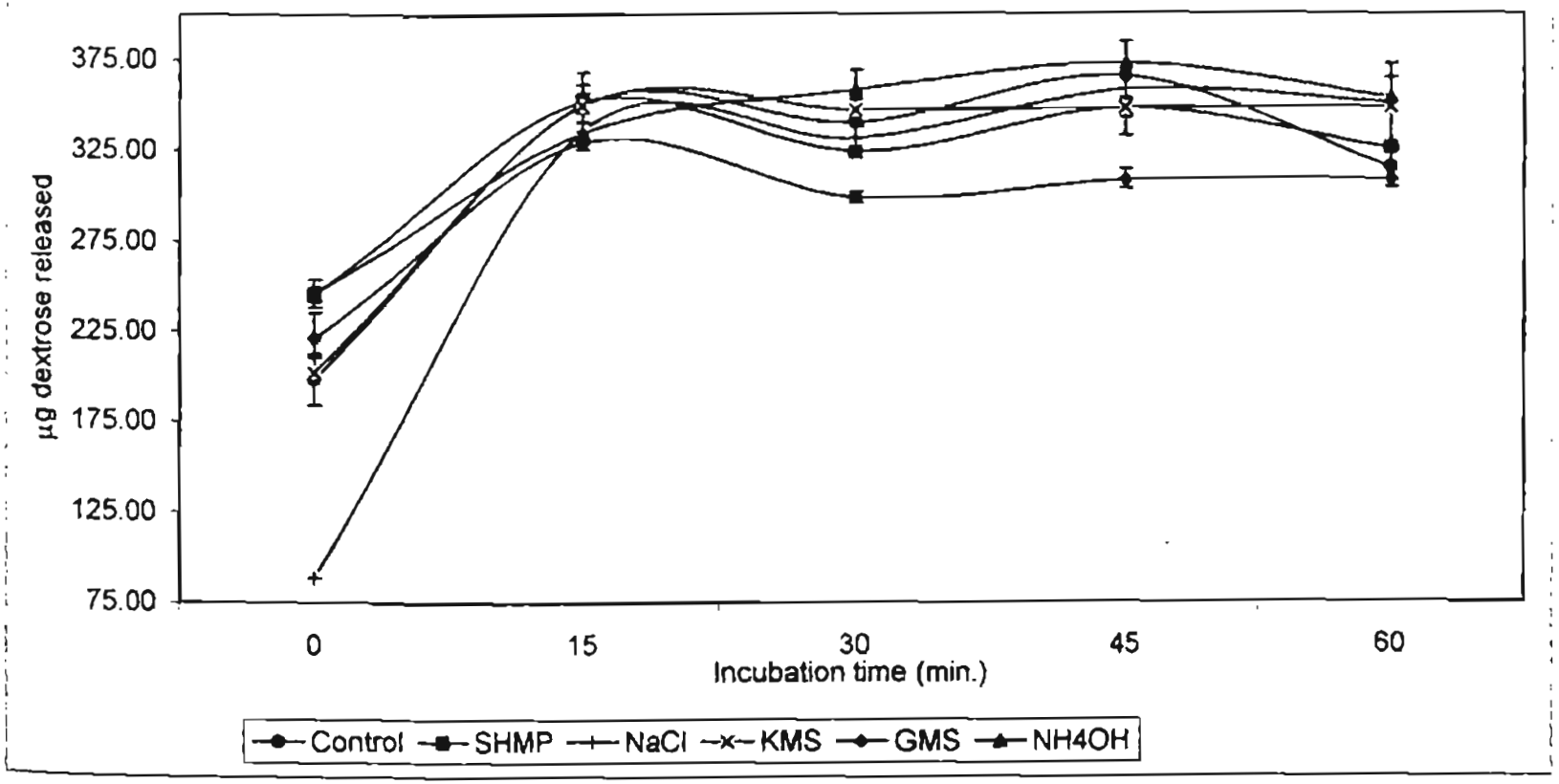
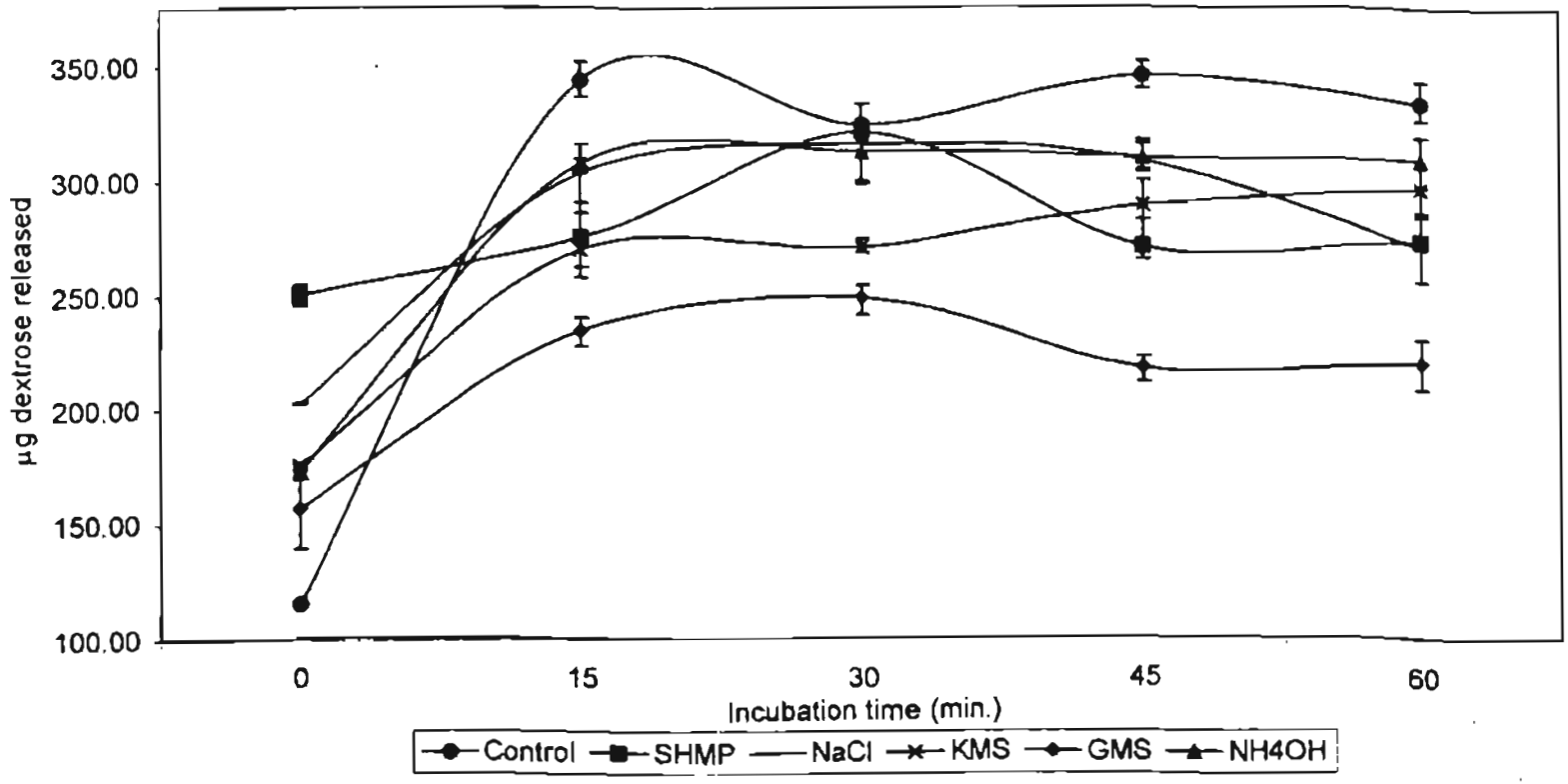


Fig.11. α-Amylase digestibility pattern of *D. esculenta* starch samples



lower the enzyme action. A noticeable decrease in enzyme action was observed in the case of starch sample prepared from roots pretreated with GMS (Fig.9) while pretreatment with NH_4OH enhanced the enzyme susceptibility.

In the case of *D. rotundata* starch samples (Fig.10), control as well as samples prepared from NaCl and NH_4OH pretreated tubers showed highest enzyme susceptibility at 45 minutes of incubation. On the other hand, starch samples prepared from roots pretreated with GMS, KMS and SHMP exhibited maximum susceptibility at 15 minutes of incubation beyond which there was a decline in sugar content reflecting lower level of enzyme susceptibility. As in the case of *D. alata* tubers, a decline in the enzyme susceptibility was observed for GMS treated *D. rotundata* starch sample while an increase in enzyme susceptibility was observed in the case of NH_4OH treated sample.

It can be seen from Fig.11 that the control sample of *D. esculenta* starch also showed highest activity at 45 minutes of incubation. For starch samples prepared from roots pretreated with SHMP, GMS, NaCl and NH_4OH, the highest activity was observed at 45 minutes of incubation. There was a time lag in attaining the highest activity for starch samples prepared from roots pretreated with KMS showing maximum susceptibility at 60 minutes of incubation. Similar to *D. alata* and *D. rotundata* tubers, noticeable reduction in enzyme action was observed in the case of starch extracted from *D. esculenta* tubers pretreated with GMS. For *D. esculenta* samples, it is also noticed that pretreatment slightly suppressed the enzyme susceptibility.

The enzyme digestibility profile of the three *Dioscorea* native starches was more or less similar. A major observation was that starch samples prepared from GMS pretreated *D. alata*, *D. rotundata* and *D. esculenta* tubers showed a fall in the enzyme activity. A similar fall in enzyme activity has been reported in the case of starch samples from GMS pretreated *Amorphophallus* tubers[177]. According to Svensson[190] hydrolysis of starch depends on the ability of the amylase to adsorb on the surface of the granules. Hence, low levels of

enzyme susceptibility observed in the starch sample as a result of GMS pretreatment may be attributed to less number of sites available for fixation of the enzyme. Linking of GMS with starch matrix could give rise to a partial shielding effect for the enzyme attack. Several researchers[191-196] have shown that amylose complexed with lipid is more resistant to enzyme hydrolysis than free amylose. Eliasson and Krog[191] have already reported that amylose complexed with saturated monoglycerides is more resistant to enzymic breakdown than unsaturated monoglycerides. However, it has been reported recently that native starches isolated from sarghum presoaked in SDS-sulphite showed increased susceptibility to enzymolysis as a result of loosened granular structure, thereby increasing the effective area for α-amylase to adsorb[197]. Enzymatic hydrolysis of native starch with α-amylase is a reaction between liquid and solid phases and consists of several steps such as, enzyme diffusion to the solid surface, adsorption, orientation and finally catalysis[198]. Alteration of granular structures can change some or all these steps and hence the overall susceptibility to hydrolysis. As mentioned earlier, an increase in enzyme susceptibility, although only to a less extent, was observed in starches extracted from *D. alata* and *D. rotundata* tubers pretreated with NH_4OH. This presumably suggests that some disruption of the granular structure might have occurred so that there is more effective area for α-amylase to adsorb and thus result in increased enzymatic hydrolysis.

