ENHANCING SWEET POTATO (*Ipomoea batatas* Lam.) STARCH EXTRACTABILITY USING ENZYMES

By

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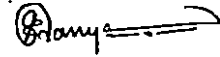
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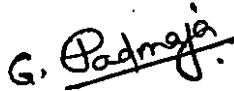Sreekariyam,

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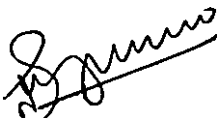
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LIST OF ABBREVIATIONS

μl	microlitre
cP	centipoise
DPPH	2,2-diphenyl-1-picrylhydrazyl
DSC	Differential Scanning Calorimetry
FAO	Food and Agriculture Organisation
FAOSTAT	Food and Agriculture Organisation Statistical Databases
g	gram
HCl	Hydrogen Chloride
KCl	Pottasium Chloride
kV	kilovolts
M	Molar
mA	milliamperes
mg	milligram
ml	millilitre
Mt	Milliontonnes
N	Normal
NaOH	Sodium Hydroxide
nm	nanometre
Pa	Pascal
RDS	Rapidly Digestible starch
RS	Resistant starch
RVA	Rapid Visco Analyser

SDS	Slowly digestible starch
SEM	Scanning Electron Microscopy
SPF	Sweet potato flour
SPS	Sweet potato starch
SR	Starch recovery
SY	Starch yield
TCD	Total Colour Difference

Introduction

1. INTRODUCTION

The sweet potato, *Ipomoea batatas,* belongs to the family *Convolvulaceae.* It is a tuberous rooted perennial, usually grown as an annual, which is now grown extensively in a wide range of environments. Sweet potato was brought to Europe by Columbus and subsequently introduced to Africa and Asia by the Portuguese and Spanish traders (Salawu and Mukhtar, 2008). It is adaptable and can grow under many different ecological conditions. It has a shorter growth period than most other tuber crops (three to five months) and under suitable climatic conditions it shows no marked seasonality. Sweet potato is cultivated throughout the tropics and warm temperate regions of the world for its starchy roots which can provide nutrition, besides energy. The edible tuberous root is either long and tapered, ovoid or round with a skin colour ranging from white, brown or purple and the flesh colour ranging from white, pale cream, purple or orange. Besides, the plant is also much valued for its green tops, which are a concentrated source of many essential vitamins and minerals. Worldwide, sweet potato is the sixth most important food crop after rice, wheat, potatoes, maize, and cassava. China is the largest producer of sweet potatoes, accounting for more than 80 per cent of the world supply, of which only 40 per cent of the production is used for human consumption and industrial uses, while rest goes as animal feed. Sweet potato roots with 18-30 per cent (on fresh weight basis) starch are one of the major sources for the commercial extraction of starch. The roots are extensively used for starch extraction in China, Japan, Korea and Taiwan. Most studied nutraceuticals in sweet potato are carotenoids and anthocyanins. Suda *et al.* (2003) reported that the sweet potato anthocyanins have multiple physiological functions such as radical scavenging, anti-mutagenic, hepato protective, anti hypertension and hypoglycaemic activities. The basic process for manufacture of starch is similar to that for

other sources. Sweet potato starch finds commercial application in the production of noodles, sugar syrups, thickener etc.

Sweet potato is a natural health food because of its high energy, dietary fibre, vitamins and mineral content (Padmaja, 2009). Experts feel that further research is needed to standardize technologies to make sweet potato a commercial crop in India and to utilize it for feed and for production of starch as is done in China and Japan.

Starch is the main component of a food diet due to its functional properties. Both in the raw and modified form, it is widely used in food production on account of its low cost. It is a renewable, degradable and non-toxic raw material, made essentially of α-D glucose polymers (Fenema, 1996).

