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Characteristics of acetylated and enzyme-modified potato and sweet potato flours

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Abstract

The properties of modified potato and sweet potato flours have been determined by incorporating acetyl groups (acetylation) and by treating with glucoamylase (enzymatic modification). Fractionation studies on Sepharose CL-2B showed that the content of high molecular weight fraction decreased, with a proportionate increase in the lower molecular weight carbohydrate fraction, whereas FT-IR indicated changes in crystallinity of the modified starches. The data showed that the degradation of starch is dependent on the type of modification which, in turn, determined its crystallinity and digestibility. The swelling power and solubility patterns of modified flours indicated a greater degree of associative forces in the starch granules. Scanning electron microscopy revealed indentation on acetylated starch granules, and the granules appeared as bunches/clusters, whereas surface erosion was observed in the enzyme-treated samples. The presence of substituent groups in acetylated flour influenced digestibility inversely, whereas improved digestibility was observed in enzyme-modified samples.

Keywords: Potato; Sweet potato; Starch; Acetylation; Enzyme-modification; Gel permeation chromatography; Infra red spectroscopy; Scanning electron microscopy

1. Introduction

Potato ranks fourth after wheat, maize and rice in global production. In developing countries, such as India, with an average annual production of 25 million tonnes of potato (FAO, 2005), the cold storage facilities available in the country are insufficient to accommodate the surplus produce during a glut season, whereby the value addition to potato becomes inevitable (Ezekiel, Verma, Sukumaran, & Shekhawat, 1999). Sweet potato (*Ipomoea batatas* L) is being used in starch noodles, bakery foods, snack foods, confectionery products and for alcohol production by the brewing industries (Chen, Schols, & Voragen, 2003; Montreka & Adelia, 2003). In spite of the fact that sweet potato is cheaper than other crops, this abundant resource is still poorly utilized. Sweet potato roots can be processed into products with a longer shelf life and improved characteristics. Flour is one such product, which is stable, versatile and can be used round the year. It can be used as a thickener in soup, gravy, fabricated snacks and bakery products (Kulkarni, Govindan, & Kulkarni, 1996; Marwaha & Sandhu, 1997). A soup mix using physically modified potato flour as starch base (Rekha, Chauhan, Ramesh Yadav, Guha, & Ramteke, 2005) and puffed cubes containing no oil/fat, by subjecting potato/sweet potato to high temperatures has been developed, which can be used as a ready-to-eat snack (Chauhan et al., 2004).

Potato/sweet potato flours are prepared by drum-drying or hot air-drying techniques (Willard & Hix, 1987; Woolfe, 1992). During the drying process, starch undergoes changes in its structural features, thereby influencing its functional properties, such as pasting viscosities and solubility (Ramesh Yadav, Guha, Tharanathan, & Ramteke, 2006a,

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2006b). Thus, the functional properties of such flours are particularly dependent on the method of preparation. Though different workers have reported the possible use of potato flours for product development, the properties of the flour desired for the preparation of various products, such as noodles, pudding, gravy, sauces, gruel and bakery products, vary (Sajilata & Singhal, 2005). Several basic properties of flours of concern to food processors are improved by starch modification; among these are improvement in heat, shear and acid stability.

With a view to providing simple methods of choice to processors, acetylation and enzyme-modification of potato/sweet potato flours were attempted. Acetylated starches with low degree of substitution (DS) have been widely used in food industries for many years because of their unique characteristics, such as low gelatinization temperature, high swelling and solubility, and good cooking and storage stability (Liu & Corke, 1999; Wang & Wang, 2001). Acetylated potato starch could significantly improve the quality of white salted noodle by replacing commonly used wheat flour. The DS for a starch derivative is defined as the number of hydroxyl groups substituted per D-glucopyranosyl unit of the starch polymer. Since each D-glucose unit possesses three reactive hydroxyl groups, the maximum possible DS value is three. Therefore, the reaction to prepare acetylated starches of any desirable DS value can be controlled with high accuracy by adjusting the molar ratio of the reagent and catalyst in the reaction mixture.