4.4. X-ray Diffraction

Starch granules possess a definite crystalline nature and the crystallinity has been assigned to the well ordered structure of the amylopectin molecules. The data of X-ray powder diffractograms of native *D. alata*, *D. rotundata* and *D. esculenta* starches are presented in Table 19. Native granular starches display X-ray diffraction patterns that have been classified as A (eg.maize), B (eg.potato) and C (eg.cassava)[199]. *Dioscorea* starches exhibited maximum peaks at diffraction angles 17° 2θ. Other significant peaks were around 5.6°,

15°, 23° and 26°. The results confirm that *Dioscorea* starches are B-type and its pattern appears to be similar to that of potato starch.

Table 19: X-ray Diffraction data of *Dioscorea* starches

Starch Sample	Diffraction Data		
	'd' spacing	Angle (2θ)	Intensity* (I_o/I_{max})
D.alata	15.48	5.71	28.49
	6.13	14.44	36.16
	5.87	15.09	51.30
	5.20	17.03	100.00
	3.98	22.30	30.70
	3.68	24.19	34.56
	3.38	26.33	14.83
	2.57	34.86	21.74
	2.34	38.44	10.52
D.rotundata	15.77	5.61	13.55
	6.19	14.30	39.30
	5.75	15.41	62.33
	5.09	17.41	100.00
	3.95	22.50	60.30
	3.80	23.40	70.19
	3.66	24.30	66.40
	2.87	31.21	33.20
	2.34	38.50	28.46
D.esculenta	15.77	5.61	13.55
	6.19	14.30	39.30
	5.75	15.41	62.33
	5.09	17.41	100.00
	3.95	22.50	60.30
	3.80	23.40	70.19
	3.66	24.30	66.40
	2.87	31.21	33.20
	2.59	34.60	32.52

*Values expressed as percentage

A comparison of the specific data on 'd' spacing, diffraction angle (2θ) and peak intensity (I_o/I_{max}) of the control and experimental samples from all the three tubers reveals that starches from control as well as chemically pretreated samples did not show any conspicuous differences. A similar observation has

also been made earlier with chemically pretreated *Amorphophallus* starches[177]. X-ray diffraction data of starches isolated from untreated as well as chemically pretreated tubers of *D. rotundata* are presented in Table 20. From the above data, it could be noticed that starches prepared from chemically pretreated root samples did not show any significant change. However, there was some minor shift in the peaks with respect to both diffraction angle (2θ) as well as intensity (I_o/I_{max}) indicating a partial shift or change in the orientation of crystalline phases, rather a total destruction.

4.5. Rheological Properties

The basic rheology parameters viz., storage modulus G', tangent of the loss angle, tan δ (G"/G') and phase angle δ summed over all the starch samples are shown in Tables 21, 22 and 23. In the case of *D. alata* starch samples (Table 21) the highest value for G' was noticed after holding the paste at 35°C for one hour, except in the case of sample pretreated with KMS. In the latter case, the maximum value for G' was observed at 95°C and when cooled to 35°C, the value showed a decrease. However, when the paste was held at 35°C for one hour an increase in G' value was observed, similar to other samples of *D.alata*. For NaCl treated sample, a continuous increase in G' during heating from 75 to 95°C and then cooling to 35°C was noticed similar to control sample. It was noticed that in the case of control as well as chemically pretreated samples, G' predominates over G" indicating that elastic nature of the material exceeds the viscous nature of the paste. As a rule, the magnitude of G' is not enough to estimate whether the elastic properties predominate over the viscous ones. However, the low values of tan δ or G"/G' indicate that the system is predominantly elastic. In the case of *D. alata* starch samples, the starch sample prepared from tubers pretreated with NH_4OH showed the highest values for G"/G' at every stage indicating a reduction in the elasticity of the system.

Table 20: X-ray diffraction data of *D.rotundata* starch samples

Sample	Diffraction data		
	d' spacing	angle (2θ)	I_o/I_{max} (%)
Control	16.37	5.40	5.00
	7.63	11.60	19.33
	5.77	15.35	64.33
	5.11	17.40	100.00
	3.80	23.40	70.33
	3.34	26.70	29.33
SHMP	15.25	5.80	15.00
	7.63	11.60	18.75
	5.68	15.60	58.75
	5.04	17.59	100.00
	3.69	24.10	56.88
	3.35	26.60	29.68
KMS	15.57	5.70	6.43
	7.38	12.00	16.07
	5.83	15.20	57.14
	5.10	17.39	100.00
	3.80	23.40	67.86
	3.00	29.80	24.28
NaCl	15.76	5.60	7.86
	7.66	11.55	17.86
	5.80	15.30	60.71
	5.14	17.30	100.00
	3.82	23.30	60.71
	3.35	26.60	22.14
NH_4OH	15.76	5.60	9.06
	7.57	11.70	17.80
	5.69	15.58	64.08
	5.07	17.50	100.00
	3.69	24.10	67.96
	3.38	26.40	32.36
GMS	15.76	5.60	11.31
	7.83	11.30	20.07
	5.83	15.20	62.40
	5.14	17.29	100.00
	3.83	23.20	69.71
	3.38	26.40	30.29

Table 21: Rheology data - D.alata starch samples

Temp. (oC)	Control			SHMP			NaCl			KMS			GMS			NH_4OH		
	G' (Pa)	G"/G'	δ	G' (Pa)	G"/G'	δ	G' (Pa)	G"/G'	δ	G' (Pa)	G"/G'	δ	G' (Pa)	G"/G'	δ	G' (Pa)	G"/G'	δ
75	49.90	0.2244	12.6	9.27	0.2999	16.7	58.40	0.2740	15.3	60.80	0.1186	6.8	72.80	0.0940	5.4	53.00	0.2434	13.7
85	51.00	0.1955	11.1	15.00	0.3067	17.1	63.20	0.1788	10.2	72.70	0.0853	4.9	75.60	0.0815	4.7	46.60	0.3562	19.6
95	68.70	0.2213	12.4	97.30	0.2240	12.6	65.20	0.1626	9.3	94.60	0.1195	6.8	91.60	0.0739	4.2	57.20	0.4913	26.1
95 (5)	68.30	0.2108	11.9	111.00	0.1729	9.8	63.10	0.2314	13.0	88.30	0.1069	6.1	94.90	0.0954	5.4	69.50	0.3655	20.1
95 (10)	69.40	0.1628	9.3	115.00	0.1426	8.1	73.40	0.2698	15.1	84.80	0.0950	5.4	93.80	0.0941	5.4	75.70	0.2351	13.3
85	65.30	0.2634	14.7	109.00	0.2073	11.7	76.70	0.2308	13.0	85.50	0.1088	6.2	91.30	0.1150	6.6	58.90	0.4941	26.2
75	68.10	0.2423	13.6	109.00	0.2064	11.6	78.00	0.2231	12.6	84.40	0.1113	6.3	89.40	0.1544	8.7	63.40	0.4306	23.3
65	69.40	0.2406	13.5	115.00	0.2026	11.4	78.00	0.2461	13.8	83.00	0.1160	6.6	87.30	0.1615	9.2	62.00	0.4694	25.2
55	71.40	0.2395	13.4	115.00	0.2070	11.6	80.40	0.2425	13.6	82.40	0.1262	7.2	86.00	0.1570	8.9	64.60	0.4659	25.0
45	73.70	0.2307	13.0	118.00	0.2263	12.7	83.90	0.2372	13.3	82.60	0.1320	7.5	87.90	0.1524	8.7	66.60	0.4699	25.1
35	74.90	0.2443	13.7	124.00	0.2185	12.3	87.00	0.2299	13.0	83.70	0.1362	7.8	86.70	0.1638	9.3	76.20	0.4121	22.4
35 (15)	84.60	0.1560	8.9	132.00	0.1811	10.3	94.30	0.1707	9.7	85.20	0.1232	7.1	91.30	0.1260	7.2	84.20	0.3124	17.4
35 (30)	93.80	0.1247	7.1	140.00	0.1407	8.0	101.00	0.1455	8.3	86.20	0.1183	6.8	94.10	0.1116	6.4	90.80	0.2412	13.6
35 (45)	112.00	0.1000	5.7	142.00	0.1359	7.7	114.00	0.1246	7.1	86.90	0.1162	6.6	97.50	0.1046	6.0	93.40	0.2270	12.8
35 (60)	146.00	0.0648	3.7	145.00	0.1303	7.4	144.00	0.0888	5.1	87.50	0.1135	6.5	101.00	0.1000	5.7	95.60	0.2134	12.0

Figures in brackets indicates the time in minutes, the starch gel was kept at this temperature

Table 22: Rheology data - *D.rotundata* starch samples

Temp. (oC)	Control			SHMP			NaCl			KMS			GMS			NH_4OH		
	G' (Pa)	G"/G'	δ	G' (Pa)	G"/G'	δ	G' (Pa)	G"/G'	δ	G' (Pa)	G"/G'	δ	G' (Pa)	G"/G'	δ	G' (Pa)	G"/G'	δ
75	60.40	0.1238	7.1	50.20	0.3048	17.0	56.80	0.1813	10.3	63.40	0.1435	8.2	77.90	0.0661	3.8	36.50	0.2822	15.8
85	76.10	0.0728	4.2	54.30	0.2339	13.2	62.10	0.1272	7.2	70.60	0.1299	7.4	109.00	0.0419	2.4	36.60	0.2842	15.9
95	106.00	0.0692	4.0	61.30	0.2936	16.3	75.00	0.1059	6.0	77.70	0.1351	7.7	166.00	0.0295	1.7	46.90	0.3241	17.9
95 (5)	103.00	0.0888	5.1	62.00	0.2968	16.5	78.70	0.1090	6.2	72.10	0.1171	6.7	157.00	0.0627	3.6	55.40	0.2924	16.3
95 (10)	99.00	0.0863	4.9	59.70	0.2395	13.5	77.70	0.1032	5.9	67.50	0.1004	5.7	150.00	0.0472	2.7	56.10	0.2513	14.1
85	95.90	0.1105	6.3	54.30	0.3168	17.6	73.80	0.1504	8.6	62.60	0.1613	9.1	141.00	0.0809	4.6	48.40	0.3450	19.0
75	95.90	0.1116	6.4	53.30	0.2908	16.2	70.40	0.1776	10.0	62.20	0.1569	8.9	136.00	0.0704	4.0	44.20	0.3846	21.0
65	93.30	0.1275	7.3	54.40	0.2886	16.1	68.30	0.2035	11.5	62.30	0.1488	8.5	132.00	0.0692	4.0	48.00	0.3354	18.5
55	91.20	0.1393	8.0	54.20	0.2749	15.4	68.00	0.2191	12.4	62.70	0.1563	8.9	130.00	0.0719	4.1	48.00	0.3458	19.1
45	89.10	0.1684	9.6	56.50	0.2655	14.9	70.00	0.2157	12.2	62.40	0.1635	9.3	128.00	0.0738	4.2	47.70	0.3606	19.8
35	90.40	0.1704	9.7	56.30	0.2700	15.1	72.20	0.2161	12.2	63.80	0.1583	9.0	127.00	0.0746	4.3	52.20	0.3487	19.2
35 (15)	91.10	0.1339	7.6	58.90	0.2377	13.4	73.60	0.1997	11.3	64.80	0.1427	8.1	127.00	0.0646	3.7	58.80	0.2738	15.3
35 (30)	91.70	0.1265	7.2	59.30	0.2243	12.7	74.90	0.1789	10.2	65.70	0.1314	7.5	128.00	0.0627	3.6	64.10	0.2356	13.3
35 (45)	92.10	0.1249	7.1	59.40	0.2189	12.3	75.20	0.1702	9.7	66.00	0.1259	7.2	128.00	0.0607	3.5	66.90	0.2212	12.5
35 (60)	92.40	0.1245	7.1	59.60	0.2114	12.0	75.60	0.1680	9.5	66.00	0.1261	7.2	129.00	0.0590	3.4	68.40	0.2120	12.0