However, poor extractability of starch as well as the discolouration limits the use of sweet potato starch. The starch granules present in sweet potato are embedded in cellulosic fibers and held together by pectic substances (Rahman and Rakshit, 2004). As a result, although sweet potato tubers contain 18-30 per cent starch, the yield is low ranging from 15-20 per cent. The conventional starch extraction from tuber crops involves mechanical rupture of cell walls using rasping method and washing out the starch with adequate quantity of water (Joshi and Kulkarni, 1993). However, the process is often energy intensive and requires large quantities of water (1.0 tonne of tubers requires 4000 litres of water). Due to the trapping of starch in cellulose-hemicellulose-pectin matrix, these methods often result in low starch recovery from sweet potatoes. Besides, rasping or crushing in blenders could alter the integrity of starch granules and change its properties. High levels of phenols and presence of polyphenol oxidase and peroxidase are major issues leading to discolouration of starch in sweet potato. In order to tackle these issues, usually chemicals such as sodium metabisulphite or organic acids are used (Jyothi Krishnan, 2012). Enzyme mediated extraction of starch has been attempted from tubers such as potato, sweet potato and cassava by various workers (Kallabinski and Balagopalan, 1994; Rahman

and Rakshit, 2004; Espino *et al.*, 2005; Nandan *et al.*, 2011). The distinctive feature of enzymes is that they can operate at mild conditions, at atmospheric pressure and at reasonably low or ambient temperatures. In view of the availability of improved cellulolytic enzymes with higher activity, it was thought worthwhile to study the possibility of using enzymes such as cellulases, pectinases, hemicellulases either alone or in combination, for improving the extractability of starch from sweet potato. Further, the effect of enzyme mediated extraction in improving the colour of starch has not been studied in detail. The present study is expected to lead to the perfection of a technology to maximize the extraction of starch from sweet potatoes and also to enhance the utilization prospects of sweet potato starch for food product development.

Review of Literature

2. REVIEW OF LITERATURE

2.1 Sweet potato

Sweet potato is cultivated as a perennial in tropical and subtropical lowland agro- ecologies, although it is well adapted to other zones and can be grown over widely different environments (Plate 1). The sweet potato contains between 16 – 40 per cent dry mass, of which 75 – 90 per cent are carbohydrates made up of starch, sugar, cellulose, pectin and hemi-cellulose. Sucrose is the most abundant sugar in the raw sweet potato, with a small amount of glucose and fructose (Graziella *et al.*, 2011). Its consumption is threefold: human food, animal feed and the production of alcohol and starch. According to FAO (2010), Sweet potato production from 115 countries was 106,569,572 tonnes. However supply remains very concentrated, 82.3 per cent of global production being in Asia. With 81,175,660 tonnes, China produces by far the largest part and possesses a little less than half the global acreage dedicated to the sweet potato. Far behind, Indonesia is the 2nd Asian producing country and the 4th in the world with more than 2 million tonnes of production. It is also an alternative source of bio-energy as a raw material for fuel production (Zang *et al.*, 2009). 60 per cent of sweet potato production in China is used for feed or processed into starch. In Japan, and Korea also, sweet potatoes are used for starch extraction.

In India, tuber crops are cultivated mainly in the Southern, eastern and North-eastern States, where they play a vital role in alleviating the hunger of a great majority of population. Cassava production is concentrated mainly in the Southern States of Kerala, Tamil Nadu and Andhra Pradesh. This is mainly used as a food or feed in Kerala, while it is almost exclusively an Industrial crop in the other two States. Of late, the cultivation is taking off in the non-traditional States like Maharashtra and Madhya Pradesh, owing to its recently realized importance as a bioethanol crop. Although sweet potato is cultivated in around 0.12 million

hectares in India, with a total production of 1.09 million tonnes, its productivity *viz.*, 9.08 t/ha is lower than the world average of 13.14 t/ha (FAOSTAT, 2012).

Sweet potato roots are classified into two general types: dry-fleshed cultivars with mealy, light yellow or white flesh (Plate 2) and the moist - type cultivars with soft, gelatinous, bright orange flesh. The average dry matter content is 30 per cent, but varies depending on such factors as cultivar, location, climate, day length, soil type, incidence of pests and diseases and cultivation practices. Sweet potato is a nutritive vegetable, being an excellent source of vitamin A precursor, certain other vitamins and minerals, energy, dietary fiber and protein (Collins and Washman, 1987). In addition, sweet potato consists of more than 80 per cent carbohydrates on dry basis, of which a major portion is starch (Table 1).