In the *in vitro* alpha amylolysis of different starch granules, the enzyme attack is rather restricted and is usually from outside inwards (Bhat, Paramahans, & Tharanathan, 1983). The smaller starch granules (<5 μm) are rather more susceptible to amylolysis than are the large granules (MacGregor & Matsuo, 1982). The enzymatically modified flour is a useful adjunct in the manufacture of low energy, dietetic foods. Absorption of oil, emulsifying capacity and the solubility of wheat flour are considerably altered by enzyme-modification (Taufel, Ruttloff, & Kodet, 1992). Such modified flours are useful in the manufacture of low fat and low sugar wafers and other bakery products. However, the available information on functional properties of potato/sweet potato flours essentially refers to their starch instead of flour, though the functional properties of flour are influenced, not only by the starch, but also by other flour components (protein, fat), which restrict access of water into the starch granules. Hence, the present investigation highlights the changes in properties of modified potato and sweet potato flours, as a result of acetylation and enzyme-modification.

2. Materials and methods

2.1. Material

Potato (*Solanum tuberosum* L var. Kufrijyothi) and sweet potato (*Ipomoea batatas* L) were procured locally, cleaned under running tap water, air-dried and stored at 12 °C until analyzed.

2.2. Preparation of native flour

Potatoes/sweet potatoes were peeled manually with a hand peeler, cut into 10 mm thick cubes with a dicing machine RG-400, AB Hallde Maskiner, Sweden and dried at 40 ± 2 °C, ground in a hammer mill (APEX, Apex Construction Ltd., England) and sieved through a 189 μm sieve. The powder was packed in polyethylene bags and stored at 12 °C prior to further use.

2.3. Acetylated flour

The flour sample (2 g), mixed with solid $NaHCO_3$ (1 g), was wetted with distilled water (1 ml), followed by addition of acetic anhydride (glass-distilled, 4 ml). The mixture was allowed to react for 2 h at 40 °C (Tharanathan, Susheelamma, & Ramesh, 2004), and later was washed thoroughly with aqueous alcohol (80%) and dried at 40 °C overnight. The degree of substitution (DS) was determined by the ratio of absorbance A1733 cm^{-1}/A3450 cm^{-1} (Kittur, 2000). The absorbance was determined from the IR spectra, recorded in KBr discs using a FT-IR spectrometer (Model SPECTRUM-2000, Perkin–Elmer, USA) under dry air at room temperature. Approximately 4 mg of dried flour sample were blended with 100 mg of potassium bromide (IR grade) and about 40 mg of the mixture were then used to prepare a pellet. The DS was determined by considering the OH band at 3450 cm^{-1} as a reference. The DS was defined as the ratio of absorbance: (A1733 cm^{-1}/A3450 cm^{-1}). IR spectra of native and enzyme-modified samples were also recorded using the FT-IR spectrometer.

2.4. Enzyme-modified flour

To the flour sample (1 g) in acetate buffer (0.05 M, pH 4.6, 6 ml) were added 10 mg glucoamylase (from *Rhizopus* mold, 21,100 units g^{-1} solid, Sigma, USA). The reaction mixture was incubated at 60 °C for 90 min for potato and 120 min for sweet potato flour. It was centrifuged and the sediment was washed with alcohol repeatedly and dried. Reducing sugars in enzyme-treated samples were estimated by the dinitrosalicylic acid (DNS) method (Miller, 1959). To the sample or standard (1.0 ml, 100–1000 μg glucose), 1 ml of DNS reagent (1 g of dinitrosalicylic acid and 30 g of sodium potassium tartarate in 0.4 N NaOH) were added and the mixture heated for 15 min in a boiling water bath. After cooling, 3.0 ml of double-distilled water were added and the absorbance was measured at 530 nm. The calibration curve was constructed using glucose as standard.

2.5. In vitro digestibility

Potato or sweet potato flour (50 mg), suspended in sodium acetate buffer (pH 4.6, 0.05 M, 4 ml) was

gelatinized by keeping in a boiling water bath for 15 min, cooling to 60 °C and incubating with glucoamylase (50 units) for 30 min. The enzyme was inactivated by heating the digest in a boiling water bath for 10 min. The mixture was centrifuged (5000 rpm for 15 min) and the residue was washed with water. The supernatant, with all washings, was made up to 15 ml and analyzed for released glucose (Dahlqvist, 1964).