Figures in brackets indicates the time in minutes, the starch gel was kept at this temperature

Table 23: Rheology data - *D.esculenta* starch samples

Temp. (oC)	Control			SHMP			NaCl			KMS			GMS			NH_4OH		
	G' (Pa)	G"/G'	δ	G' (Pa)	G"/G'	δ	G' (Pa)	G"/G'	δ	G' (Pa)	G"/G'	δ	G' (Pa)	G"/G'	δ	G' (Pa)	G"/G'	δ
75	32.40	0.1954	11.1	53.10	0.3202	17.7	12.10	0.1917	10.9	16.00	0.3581	19.8	0.09	0.6828	34.4	37.00	0.3378	18.6
85	29.20	0.1798	10.2	73.00	0.2932	16.3	12.90	0.2302	13.0	26.70	0.3161	17.6	0.75	0.2895	16.1	32.70	0.3211	17.8
95	26.10	0.1966	11.1	77.50	0.3484	19.2	13.90	0.3043	16.9	51.90	0.2563	14.4	12.20	0.1238	7.1	36.30	0.3691	20.3
95 (5)	23.00	0.1930	10.9	73.70	0.2320	13.0	12.60	0.2627	14.7	45.00	0.2911	16.2	22.70	0.1026	5.9	41.20	0.3617	19.9
95 (10)	21.20	0.1892	10.7	70.80	0.1808	10.2	11.70	0.2308	13.0	41.90	0.2625	14.7	20.90	0.1115	6.4	43.10	0.3132	17.4
85	19.10	0.2298	13.0	62.80	0.2930	16.3	9.96	0.3414	18.8	38.80	0.3889	16.1	19.90	0.1156	6.6	43.60	0.2638	14.7
75	19.00	0.2232	12.6	63.90	0.2582	14.4	10.40	0.3144	17.4	39.30	0.2595	14.5	20.50	0.1117	6.4	43.80	0.2265	12.8
65	19.30	0.2311	13.0	65.90	0.2322	13.1	10.90	0.2908	16.3	39.20	0.2628	14.8	26.00	0.0896	5.1	43.60	0.1945	11.0
55	19.70	0.2365	13.3	67.50	0.2296	13.0	11.40	0.3035	16.9	40.30	0.2556	14.4	32.20	0.0773	4.4	43.00	0.1798	10.2
45	20.20	0.2515	14.1	68.60	0.2216	12.5	12.00	0.3167	17.6	41.40	0.2657	14.8	36.60	0.0773	4.4	42.30	0.1716	9.7
35	20.90	0.2598	14.6	71.30	0.2216	12.5	12.50	0.3248	18.1	43.10	0.2599	14.6	39.50	0.0794	4.5	41.70	0.1734	9.9
35 (15)	21.30	0.2553	14.3	73.40	0.2016	11.4	13.10	0.3176	17.6	45.20	0.2412	13.5	41.60	0.0772	4.4	52.10	0.2399	13.5
35 (30)	21.60	0.2528	14.2	74.30	0.1952	11.0	13.10	0.3145	17.3	45.70	0.2298	13.0	42.10	0.0772	4.4	59.70	0.2178	12.2
35 (45)	21.60	0.2537	14.2	74.50	0.1946	11.0	13.20	0.3068	17.0	46.00	0.2261	12.8	42.10	0.0758	4.3	60.90	0.2036	11.5
35 (60)	21.60	0.2523	14.1	75.00	0.1907	10.8	13.30	0.3060	17.1	46.20	0.2251	12.7	42.40	0.0741	4.2	60.20	0.1944	11.0

Figures in brackets indicates the time in minutes, the starch gel was kept at this temperature

As shown in Table 22, the control and experimental samples of *D. rotundata* showed an increase in G' during heating and the maximum value was observed at 95°C except for NH_4OH treated sample. In the latter case, although an increase in G' was observed during heating, the maximum value for G' was noticed when, the starch paste was held at 35°C for 45 minutes. The general pattern observed was that G' increased during heating to 95°C, after which the value declined when the system cooled to 35°C. Subsequently when the paste was held at 35°C for 60 minutes, an increase in G' was observed. As seen from Table 22, starches extracted from chemically pretreated tubers generally lowered the value for G' except for the GMS treated sample. In the latter case, a significant increase in G' value from 106 of the control sample to 166 Pa was observed (Fig.12, Table22). In conjunction with the above increase in G' value a decrease in G"/G' or phase angle could also be noticed. Among the other samples, SHMP and NH_4OH pretreated samples showed relatively lower values for G'. In both the above cases, a corresponding increase in G"/G' ratio or phase angle could be observed. For NaCl and KMS treated samples also, a decrease in G' value was observed although to a less extent compared to NH_4OH and SHMP treated samples.

In the case of control sample of *D. esculenta*, during heating from 75-95°C, some discrepancy in the values of G' was observed, which may be due to the fact that during the measurements in the Bohlin rheometer, the samples are heated prior to loading on the rheometer and hence the uniformity of the suspension is not ensured. However, the data obtained during cooling to 35°C and holding the paste for one hour at the above temperature are found to be logical (Table 23). An increase in G' was observed for all the treated samples during heating. For SHMP, NaCl and KMS treated samples, values for G' reached the maximum at 95°C and upon cooling the gel to 35°C the value decreased. When the cooled system was subsequently held at 35°C for one hour, G' value again showed an increase indicating the formation of an elastic

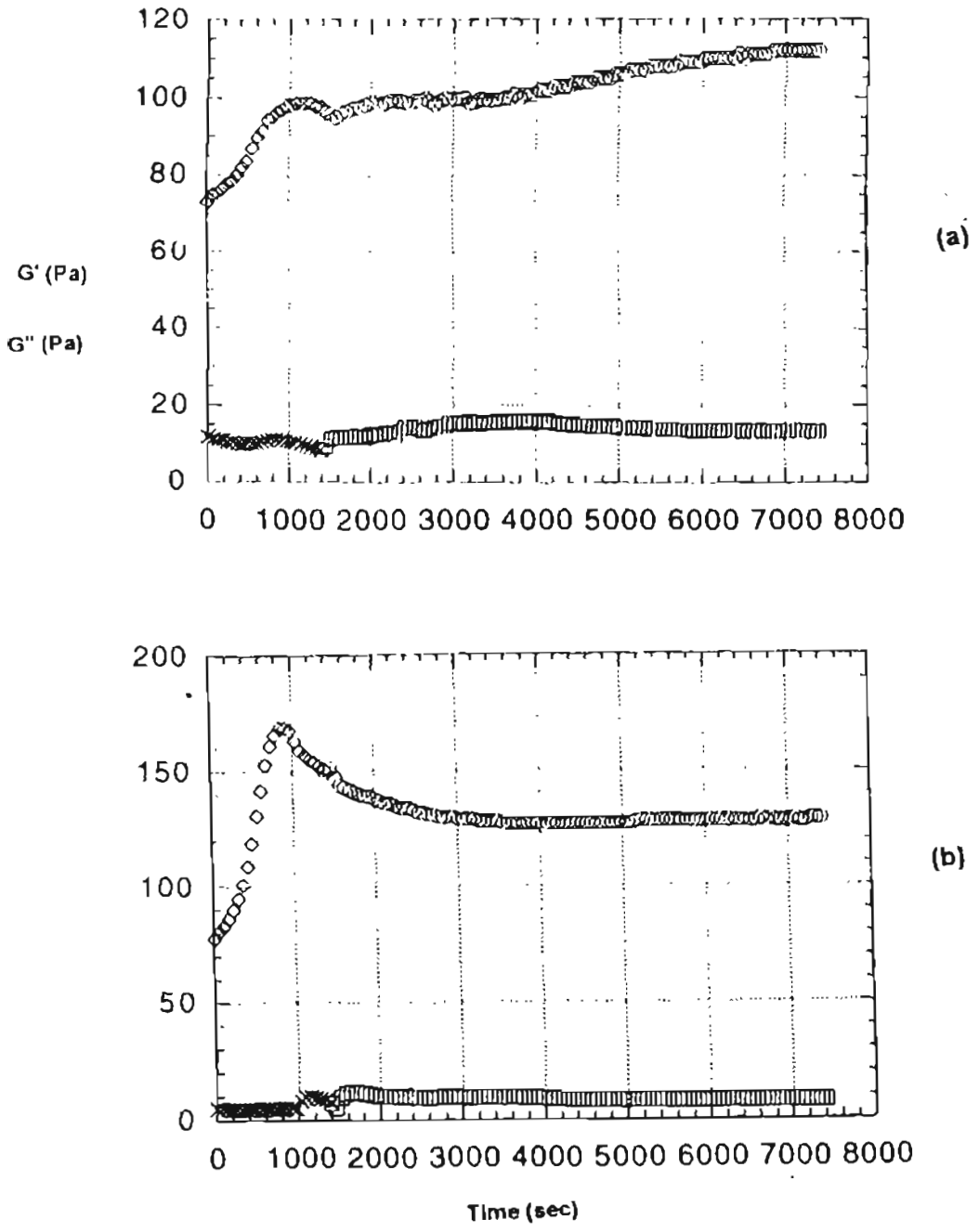


Fig 12: Storage (G') and Loss moduli (G") as a function of time for *D. rotundata* starches.

(a) Control (b) GMS treated sample.

gel system. For GMS and NH_4OH treated samples, although an increase in G' value was observed during heating, the maximum value for G' was shown, when the cooled paste was held at 35°C for 60 and 45 minutes respectively. A comparison of the G' value of the treated samples revealed that the NaCl treated sample generally possesses lowest values for G' with corresponding increase in the G"/G' ratio or phase angle suggesting there by the formation of a relatively less elastic gel. As in the case of *D. alata* and *D. rotundata*, NH_4OH treated samples of *D. esculenta* also showed a relatively higher ratio of G"/G' suggesting that the gel formed is less elastic. For GMS treated sample, although the value of G' was relatively low, the tangent of the loss angle or the ratio of G"/G' showed lower values indicating the formation of an elastic gelled system.