Table 1. Composition of sweet potato roots (g / 100 g)

Major nutrients (g/100g f. w)	
Moisture	71.0
Starch	20.0
Sugar	2.4
Protein	1.4
Lipid	0.2
Dietary fiber	1.6
Ash	0.7
Minor nutrients (mg/100g)	
Calcium (mg)	29
Phosphorous (mg)	51
Iron (mg)	0.5
Magnesium (mg)	26
Potassium (mg)	260
Zinc (mg)	0.6
Copper (mg)	0.2
Vitamin A (mg)	0.01

Table 1. Continued

Thiamine (mg)	0.08
Riboflavine (mg)	0.03
Niacin (mg)	0.6
Ascorbic acid (mg)	24

Source: Woolfe, 1992

The starch content of fresh sweet potato roots varies between 6.9 - 30.7 per cent. Starch as a component of sweet potato can be prepared to exhibit functional properties with potential utility in certain food applications. These properties can be developed by controlling the rate of heating during cooking which activates endogenous amylolytic enzymes of the sweet potato root to convert a portion of the starch to dextrins (Hoover, 1967). Dextrins form an adhesive material that could function as a binding agent in food products. Sweet potato roots are one of the major food resources of carotenoids along with apricots, carrots and peaches. The significance of carotenoids is that some are converted into vitamin A. Beta-carotene has the highest (100 %) provitamin A activity, followed by alpha and gamma carotenes with 50 per cent activity. Orange fleshed sweet potatoes obtain their colour through the presence of carotenoid pigments, with the flesh colour of the sweet potato root largely a function of the concentration of beta-carotene. Sweet potatoes are substantial sources of ascorbic acid (vitamin C) and contain moderate amounts of thiamine (B1), riboflavin (B2) and niacin as well as pyridoxine and its derivatives (B6), pantothenic acid (B5) and folic acid. They have been reported to contain satisfactory quantities of vitamin E. A 130 g serving of sweet potato provides 320 per cent of the daily minimum requirements (DMR) for vitamin A, 70 per cent of the vitamin C, and appreciable quantities of thiamine, riboflavin, niacin, phosphorous, iron and calcium. Starch manufacture is the main industrial utilization of sweet potatoes which has been used in starch noodles, bakery foods, snack foods, confectionery products and by the alcohol production and brewing industries (Montreka and Adelia, 2003).