2.6. Gel permeation chromatography (GPC)

Sepharose CL-2B was packed into a glass column (1.7×92 cm) and equilibrated with the running eluent (water) overnight. The sample (10 mg) of native and treated flours, dispersed in aqueous DMSO (85%, 1 ml), was applied over the column bed and eluted, by the descending method, with water containing 0.02% sodium azide, at a constant flow rate (18 ml h^{-1}). Fractions (3.0 ml) were collected and an aliquot (0.5 ml) of the fraction was analyzed for total sugars (OD at 480 nm) (Rao & Pattabhiraman, 1989).

2.7. Molecular weight determination

The approximate molecular weight (MW) was determined from a calibration curve prepared for standard dextrans (T-10, T-20, T-70, T-500 and T-2000) of known MW on a Sepharose CL-2B column (Brown & Volence, 1989). The void volume (Vo) was determined by using a predialyzed blue dextran solution (5 mg/0.5 ml water). The MW values were computed from the standard plot of log MW vs. Ve/Vo, where Ve was the elution volume of the respective starch fraction.

2.8. Swelling power and solubility

Swelling power and solubility patterns were determined as described by the method of Unnikrishnan and Bhattacharya (1981).

2.9. Scanning electron microscopy

Samples were mounted on metallic stubs, gold coated (~100 Å) with a sputter coater (Polaron Sputter Coat System, Model 5001, England) and viewed under SEM 435 VP (Leo 40 Electron Microscopy Ltd., Cambridge, UK).

3. Results and discussion

3.1. Fractionation studies

The flour samples, solubilized in aqueous DMSO, were subjected to GPC on a Sepharose CL-2B column to check for their homogeneity. GPC on Sepharose CL-2B fractionates molecules in a way opposed to that by hydrodynamic volume. Amylose, amylopectin as well as the intermediate fraction have wide variations in their elution volumes (Ve), and accordingly they show different MW values. The latter is deducible from a pre-calibrated GPC column. The GPC of native flour comprised two major peaks, the first peak (Fr-I) corresponding to amylopectin with a high molecular weight ($>20 \times 10^6$ Da), appearing in the void volume. The second peak (Fr-II), generally considered as amylose (MW 2.4×10^5 Da), entered the gel and was eluted over a wide range of MWs. The degraded products in the modified flour samples entered the gel and were eluted together in Fr-II, thereby increasing the proportion of carbohydrates in this fraction (MW $\sim 4.0 \times 10^5$ Da). The recovery of carbohydrates in the fractions ranged between 82% and 96.6%, with a mean of 89.3%. Further, among the flour samples, the degradation of Fr-I and the shift of carbohydrate content in Fr-II were greater in enzyme-modified samples. The formation of linear oligosaccharides in modified samples may be the reason for the reduction in intensity of the first peak and increase of subsequent peaks (Fig. 1).

The GPC profiles of native and modified sweet potato flours were comparable with those of potato flour. The major peak of native sample was obtained in the void volume and its second fraction (MW 6.5×10^5 Da) was eluted later (Fig. 2). The degradation of starch in modified samples led to the formation of a low molecular weight first fraction with proportionate increase in the subsequent fractions. The degraded products of acetylated (MW: Fr-II 18.3×10^5 Da and Fr-III 3.1×10^5 Da) and enzyme-modified samples (Fr-II 9.2×10^5 Da) entered the gel and were eluted as Fractions II and III.