Studies carried out on the rheological properties of native as well as the treated samples of *Dioscorea* starches have thus revealed that chemical pretreatment of tubers affect the visco-elastic properties of the starch gel in a noticeable manner. However in the case of all samples, it was noticed that the elastic nature of the material exceeds the viscous nature of the paste as indicated by a low value for G"/G'. This is in agreement with previous reports, that describe starch pastes like soft solids in which the deformations within the linear range imposed by low strain will be essentially recoverable[42,200]. In general, during heating the storage (G') and loss moduli (G") increased and the phase angle decreased, indicating a phase change from sol to gel which occurred during heating. This increase in G' and G" is due to progressive swelling of the starch granules so that they finally became close-packed. As mentioned earlier, an increase in the elasticity of the system was noticed in GMS pretreated samples of all the three *Dioscorea* starches. The above effect was however more pronounced for *D.rotundata* starch. Eliasson et al.[46] have reported an increase in G" of hot starch pastes of 10% w/w of corn, potato and waxy barley in presence of GMS, Sodium Stearoyl Lactylate (SSL) and SDS at

1% concentration. In a subsequent study[47], they have observed that presence of saturated monoglycerides increased G' for pastes of native corn starch. Biliaderis et al[201] have observed an increase in G' values when monoacyl lipids are included in rice and wheat starch gels while smaller changes in viscoelastic properties were observed for legume starch gels. Unlike GMS treated sample, NH_4OH treated samples showed a noticeable loss in the elastic character of the system as reflected from the relatively higher values of G"/G'. This indicates a higher contribution of viscous components to the properties, which may be ascribed to the action of NH_4OH acting as lubricating agent in the dispersion.

CHAPTER V

EFFECT OF ACID TREATMENT ON PROPERTIES OF DIOSCOREA STARCHES

INTRODUCTION

Starches are invariably modified prior to their use either for industrial purposes or for direct consumption as a food item. Hydrolysis is one of the common methods carried out in presence of either acid or enzyme or a combination of the above two in order to modify the starch granules. Treatment of starches with sulphuric acid (1.7 M) at room temperature results in formation of Nageli amylodextrins[202] while treatment with 2.2 M HCl at slightly elevated temperatures, around 30-40°C produces litnerized starch[203]. In this chapter, results of the study carried out to investigate the effects of treating *Dioscorea* starches viz., *D.alata, D.rotundata* and *D.esculenta* with 2.2 M HCl at room temperature of 28-30°C upto a period of 72 h, on some of the physico-chemical and enzyme digestibility properties are discussed.

5.0. Results and Discussion

5.1. Compositional Characteristics of Native *Dioscorea* Starches

Compositional characteristics, covering moisture (%), starch (%), protein (%), free fat (%) and ash content (%) of the unmodified starches extracted from *D. alata, D. roundata* and *D. esculenta* were determined (Table 24). All the dried native starch samples had a moisture range of 14-15% and the actual starch content (%) of the samples varied between 92-98% showing that they are relatively pure. While protein content in the starch samples ranged between 0.03 to 0.05%, the free fat content in the sample were in the range of 0.16 to 0.64%. Ash content was found to be in the range of 0.13-0.22%.

Table 24: Compositional characteristics of Dioscorea starches

Sample	Moisture* (%)	Starch* Content (%)	Protein* (%)	Lipid* (%)	Ash* (%)
D.alata	15.4	95.9	0.04	0.16	0.22
D.rotundata	14.3	97.8	0.03	0.64	0.13
D.esculenta	14.4	91.9	0.05	0.50	0.23

* Mean of 3 determinants

5.2. Free Reducing Sugars

The results of the study indicated that there is a slow but progressive accumulation of free sugars immediately after starch granules came into contact with acid, i.e. 0 h reaction onwards (Table 25). Native *D. alata* starch contained about 0.02% free sugar, whereas after acid treatment for 72 h, the free sugar content showed a high value reaching 0.4%, i.e. almost 20 fold increase was observed. In a similar manner, *D. rotundata* starch samples also, as a result of acid treatment for 72 h, showed about 16 fold higher sugar concentration compared to that of the control sample. Compared to *D. alata* and *D. rotundata*, the increase in the sugar concentration as a function of the duration of acid treatment was more pronounced in the case of *D. esculenta* starch. In the above case, acid treatment even for 56 h raised the free reducing sugar content to 0.85% from the initial value of 0.035% of the control sample, i.e., almost 24 fold increase was observed.

Table 25: Free sugar contents and cold water solubles of acid hydrolysed Dioscorea starches

Sample	Free reducing sugar* (%)			Cold water solubles* (%)		
	D.alata	*D.rotundata*	*D.esculenta*	*D.alata*	*D.rotundata*	*D.esculenta*
Control	0.02	0.1	0.035	1.5	3.25	2.25
0h	0.08	0.2	0.430	3.9	4.20	4.85
8h	0.09	0.3	0.440	6.9	5.45	6.35
24h	0.10	0.8	0.460	11.5	10.80	8.97
32h	0.15	1.3	0.560	13.25	11.37	10.70
48h	0.20	1.4	0.740	14.70	13.15	15.75
56h	0.30	1.5	0.850	16.05	15.75	17.15
72h	0.40	1.6	ND	ND	18.40	ND

* Mean of 3 determinants; ND-Not determined

Studies by Robin et al.[140,204] on acid treatment of potato starch for 40 days revealed that solubilization of carbohydrate during acid treatment occurs in two stages, the first one being relatively faster. Further, the faster pattern of solubilization occurring during the initial phase of acid treatment was attributed to the hydrolysis of the amorphous part of the starch granule which was followed by a slower rate of hydrolysis occurring in the crystalline region during the second stage (8-40 days). Wolfrom et al[205] have also supported the above view of the preferential action of acid on the linear region of the starch *compared to the vicinity of the branching cites during the initial stage of the* starch acid interaction. As there is enough evidence, which establishes that it is the amylopectin that builds up the crystalline regions of starch[206], it should be inferred that the earlier action during acid treatment at room temperature generally remains targeted towards the linear amylose sub-unit or long chains with limited branching. Studies on acid treated samples of cassava and maize[141] have suggested an increase in dissolution of carbohydrates up to 20 days, subsequent to which the samples showed a tendency to decline. The presently observed increase in dissolved sugars as a result of acid treatment hence indicates that the action of acid is targeted towards the amorphous region which is further supported by a progressive decrease in amylose content observed in the acid-treated starches.

5.3. Cold water solubles

As could be observed from Table 25, among the three native starches, the percent cold water solubles was found to be the highest for *D. rotundata* (3.25%) while the corresponding values for *D. alata* and *D. esculenta* were *1.5% and 2.25% respectively*. *There was found to be a conspicuous increase in* percentage of cold water solubles as a result of acid-treatment for all the three starch samples (Table 25).

It has been reported that the cold water solubility of acid-treated cassava and maize starches showed a three-stage pattern[141]. The highest solubility of cassava and maize starches as a result of acid treatment was noticed between 9 and10 days, and between 14 and 24 days respectively. In the present study it has been found that the percentage of cold water solubles incrementally changed with duration of acid treatment. The above increase in cold water solubles as a result of acid treatment again supports the possibility of the action of acid on the amorphous region. During acid hydrolysis, the glucosidic bonds are ruptured resulting in the formation of short molecular segments having more solubility in water.

5.4. Intrinsic Viscosity

There was a progressive decline in the intrinsic viscosity of the acid-treated samples of all the three *Dioscorea* starches commencing from 0 h treatment. The levels of % reduction in intrinsic viscosity calculated for zero hour treated samples were 59.78, 52.65 and 60.78% for *D. alata, D. rotundata* and *D. esculenta* starches respectively (Table 26). Along with the increase in the duration of acid-treatment, there was a progressive reduction in intrinsic viscosity, so that the samples acid-treated for the longest period of 72 h, evinced a reduction by 90.76%, 92.14% and 89.54 for the above three samples compared to their respective control. The above decline in the values of intrinsic viscosity was more prominent in the samples acid-treated for 8 h and beyond. Among the three *Dioscorea* samples, *D. rotundata* starch sample acid-treated for 72 h showed greatest percent reduction in viscosity (Table 26). A similar effect was observed with acid hydrolysed arrow root starch[144] in which the intrinsic viscosity for the sample acid-treated for 72 h showed a decline by about 94% compared to that of the control sample.

Table 26: Intrinsic viscosity* data of acid hydrolysed *Dioscorea* starches

Sample	Intrinsic Viscosity		
	D.alata	*D.rotundata*	*D.esculenta*
Control (Unmodified)	1.84±0.015	1.91±0.005	1.53±0.020
Acid-treated			
0 h	0.74±0.020	0.91±0.005	0.60±0.010
8 h	0.45±0.000	0.41±0.010	0.26±0.005
24 h	0.30±0.010	0.23±0.050	0.24±0.000
32 h	0.28±0.020	0.21±0.040	0.23±0.005
48 h	0.24±0.005	0.19±0.010	0.19±0.010
56 h	0.20±0.010	0.17±0.010	0.18±0.010
72 h	0.17±0.005	0.15±0.000	0.16±0.010

*Mean of 3 determinants were taken and the dispersion is indicated by Standard Deviation

Reduction in the intrinsic viscosity of starch samples could generally be attributed to the progressive cleavage of glucosidic bonds of the starch molecules during hydrolysis leading to formation of short molecular segments, which are more soluble. Betancur and Chel have reported a reduction in viscosity of starch associated with an increase in the alkali number in the case of acid hydrolysed *Canavalia ensiformis* starch[143]. According to the above authors, factors such as strength of acid, temperature and reaction time significantly influence the modification of native starch. The stronger the severity of the treatment, the higher the degree of hydrolysis attained, which in turn results in lowering the viscosity.

5.5. Amylose content

Compared to the rate of decline in the intrinsic viscosity, fall in amylose blue value appeared to be more gradual. It was observed that progressive reduction in amylose content in the samples was directly related to duration of acid treatment. Thus the starch samples acid-treated for 72 h showed the highest reduction in blue value by 35.90, 41.25 and 55.50% for *D. alata*, *D. rotundata* and *D. esculenta* starches respectively (Table 27). As could be seen from the data, among the above starch sources, the maximum reduction in the amylose blue value as a result of acid treatment was observed for *D. esculenta* starch.

Table 27: Amylose content* of acid-hydrolysed *Dioscorea* starches

Sample	Amylose blue value		
	D.alata	*D.rotundata*	*D.esculenta*
Control (Unmodified)	0.156± 0.0001	0.0160±0.0003	0.0126±0.0004
Acid-treated			
0 h	0.0154± 0.0004	0.0156±0.0002	0.0108±0.0002
8 h	0.0150 ±0.0001	0.0152±0.0002	0.0096±0.0001
24 h	0.0148 ±0.0005	0.0148±0.0003	0.0094±0.0009
32 h	0.0136 ±0.0003	0.0130±0.0000	0.0076±0.0006
48 h	0.0124 ±0.0003	0.0110±0.0007	0.0072±0.0006
56 h	0.0114 ±0.0000	0.0100±0.0006	0.0068±0.0004
72 h	0.0100 ±0.0002	0.0094±0.0006	0.0056±0.0001

*Mean of 4 determinants were taken and the dispersion is indicated by Standard Deviation

The above pattern of decrease in amylose content noticed as a result of acid-treatment suggests that the action of acid on amylose region, takes place possibly by disrupting the hydrogen bonds, the presence of which otherwise would have facilitated complexation with iodine molecule. Betancur and Chel[143] have, however, reported an increase in apparent amylose during acid hydrolysis of *Cannavalia ensiformis* starch. According to the above authors, increase in amylose was attributed to faster rate of amylopectin depolymerization and liberation of more and more linear fragments. But many of the earlier studies carried out in this area[139,141,204] have revealed a decrease in amylose, especially during the earlier phase of starch-acid interaction at ambient temperature. The work on acid treatment of arrowroot starch[144] has also shown a similar trend. It is, however, possible that at elevated temperatures i.e. 45 and 55°C as tried by Betancur and Chel[143], a heterogeneous hydrolytic degradation would be possible resulting in the cleavage and depolymerization of amylopectin sub-unit leading to the formation of linear fragments within a short period of 4 and 6 h, unlike the pattern of cleavage that occurs at ambient temperature affecting the amorphous region.