| Plate 1: Sweet potato plant | Plate 2: Sweet potato tuber (white variety) |
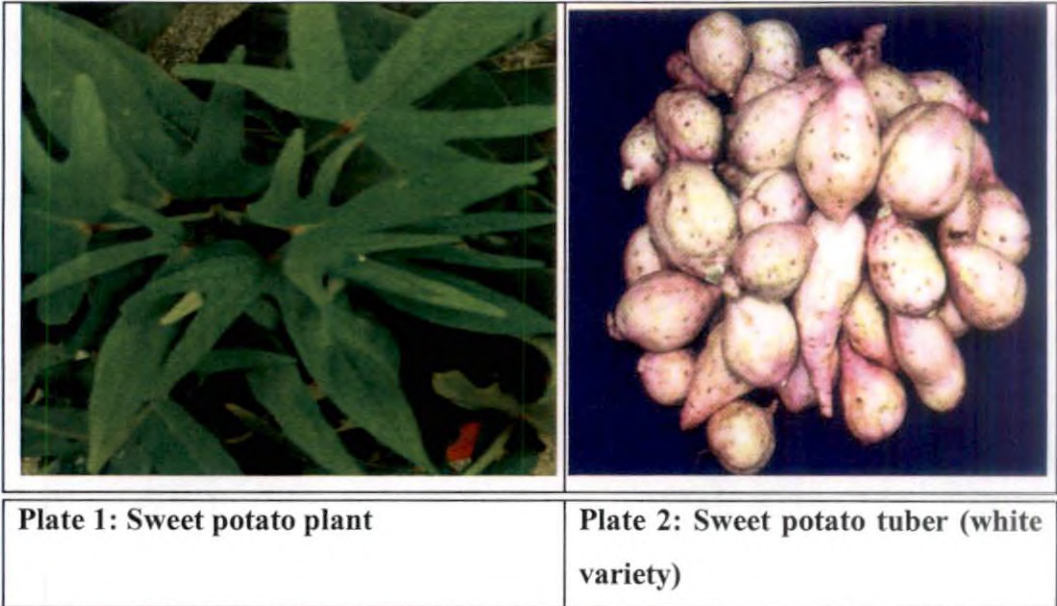
The crop has limited production costs and does well even under marginal conditions (poor soils with limited water supplies). Among the world's major food crops, sweet potato produces the highest amount of edible energy, per hectare per day. For example, in the Papua province in Indonesia, 90 per cent of dishes contain sweet potato; it is also the staple food for pigs. Far behind but ranked the second continent in the world, Africa contributes up to 14 per cent of global production with more than 14.2 Mt. Contrary to the main producing countries which have seen their offer decline over the years, such as China, Indonesia, Brazil and the Philippines, some African countries have increased their production. Today, the most important is Uganda with a harvest that increased from around 2 million in 1999 to 2.83 Mt in 2010. The sweet potato is the 3rd most important agricultural product in terms of volume after the plantain and the cassava. Sweet potato is a natural health food because of its high energy, dietary fibre, vitamins and mineral content (Padmaja, 2009). The tender shoots of sweet potato serve as an important source of vegetable fibre and food. The high level of phytonutrients in sweet potato leaves is promising of a new food additive product for use as a functional food. The deficiency of vitamin A is associated with an increased risk of Night blindness, Bitot spots, and Keratomalacia. One possible

solution for addressing vitamin A deficiency is through a food-based approach, using orange-fleshed sweet potato as an inexpensive source of beta-carotene (the pre-cursor to vitamin A). Sweet potato leaves are also rich source of nutraceuticals. Ishiguro and Yoshimoto (2005) reported high content of polyphenols and xanthophylls in sweet potato leaves. They reported the presence of high concentration of lutein, a member of the xanthophyll family of carotenoids, in sweet potato leaves (Variety 'Sujoh'). Lutein was present to the extent of 29.5 mg/100g (fwb) and hence the consumption of leaves of this variety were capable of mitigating eye diseases like age- related macular degeneration and cataract. Huang *et al.* (2004) reported that the sweet potato leaves contained high amounts of total phenolic and flavonoid compounds, which were responsible for its DPPH radical scavenging activity. The nutritive value of sweet potato leaves has been attributed to the high content of antioxidants especially phenolic compounds in them (Islam *et al.*, 2002; Yoshimoto *et al.,* 2005). Sweet potato is reported to have anti-diabetic property and the components contributing to this effect have been isolated and studied from white-skinned sweet potatoes (Kusano and Abe, 2000).

2.2 Starch

Starch is a biopolymer composed of anhydroglucose units and is the major storage energy in various plants in nature. It can be widely found in cereal grain seeds (e.g. corn, wheat, rice, sorghum), tubers (e.g. potato), roots (e.g. cassava, sweet potato, arrowroot), legume seeds (e.g. peas, beans, lentils), fruits (e.g. green bananas, unripe apples, green tomatoes), trunks (e.g. sago palm) and leaves (e.g. tobacco). The tropical root starches have widely varying physicochemical and functional properties unlike the cereal starches which possess almost similar characteristics (Moorthy, 1994). Their structures and the relative amount of both populations play an important role in determining starch properties. Starch is considered the main foundation of carbohydrate in the diet of humans (Ratnayake and Jackson, 2008). Starch molecules are homopolymers of D-glucose and are present in two structures within starch granules, amylose and amylopectin, which

differ in molecular size and degree of branching (Pérez *et al.*, 2009). Amylose is a linear molecule consisting mainly of α-1, 4 glucosidic linkages with a few α-1, 6 branches (Pérez *et al.*, 2009). Depending on the botanical source and genetic background of the parent plant, amylose and amylopectin content of the starch granule may vary, but generally amylose content in normal starches ranges from approximately 20 – 30 per cent (Jane, 2009).