3.2. Infrared studies

Interpretation of the IR absorption bands was achieved in the light of earlier investigations (Cael, Koeing, & Blackwell, 1975; Santha, Sudha, Vijayakumari, Nayar, & Moorthy, 1990). The IR spectra of potato/sweet potato flour samples (Figs. 3 and 4), exhibited bands that originate mainly from the vibrational modes of amylose and amylopectin which have been shown to be sensitive to changes in molecular structure, such as starch chain conformation,

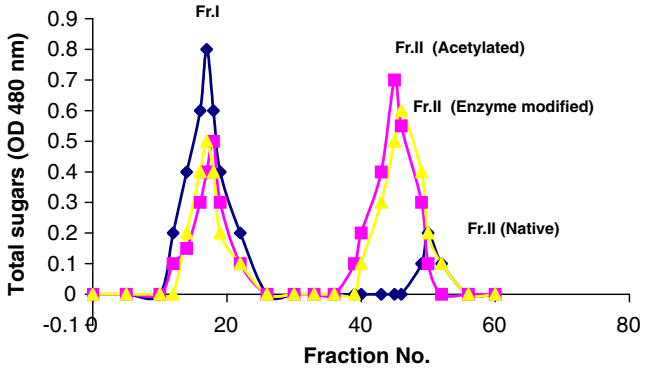

Fig. 1. GPC profile of modified potato flours.

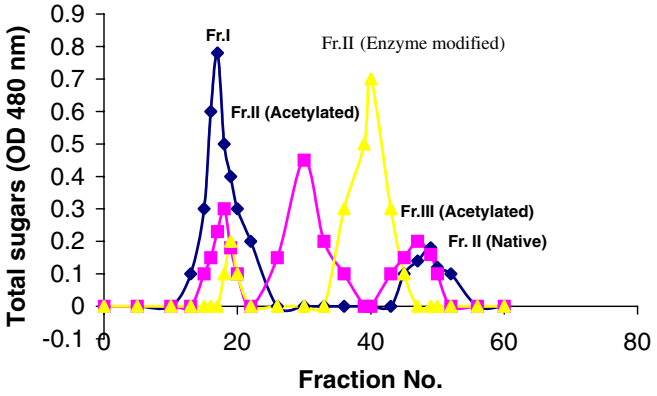

Fig. 2. GPC profile of modified sweet potato flours.

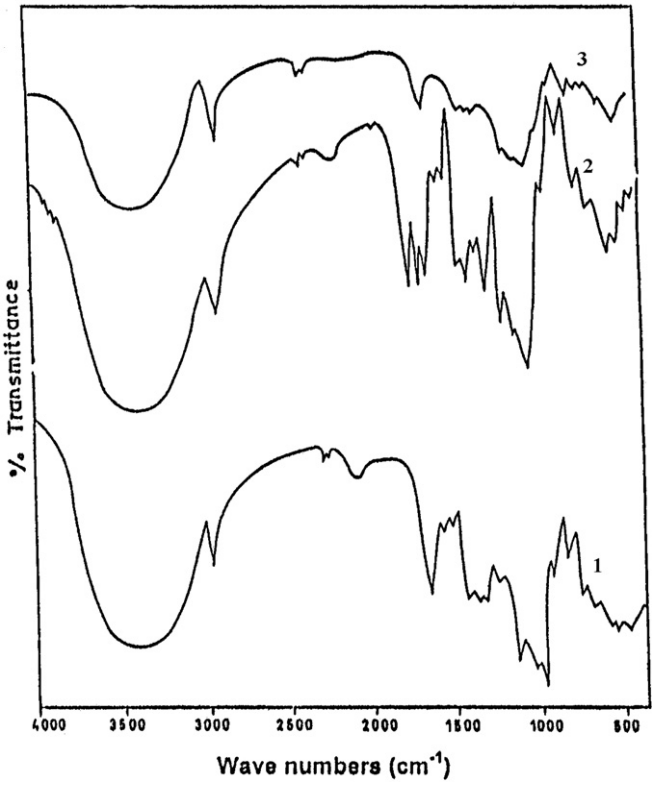

Fig. 3. FT-IR spectra of modified potato flours (1, native; 2, acetylated; and 3, enzyme-modified).

helicity, crystallinity and the retrogradation processes (Goodfellow & Wilson, 1990; Van Soest, Tournois, De Wit, & Vliegenthart, 1995). The O–H stretch (3000–3600 cm^{-1}), C–H stretch (2800–3000 cm^{-1}), and the skeletal mode vibration of the glycosidic linkage (900–950 cm^{-1}) were clearly seen for all the modified samples. The changes in intensity of some FT-IR bands and band ratios, as a function of modification, can be seen in Figs. 3 and 4.