5.6. Swelling Volume and Solubility

Results of a comparative study of swelling and solubility properties of the acid modified starches are presented in Table 28. An evaluation of swelling volume (ml/g of starch) and solubility (%) at 90°C revealed that starch samples acid treated for 24 h and beyond lost their swelling capacity and correspondingly they showed higher solubility at elevated temperature. Unmodified starch granules of *D. alata*, *D. rotundata* and *D. esculenta* showed swelling volumes of 27, 26 and 47 ml/g of starch respectively. In the case *of D. esculenta*, the starch sample acid-treated for 8 h and beyond lost the swelling capacity. *D. rotundata* starch when acid treated for 24 h showed a reduction in swelling volume by 96% whereas in the case of *D. alata* sample percent reduction was to an extent of 93% compared to the respective control

Table 28: Swelling volume* and solubility* data of *Dioscorea* starches

Sample	Swelling volume (ml/g of starch)			Solubility (%)		
	D.alata	*D.rotundata*	*D.esculenta*	*D.alata*	*D.rotundata*	*D.esculenta*
Control (Unmodified)	27.0±0.5	26.0±0.5	47.0±1.4	14.6±0.0	16.3±0.0	10.5±0.9
Acid-treated						
0 h	23.0±0.0	15.0±0.5	16.5±0.7	48.0±3.0	44.4±1.0	57.2±0.0
8 h	10.0±0.5	4.0±0.0	BD	81.0±0.0	91.9±3.0	72.5±0.1
24 h	1.8±0.5	1.0±0.0	BD	90.8±3.0	93.6±0.7	83.7±0.4
32 h	BD	BD	BD	92.5±3.5	95.0±0.5	84.5±0.6
48 h	BD	BD	BD	100.0±0.0	97.0±2.0	85.3±0.3
56 h	BD	BD	BD	100.0±0.0	100.0±0.5	84.0±1.5
72 h	BD	BD	BD	100.0±0.0	100.0±0.4	83.0±0.7

BD - Below detectable level

*Mean of 3 determinants were taken and the dispersion is indicated by Standard Deviation

sample. Beyond 24 h of treatment, all the acid-modified samples lost the swelling capacity. It could also be noticed that in association with the above loss in swelling capacity, there was a corresponding increase in solubility. While unmodified *D. rotundata* starch showed only 16.3% solubility, the sample acid-treated for 72 h showed 100% solubility. Similarly, in the case of *D. alata*, the control showed only 14.6% solubility while the sample acid-treated for 72 h showed almost 100% solubility. Among the *Dioscorea*

species, solubility of *D. esculenta* starch was found to be the lowest, showing an increase from 10.5% to 83.0% as a result of acid-treatment for 72 h. In the above case, the rate of increase in solubility beyond 32 h treatment appeared to be only marginal.

In an unmodified starch molecule, hydroxyl groups can hold water molecules by means of hydrogen bonds, causing swelling of the granule. However, in acid-treated samples, when their aqueous solutions are heated and cooled, starch granules get fragmented in a radial manner without attaining their organized structure. As a result, hydrolysed starch granules no longer can retain water inside the structure. The short molecular segments produced as a result of cleavage of glucosidic bonds are more soluble and therefore absorb less water and swell to a less extent. A similar pattern of swelling and solubility has been also observed in acid-treated arrowroot starch[144]. Further, the gel permeation chromatographic analysis of acid-hydrolysed arrowroot starch[144] has indicated the formation of higher proportions of dextrins of small and medium chain lengths. Betancur and Chel[143] also reported a similar pattern of swelling power and solubility for acid hydrolysed *Cannavalia ensiformis* starch.

5.7. Gelatinization Pattern by Differential Scanning Calorimetry (DSC)

Acid-treatment of *Dioscorea* starch samples was found to affect in a major way the thermodynamic aspects of gelatinization as reflected from T_i, T_f and ΔH values representing onset and end point of gelatinization and gelatinization enthalpy respectively (Table 29). It was apparently clear that acid-treatment caused delay in the onset of gelatinization of *D. alata* and *D. rotundata* starch samples while acid modified *D. esculenta* starch samples displayed earlier gelatinization. Further, the gelatinization temperature range got widened with duration of acid-treatment *for D. esculenta* starch. The control sample of *D. esculenta* showed a gelatinization range from 70.6 to 76.9°C and after acid-treatment for 72 h the gelatinization range was extended

Table 29: DSC data of acid hydrolyzed Dioscorea starches

Sample	T_i* (°C)	T_f* (°C)	ΔH* mj/mg
D.alata			
Control (un-modified)	74.9	81.30	14.7396
0h	75.4	81.20	12.3747
8h	77.4	83.80	13.7745
24h	79.3	85.70	12.9012
32h	80	86.30	10.7492
48h	80.3	87.80	9.7586
56h	79.6	88.40	9.7599
72h	81.7	90.50	9.0084
D.rotundata			
Control (un-modified)	73.45	84.60	15.4504
0h	74.30	84.10	14.8593
8h	77.30	85.40	14.8191
24h	79.60	87.20	11.7251
32h	80.00	87.85	7.8833
48h	80.50	89.95	6.7096
56h	80.75	88.20	9.3548
72h	71.15	89.65	5.2962
D.esculenta			
Control (un-modified)	70.60	76.90	15.2130
0h	70.10	76.90	11.7847
8h	66.50	86.80	10.1018
24h	65.10	89.50	12.9383
32h	64.70	86.60	10.6909
48h	65.10	85.30	9.3949
56h	65.10	87.40	9.1249
72h	59.50	86.35	6.5574

*Average of 2 determinations

between 59.5 and 86.35°C (Fig 13). A similar effect, although to a less extent, was observed for *D. alata* starches also (Table 29). In the case of *D. rotundata*, although the sample acid treated for 72 h showed an increase in the gelatinization range, rest of the acid treated samples displayed a marginal reduction unlike that of *D. esculenta* and *D. alata* starches. A progressive increase in T_f value was observed with increase in the duration of acid treatment in all the samples. As a result of acid-treatment, there was also a gradual decrease in gelatinization enthalpy (ΔH).

The impact of acid-treatment on gelatinization properties showed variation among the three species of *Dioscorea* studied. An increase in the

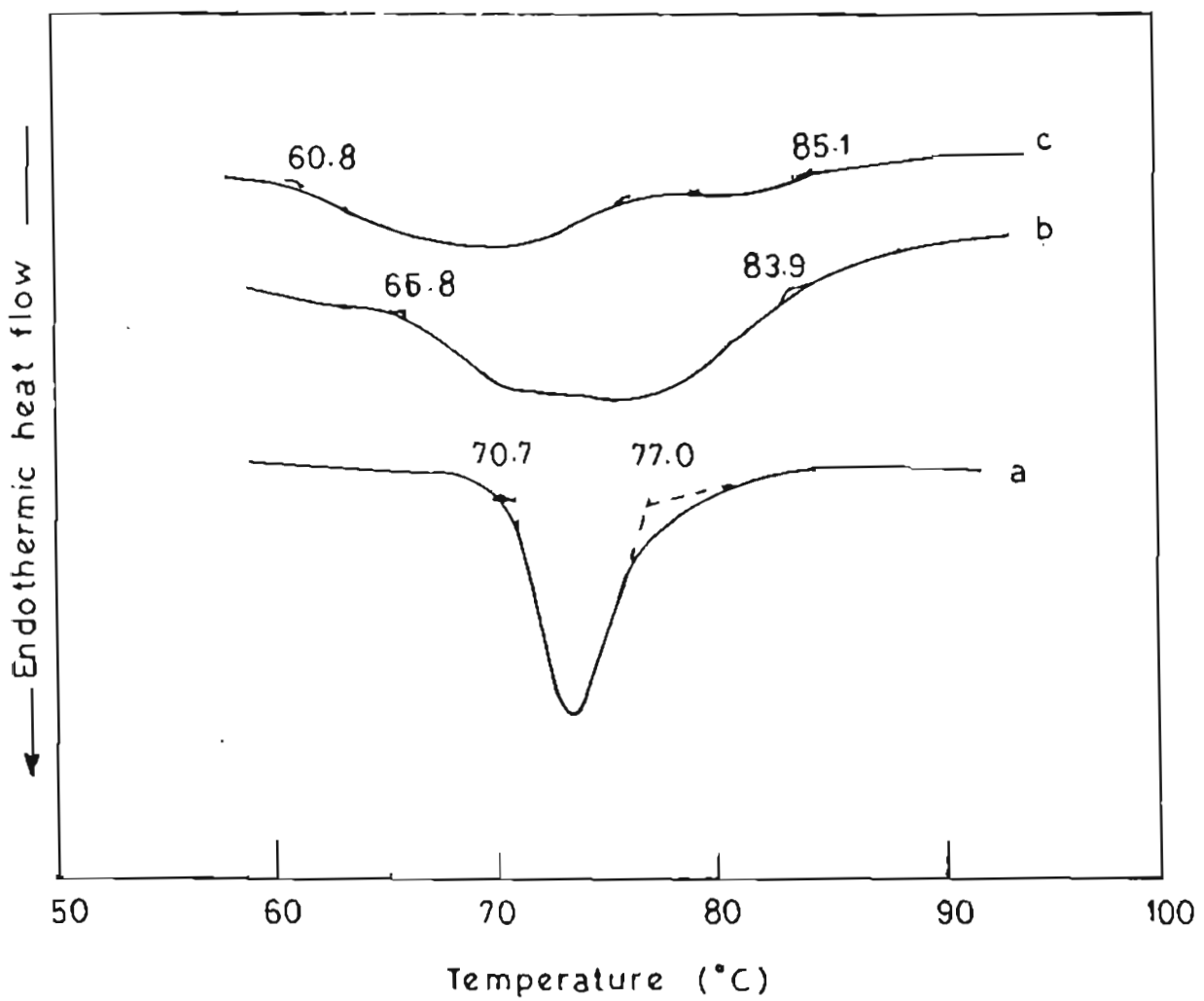


Fig 13: DSC thermogram of *D. esculenta* starch samples.
(a) native (Control) (b) acid hydrolysed for 48 h (c) acid hydrolysed for 72 h.

onset of gelatinization (T_i) temperature as a result of acid-treatment of starches has been reported by earlier workers also. Leach and Schoch[207], from their studies on acid modification of corn starch, have reported an increase in gelatinization temperature and they attributed the above increase to the increased micellar organization and internal retrogradation occurring within the starch granules. Contrary to the above, an early gelatinization resulting in lowering of T_i value was observed for acid modified *D. esculenta* starches. It is generally accepted that action of acid is predominantly directed towards amorphous region and should facilitate rapid penetration of water molecules at lower temperatures enabling earlier swelling of the granules. However, it is also possible that disruption and penetration of water through the starch crystallites take more time resulting in widening the temperature range, i.e. T_i–T_f in order to complete the gelatinization process. In the present study, it was noticed that acid hydrolysed *D.esculenta* starch showed earlier gelatinization and a corresponding widening of DSC endotherm with increase in the duration of acid treatment. More or less a similar observation has been made by Jenkins and Donald[142] and Beliaderis et al[208]. According to the latter, the acid treatment leads to weakening and destabilization of amorphous region facilitating faster penetration of water molecules and gelatinization.

5.8. X-ray diffraction

X-ray diffraction patterns of control, 0 and 72 h acid treated samples of *D. alata*, *D. rotundata* and *D. esculenta* are shown in Figs.14-16 respectively. Acid hydrolysed samples showed more intense diffraction peaks than their corresponding control samples. A similar increase in peak intensity for acid hydrolysed samples has been reported by other workers also[142,204,209,210]. To explain the above increase in the intensity of diffraction peaks, it has been suggested that the acid preferentially attack the amorphous regions of the starch granule without affecting the crystalline regions, during acid treatment for short period as adopted in the present study. The work of Robin et al.[204]

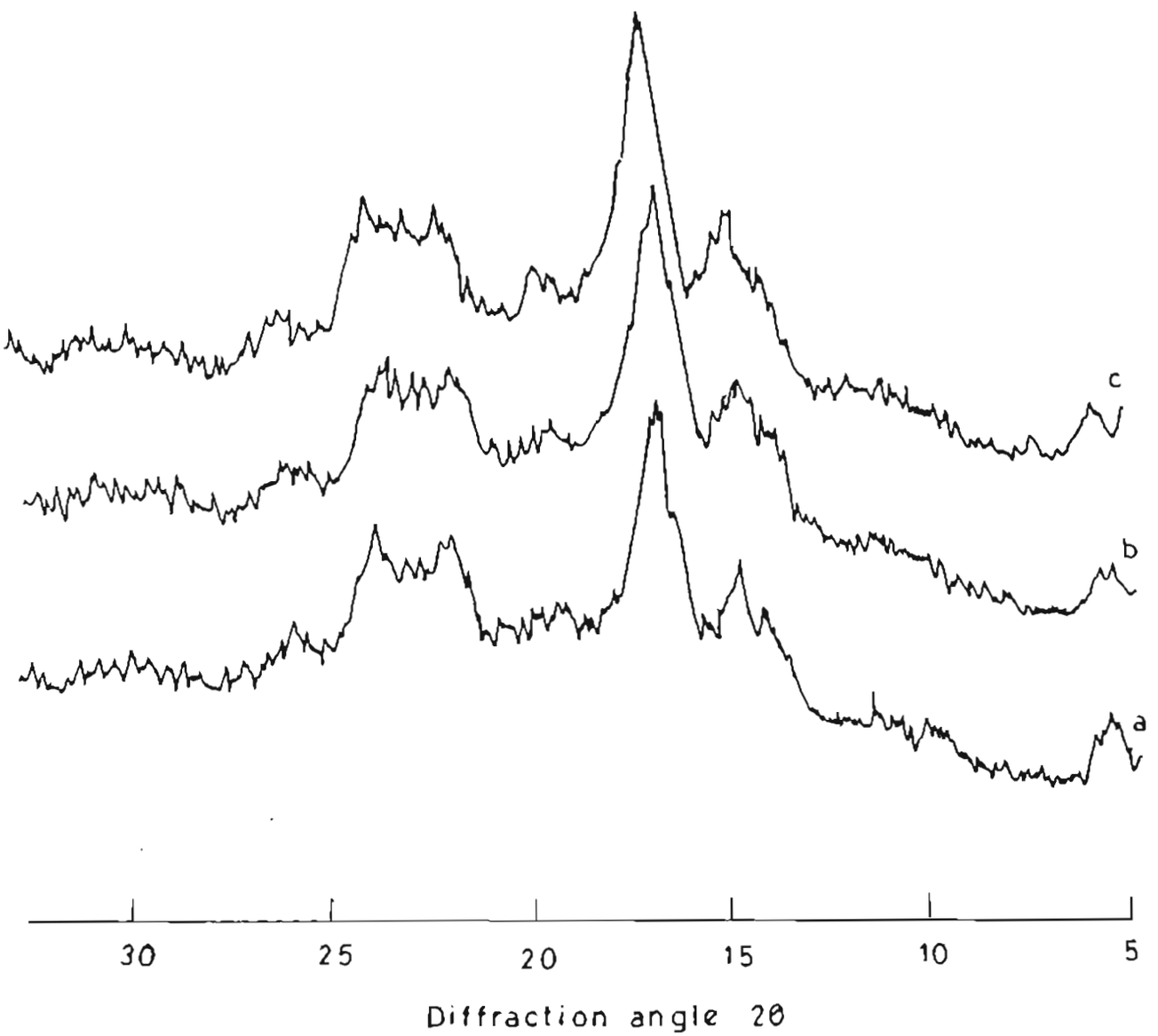


Fig 14: X-ray diffraction pattern of *D. alata* starch samples.
(a) native (Control) (b) acid hydrolysed for 0 h (c) acid hydrolysed for 72 h.

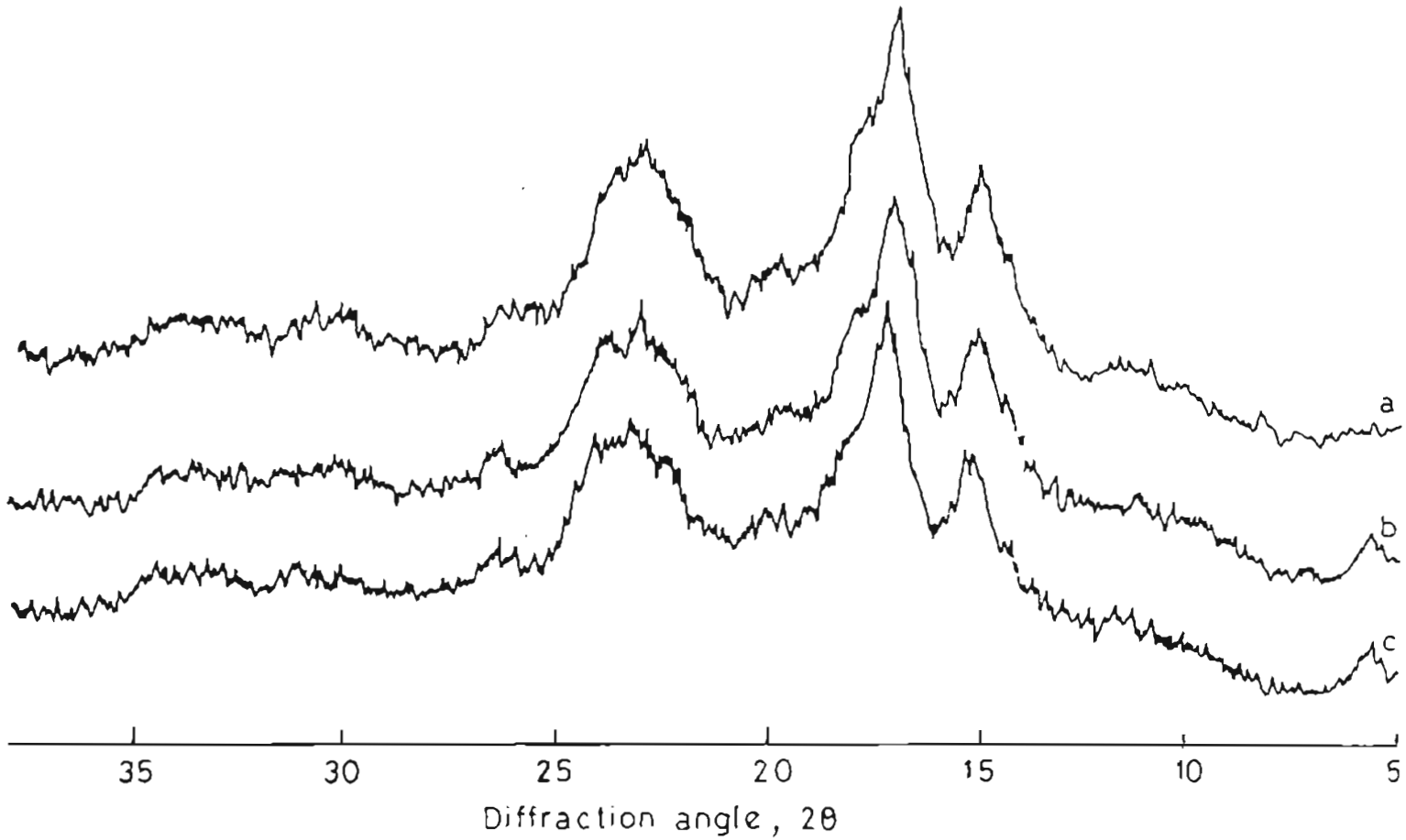


Fig 15: X-ray diffraction pattern of *D. rotundata* starch samples.
(a) native (Control) (b) acid hydrolysed for 0 h (c) acid hydrolysed for 72 h.