Native potato sample showed a band at 1648 cm^{-1} whose intensity decreased in modified samples, indicating changes in crystallinity. Such variations could also be

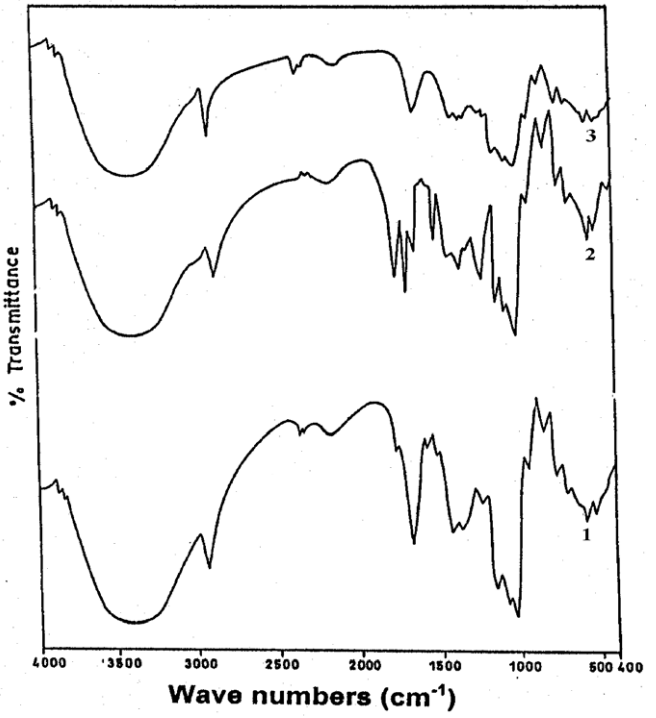

Fig. 4. FT-IR spectra of modified sweet potato flours (1, native; 2, acetylated; and 3, enzyme-modified).

attributed to the water absorbed in the amorphous region of starch granules. The change in absorbance at 1020 cm^{-1} (C–O–H bending and deformation) and 1640 cm^{-1} (O–H related vibration) indicates the rupture of the inter- and intra-molecular hydrogen bonds, and formation of intermolecular hydrogen bonds between starch and water accompanying the phase transition in modified samples. Absorbances at 1150, 1080, and 1020 cm^{-1} are more sensitive to the conformational changes during modification processes, indicating the short range order and helicity change when crystallinity and molecular orientation are lost. An extremely broad band due to hydrogen-bonded hydroxyl groups (O–H) appears at 3403 cm^{-1}, which is attributed to the complex vibrational stretches associated with free, inter- and intra-molecular bound hydroxyl groups that make up the gross structure of starch. The sharp band at 2926 cm^{-1} is characteristic of C–H stretches associated with the ring hydrogen atoms. Intensity changes in the C–H stretch range could be attributed to variations in the amount of amylose and amylopectin present in starches (Young, 1984).

The IR spectral data provided evidence for acetylation by the presence of an ester carbonyl group stretch (C=O) at 1731 cm^{-1} (Figs. 3 and 4). The DS values of potato and sweet potato flours were found to be 0.29 and 0.35, respectively. The strong band at 3403 cm^{-1} (hydroxyl groups) of native potato sample (Fig. 3) decreases in intensity after the acetylation reaction as the number of hydroxyl groups diminishes. Detection of these structural

changes, in starch subjected to chemical modifications, is an important industrial necessity in order to determine the quality of modified starches (French, 1984). Weakest hydroxyl stretching band at 3345 cm^{-1} was observed in enzyme-modification. The bands in the finger print region associated with C–O stretching (1083, 1030 cm^{-1}) decrease in intensity relative to those associated with the C–H stretch.

Sweet potato samples also showed similar types of spectra (Fig. 4). The pattern of changes resembled those of potato samples, except for a few variations, e.g., decreased intensities of 2800–3000 cm^{-1} (C–H stretch) and 1600 cm^{-1} (COO group) bands.