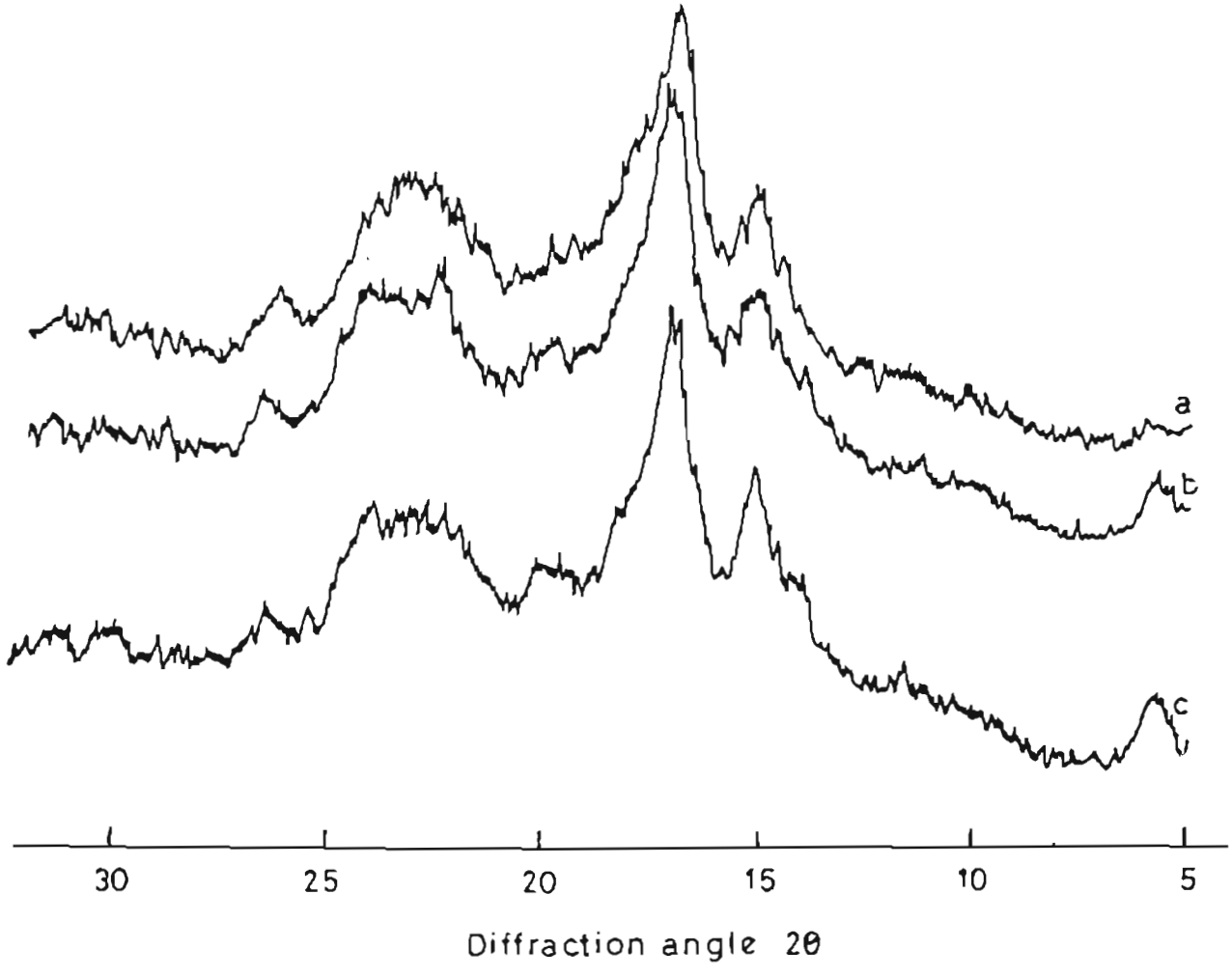


Fig 16: X-ray diffraction pattern of *D. esculenta* starch samples.
(a) native (Control) (b) acid hydrolysed for 0 h (c) acid hydrolysed for 72 h.

provides further evidence supporting the above explanation. While studying the effect of acid hydrolysis on potato starch with 2.2 M HCl at 35°C for a period of 40 days, the above workers observed a two stage hydrolysis process, an initial fast step (0-8 days) postulated to arise from hydrolysis of the amorphous regions, followed by a second slower rate (8-40 days), attributed to hydrolysis of more crystalline regions. Since in the present study, the total duration of acid hydrolysis is less than 8 days, it is likely that the crystalline regions remain unaffected by the acid. Corresponding to the increase in the extent of acid treatment, there was also an increase in the percentage of crystallinity. An earlier study, comparing the X-ray diffraction pattern of cassava and maize starches[141] subjected to acid treatment for 5 and 15 days had revealed increased crystallinity in the case of samples acid treated for 15 days. Jenkins et al[142] from their experiments, simultaneously using Small Angle and Wide Angle X-ray Scattering (SAXS/WAXS) techniques, suggested that when the starch is acid hydrolysed, the only changes are in the electron densities of the amorphous lamellae and of the amorphous background region. Both the above electron densities particularly that of the amorphous background steadily decline as the duration of hydrolysis advances. The greater destruction of the background region than of the amorphous lamellae can also be explained as the former is more accessible to attack by the acid than the narrower and more constrained lamellae. Since the acid is known to etch away the amorphous regions within the granule, a reduction in electron density for such regions is expected. The small angle peaks become less intense as a consequence of the above reduction in the electron density of the amorphous regions. Simultaneously the normalised WAXS data shows diffraction peaks with higher intensity since the amorphous regions are preferentially hydrolysed and they contribute less to the scattering. The present study on the X-ray diffraction of *Dioscorea* starches confirms the preferential attack of acid on the amorphous region.

5.9. Enzyme susceptibility

The patterns of α-amylase susceptibility of acid hydrolysed *D. alata*, *D. rotundata* and *D. esculenta* starches are shown in Figs. 17-19. Native *D. alata* starch showed maximum enzyme susceptibility at 45 minutes of incubation, whereas all acid treated samples showed maximum release of dextrose earlier between 15-45 minutes of incubation. After 45 minutes, there was a tendency towards a decline in enzyme susceptibility for all samples. Native *D. rotundata* starch also showed maximum susceptibility at 45 minutes of incubation while the corresponding acid modified samples showed considerable variations in the enzyme susceptibility. The time required to attain maximum susceptibility was shortened with increase in the duration of acid treatment. The sample acid-treated for 0 h exhibited maximum susceptibility at 60 minutes of incubation while samples acid-treatd for 8,24 and 32 h showed highest enzyme susceptibility within 45 minutes of incubation. Samples acid-treated for 48 h and beyond displayed highest enzyme susceptibility at shorter incubation periods as presented in Fig 18. Similar to *D. alata* and *D. rotundata* starches, native *D. esculenta* starch also showed maximum susceptibility at 45 minutes of incubation. The maximum release of dextrose was observed to be at 15 minutes of incubation for the sample that was acid treated for 0 h while for 8 and 24 h samples, maximum susceptibility was extended to 30 minutes of incubation. For the samples treated for 32 and 56 h, maximum susceptibility was observed at 60 minutes of incubation, while extended acid treatment up to 72 h resulted in the highest release of dextrose at 45 minutes of incubation.

Enzymatic hydrolysis is a technique that is often used to characterise the structural properties of starch samples[211] as the enzyme action on starch granules is well regulated with the gross and fine structural make-up[111]. As the starch granules undergo changes, any significant change will result in the over all pattern of enzyme activity. As there was not much difference observed in

Fig.17. α-Amylase digestibility of acid-hydrolysed *D. alata* starch samples

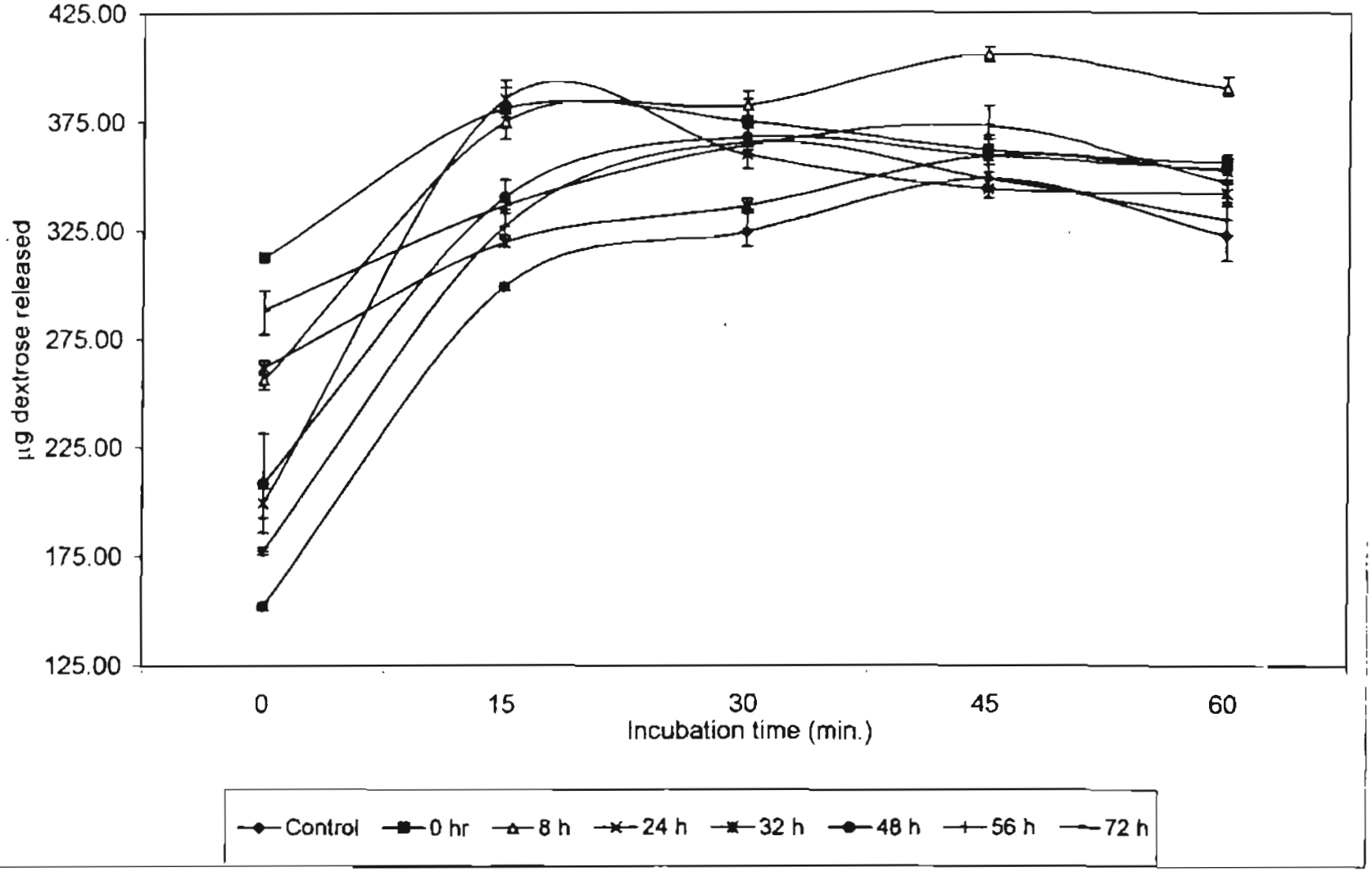


Fig.18. α-Amylase digestibility pattern of acid-hydrolysed *D. rotundata* starch sample

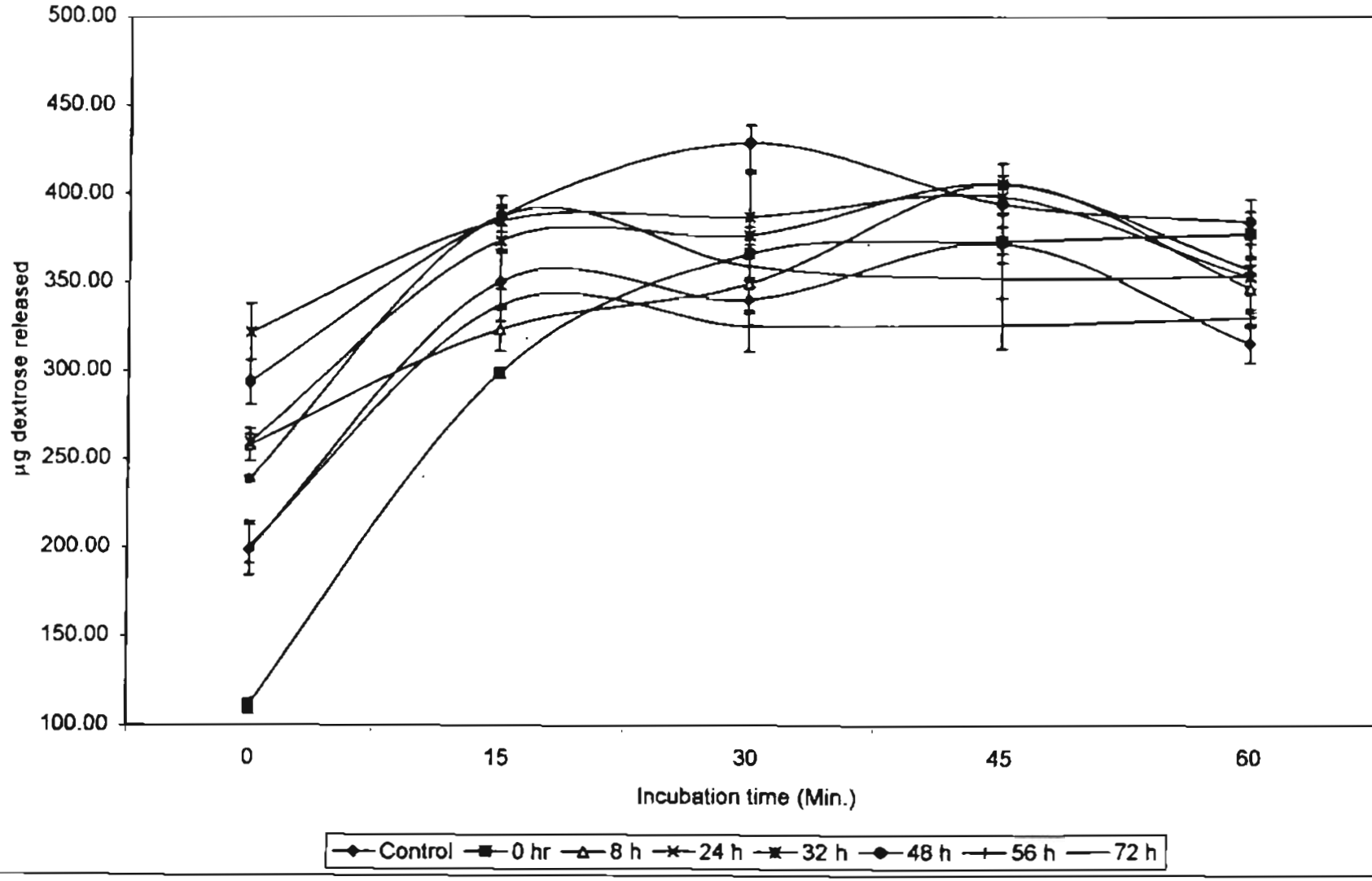
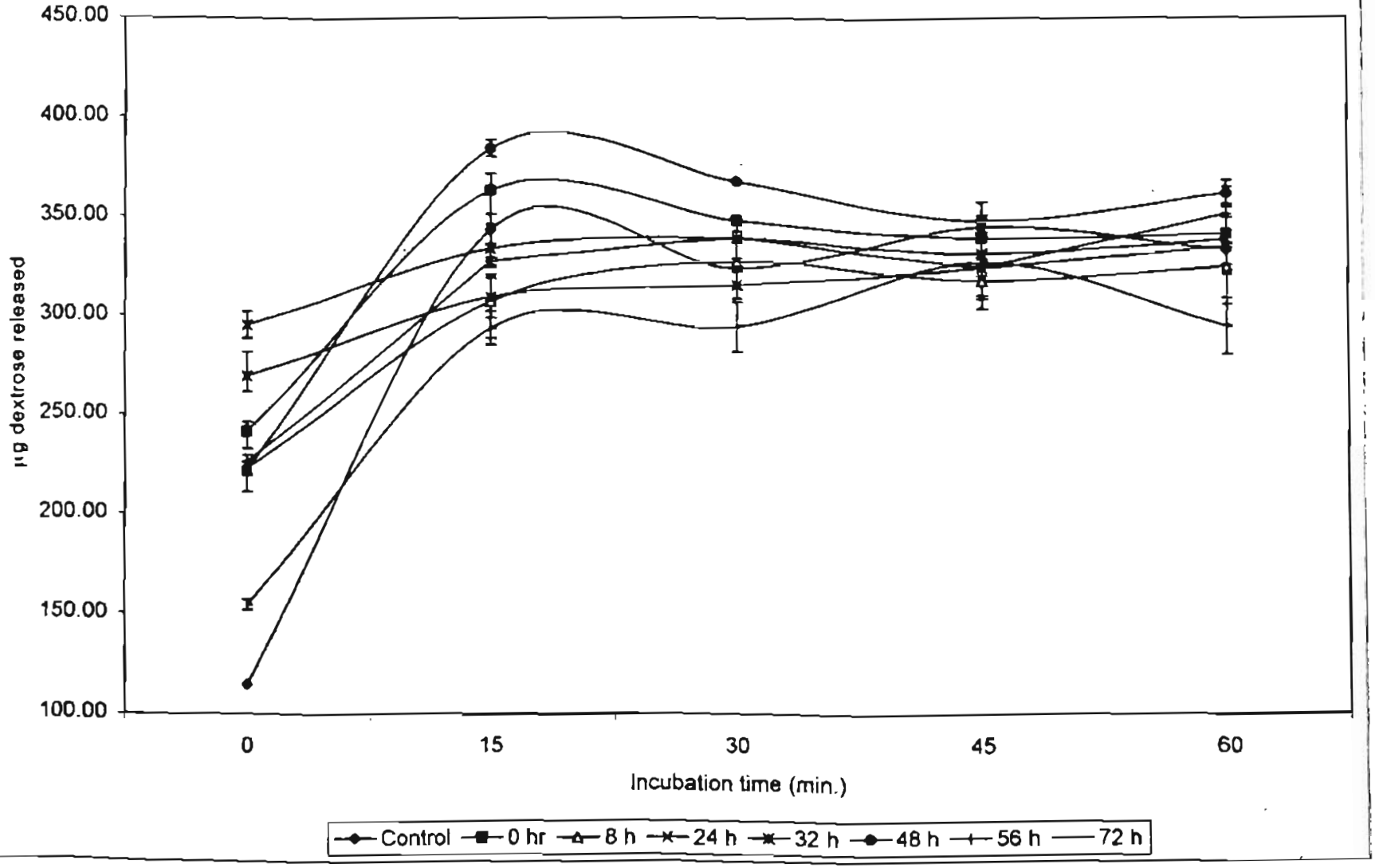


Fig.19. α-Amylase digestibility pattern of acid-hydrolysed *D esculenta* starch samples



the overall pattern in amylase susceptibility of acid treated and native starches it should be generally presumed that treatment with acid under the conditions specified does not considerably alter or affect the sites of enzyme action. However, it is to be mentioned that in the case of *D. rotundata* and to some extent also in the case of *D. alata*, the acid treatment shortened the time required for the granules to attain optimum susceptibility. A reverse trend was observed for *D. esculenta* starch. The difference in the behaviour of *D. esculenta* starch towards enzyme action may be presumed to the smaller granule size compared to *D. alata* and *D. rotundata* (Table 3).

SUMMARY

The present study on the physico-chemical and functional properties of *Dioscorea* starches was carried out to develop an efficient method for the extraction of starch from *Dioscorea* tubers which involves the pre-treatment of tubers with different chemicals. The starches thus extracted were analysed for their physico-chemical and functional characteristics and the results were compared with their corresponding control starch sample. The methodology adopted for conducting the studies has relied upon instruments such as UV-Visible spectro photometer, pH meter, Brabender visco-amylo graph, Ostwald's viscometer, Bohlin rheometer, Philip's X-ray Diffractometer and Differential Scanning Calorimeter. The chemical analyses were conducted using standard methods after appropriate modifications wherever necessary.

The main observations are summarised below:

1. Among the chemicals used for pre-treatment, NH_4OH was generally found to be beneficial for the extraction of starch from all the three *Dioscorea* tubers in terms of both starch yield and purity. However, in the case of *D.alata* tubers, pre-treatment with 1 and 2% SHMP and 3-5% KMS and for *D.esculenta* tubers, pre-treatment with 1-5% solutions of SHMP, NaCl and 0.025-0.125% solutions of GMS were also advantageous with respect to starch yield and purity.

2. Among the untreated *Dioscorea* starches, *D.esculenta* showed lowest intrinsic viscosity. Pre-treatment of the samples with NH_4OH and KMS showed a tendency to lower the intrinsic viscosity. But pre-treatment with NaCl and SHMP resulted in a marginal increase.

3. Among the control samples *Dioscorea* starches, *D.esculenta* showed highest swelling volume. Pre-treatment of *Dioscorea* tubers with both KMS and GMS suppressed the swelling volume while in the case of *D.alata*, treatment with the former showed a tendency to increase the swelling volume. The control sample of *D.alata* displayed highest solubility while *D.esculenta* starch was found to be the least soluble. Among the chemicals used for pre-treatment, GMS reduced the solubility of starch samples to a noticeable level.

4. A comparison of true, apparent and water soluble amylose contents of control samples from Dioscorea starches, in general, indicated that 93-99% of the total amylose in each of the starch samples is accounted by apparent amylose and 42-47% of the latter by water soluble fraction. In general, chemical pre-treatment particularly of *D. alata* species, lowered the total amylose blue value. Pre-treatment with GMS lowered the water soluble amylose content of all the Dioscorea starches.

5. Both DSC and Visco-amylograph data suggested that although chemical pre-treatment of fresh tubers does not influence the gelatinization and pasting temperature, there was a noticeable impact on the peak viscosity as well as hot gel stability. Of all the three Dioscorea starches, *D. esculenta* sample showed highest hot paste stability indicated by a breakdown ratio of 1.0 for control as well as experimental samples. DSC studies revealed that retrogradation of experimental starch samples resulted in lowering the onset of melting and retrogradation enthalpy.

6. An increase in the rate of moisture uptake by the starch samples, independent of their source and type was observed as the relative humidity of the environment increased. As a result of chemical

pre-treatment with SHMP and GMS, *Dioscorea* starches showed higher moisture uptake while KMS had a negative effect.

7. Among the chemically pre-treated samples, only those prepared from GMS pre-treated *Dioscorea* tubers showed a decline in the enzyme susceptibility. No major change in the crystal pattern could be inferred from the Diffraction pattern.

8. An increase in the elasticity of the system was noticed in GMS pre-treated samples and this effect was more pronounced in the case of *D.rotundata*. A decrease in elastic character of system was noticed for NH_4OH treated samples of *Dioscorea* tubers.

9. Acid-treatment of *Dioscorea* starches at ambient conditions increased the cold water soluble sugar and there was a progressive fall in the intrinsic viscosity, amylose blue value and swelling volume. Acid-treatment was found to delay the onset of gelatinization of *D.alata* and *D.rotundata* starch samples while *D.esculenta* starch samples displayed earlier gelatinization. Increase in the duration of acid treatment delayed the completion of gelatinization process for *D. esculenta* and to a less extent, for *D. alata* samples also.

10. More intense X-ray diffraction peaks of acid hydrolysed Dioscorea starches suggested an increase in the percentage of crystallinity attained as a result of acid-treatment.

11. Acid-treatment favoured the enzyme susceptibility of *D. rotundata* and *D. alata* starches, as reflected from the shorter time required to attain the optimum release of dextrose.

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