3.3. In vitro digestibility studies

The gelatinized flour samples incubated with glucoamylase were analyzed for released glucose. Both under *in vitro* and *in vivo* systems, raw starches are usually slowly digested by enzymes, but cooking increases enzyme susceptibility considerably because of the rupture and disintegration of the compact crystalline starch granule structure. The *in vitro* digestibility of acetylated flours was considerably reduced in comparison with native and enzyme-modified flours (Fig. 5). The contents of reducing sugars in enzyme-treated potato and sweet potato flour samples were found to be 7.0% and 15.0%, respectively. The low digestibility of the former may be useful in functional food formulations (Ramesh Yadav, 2005). The effect of DS on digestibility was found to be inverse. The nature of substituent groups was shown to influence the digestibility of modified wheat starches. Hydroxypropyl and acetylated wheat starches showed decreased digestibility by pancreatic amylase (Wootton & Chaudry, 1979).

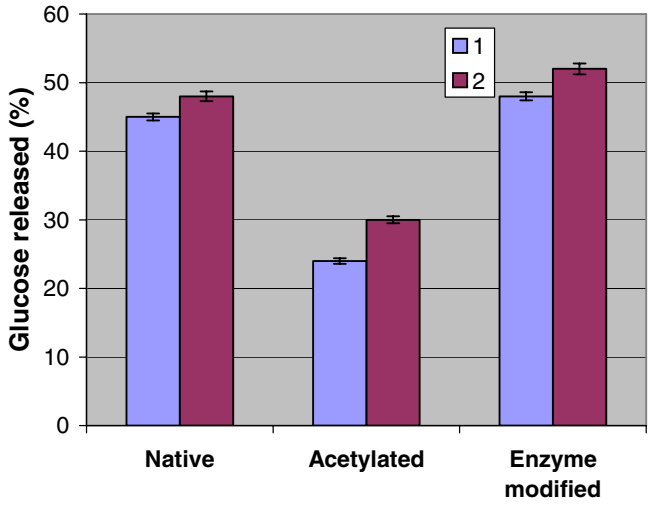

Fig. 5. Glucose (%) released from enzyme digested flours (1, potato and 2, sweet potato).

3.4. Scanning electron microscopy

Native potato starch (Fig. 6a) had oval, spherical and irregularly shaped granules of 6–60 μm size, while the acetylated samples showed indentation as a result of modification, and also the granules appeared as clusters (Fig. 6b). The fusion of starch granules in acetylated samples could be attributed to the introduction of hydrophilic groups to the starch molecules, which resulted in increased hydrogen bonding (Singh, Chawla, & Singh, 2004). Some fragmentation of the granules would have taken place during modification. The enzyme-modified potato flour showed slight surface erosion in some granules (Fig. 6c). However, a large proportion of starch granules of potato attacked by the enzyme did not show the outer striated or the inner shell structure on the same granule. The enzyme attack manifested itself in only superficial surface erosion of the granules. This might be the result of the overall composition *per se* of potato starch or due to the poor adsorption of glucoamylase onto the granule surface.

Raw sweet potato starch granules (Fig. 6d) are round, hexagonal, and spherical, of 4–26 μm size while, in modified samples, the granular characteristics partially disappeared. Indentations of acetylated flour sample (Fig. 6e) and exo-corrosion of enzyme-modified samples (Fig. 6f) were noticed. The penetration of glucoamylase was found to be greater as the granules showed serrated surfaces and breakage of outer layers in some granules. This was also evident by the release of more reducing sugars as a result of amylolysis of sweet potato samples.

3.5. Swelling and solubility

The swelling power of modified flour samples increased with temperature. The swelling power of acetylated samples was raised from 60 °C onwards (Fig. 7). Acetylated samples showed less swelling power than did other treated samples. Though the viscosity values of acetylated samples were reduced, swelling was inhibited due to esterification. High amylose content and the presence of stronger or a higher number of intermolecular bonds can reduce swelling. Formation of a lipid-starch complex can also offer low swelling volume (Swinkles, 1985) as also does the presence of non-starch carbohydrates (and other constituents) in the starch (Eliasson & Gudmundsson, 1996; Leach, McCowen, & Schoch, 1959). The low swelling power is also due to the presence of a large number of crystallites, which will increase granular stability, thereby reducing the extent of granular swelling (Hoover & Ratnayake, 2002).

Enzyme-modified samples also showed reduced swelling values compared to native sample at all the temperatures studied, though the values increased with temperature. The bonding forces within the granules of a starch affect its swelling power. Thus, highly associated starch granules with extensive and strongly bonded micellar structures display relatively great resistance towards swelling (Mariam, Abeba, & Schmidt, 1996). The presence of protein

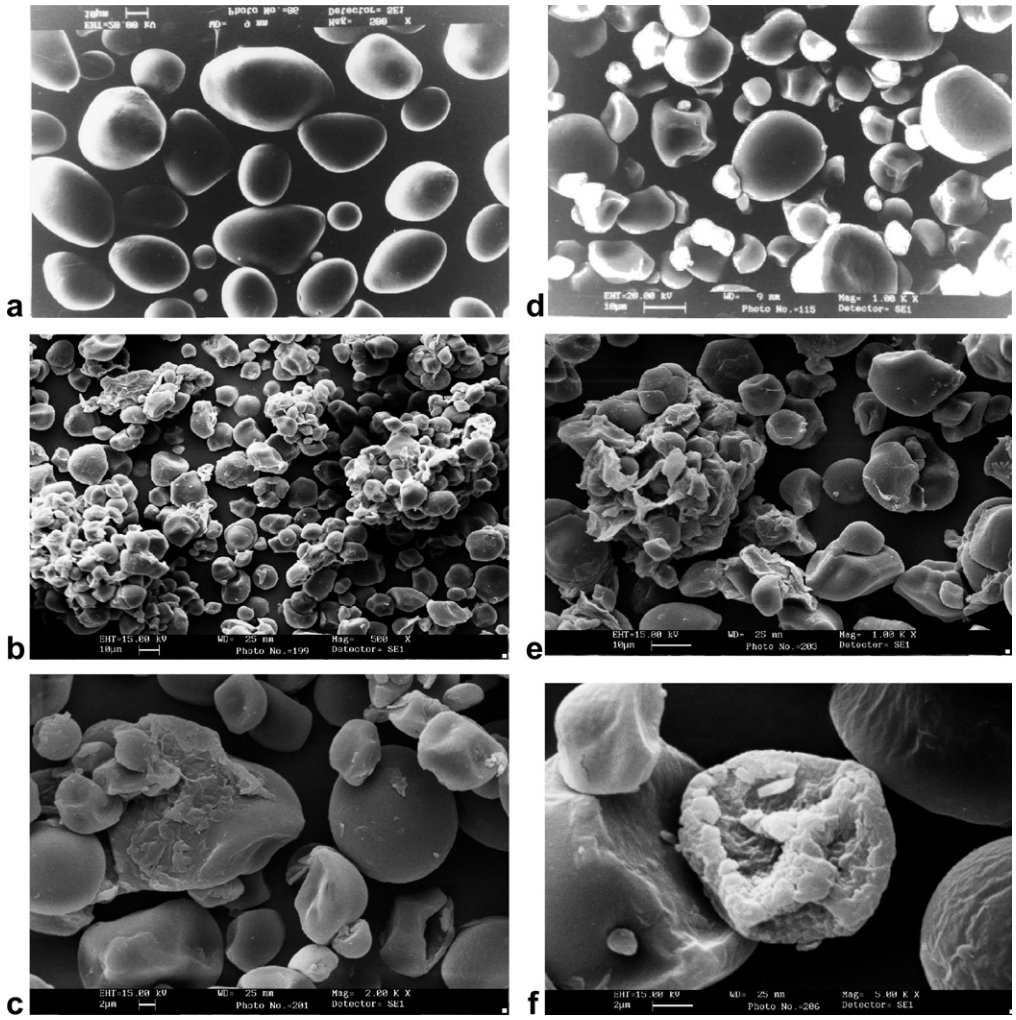

Fig. 6. Scanning electron micrographs of modified potato (a–c) and sweet potato (d–f) flours (a, native ×500; b, acetylated ×500; c, enzyme-modified ×2000; d, native ×1000; e, acetylated ×1000; and f, enzyme-modified ×5000).

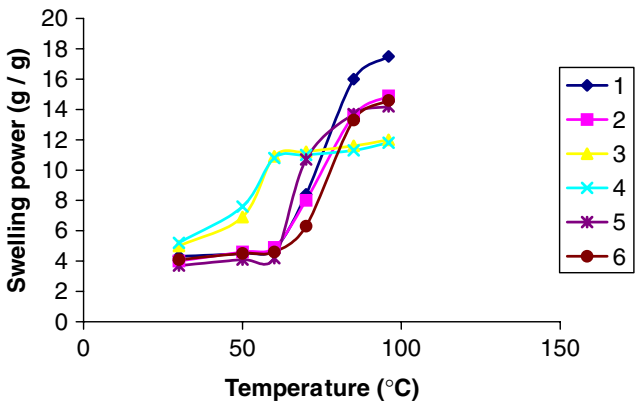

Fig. 7. Swelling power of modified potato and sweet potato flours (1and 2, native potato and sweet potato; 3 and 4, acetylated potato and sweet potato; 5 and 6, enzyme-modified potato and sweet potato).

(enzyme-starch complex) imparts rigidity, besides contributing to limiting the leaching of starch in the sample mixture (Colonna, Tayeb, & Mercier, 1976). Swelling is essentially a property of the whole amylopectin molecule, rather than parts of it, and amylose alone appears to be a diluent, while lipids (as complexes with amylose) strongly inhibit swelling (Cheng, Tsai, & Tseng, 1996). The potato flours with higher water absorption indices and lower amylose contents resulted in products with higher extensibility and lower energy to rupture.

The solubility values of acetylated potato or sweet potato flour, though slightly increased with temperature, were found to be lower than that of native samples at all temperatures (Fig. 8). The substituent groups made the associative bonds stronger. The solubility characteristics of acetylated starches depended upon degree of substitution and polymerization. The enormous differences among the modified flour samples in their swelling and solubility patterns can thus form the basis of the functional properties that determine their suitability in product development. The interference of protein and other constituents of flour with starch will also influence the flour properties (Van Hal, 2000). Peak viscosity (PV) of drum dried (physically modified) potato flour was only 363 cP, and less than that

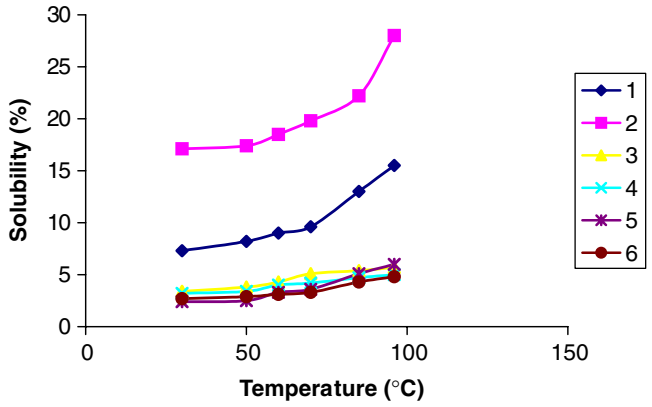

Fig. 8. Solubility of modified potato and sweet potato flours (1and 2, native potato and sweet potato; 3 and 4, acetylated potato and sweet potato; 5 and 6, enzyme-modified potato and sweet potato).

of starch (3439 cP) isolated from it. Similar findings were also obtained for sweet potato (Ramesh Yadav, 2005).

4. Conclusions

The study has provided some insights for applicable modes of processing of potato/sweet potato flours. Starch in modified potato flours was degraded to lower molecular weight components, as evidenced by GPC studies. Upon fractionation, the content of carbohydrate in the high molecular weight fraction decreased, with a proportionate increase in the lower molecular weight fraction. The data showed that the degradation of starch is dependent on the type of modification, which in turn determined its crystallinity and digestibility. This information is useful in designing food processing protocols that target consumer needs and requirements, such as for diabetics and obese people who will potentially benefit from lower levels of starch digestibility. Poorly digested starches may also function like dietary fibre and have therapeutic benefits, such as blood glucose control in diabetes, or to aid in weight control (Niba, 2003; Skrabanja, Liljeberg, Hedley, Kreft, & Bjork, 1999).

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