Starch is a versatile raw material that finds extensive use in paper, textile and food industries. Maize is the largest source for commercial extraction of starch. Cassava is also widely utilized in countries like India, Thailand and Brazil for starch extraction. Although sweet potatoes are produced in India in major states like Odisha, Uttar Pradesh, West Bengal and Jharkhand, there is a continuous fall in the total production. The utilization prospects of sweet potato in India and especially in Kerala could be enhanced only if the extractability of starch is enhanced. Rahman *et al.* (2003) found that there was no direct relationship between starch content and starch recovery and attributed this to the extent of retention of starch by the strong cellulose-hemicellulose matrix. The variation in the morphological characteristics like size and shape of starch granules is credited to the biological origin (Singh *et al.*, 2007). The major food consumed by humans is starch, providing 75- 80 per cent of the total caloric intake worldwide (Bemiller and Whistler, 1996). Starch plays a vital role in developing food products either as a raw material or as a food additive, such as thickener, stabilizer or texture enhancer (Aina *et al.*, 2010). Starch is useful in maintaining the quality of stored food products; it improves moisture retention and consequently controls water mobility in food products. It could also be used as delivery vehicle of substances of interest in food and pharmaceutical industries such as antioxidants, colourants, flavours as well as pharmaceutically active proteins (Guan *et al.*, 2000). Starch are known to differ in their physicochemical properties and these differences have been attributed to differences in mean granule size, granule size distribution, amylose/amylopectin ratio in starch granules, mineral content and the presence of naturally occurring non carbohydrate impurities in the starches (Garcia and Walter, 1998; Oduro *et al.*,

2000; Aina *et al.*, 2010). Amylose content has been shown to influence starch granule morphology and rate of starch digestion (Li *et al.*, 2008). For the 44 varieties analyzed by Collado *et al.* (1997), starch content was found to be negatively correlated with fiber, total free sugar, and ash content.

2.3 Digestible and non-digestible starch fractions

The relatively recent recognition of incomplete digestion and absorption of starch in the small intestine as a normal phenomenon has raised interest in nondigestible starch fractions (Cummings and Englyst, 1991: Englyst *et al.*, 1992). These are called "resistant starches", and extensive studies have shown them to have physiological functions similar to those of dietary fiber (Asp, 1994; Eerlingen and Decour, 1995). The diversity of the modern food industry and the enormous variety of food products it produces require starch that can tolerate a wide range of processing techniques and preparation conditions (Visser *et al.*, 1997). These demands are met by modifying native starch with chemical, physical, and enzymatic methods (Betancur and Chel, 1997), which may lead to the formation of indigestible residues. The availability of such starch therefore deserves consideration. Based on the action of enzymes starch can be classified to Rapidly digestible starch (RDS), Slowly digestible starch (SDS) and Resistant starch (RS).

RDS consists mainly of amorphous and dispersed starch and is found in high amounts in starchy foods cooked by moist heat, such as bread and potatoes. It is measured chemically as the starch, which is converted to the constituent glucose molecules in 20 min of enzyme digestion. Like RDS, SDS is expected to be completely digested in the small intestine, but for one reason or another, it is digested more slowly. It is measured chemically as starch converted to glucose after a further 100 min of enzyme digestion. RS is indigestible by body enzymes.

2.4 Resistant Starch

The greater awareness on the part of consumers of the relationship between a nutritious diet and health and well-being has been one of the reasons for the increase in popularity of novel foods with good nutritional properties (Pérez-Alvarez, 2008, Sanz *et al.*, 2008). Resistant starch refers to the portion of starch and starch products that resist digestion as they pass through the gastrointestinal tract. RS is an extremely broad and diverse range of materials and a number of different types exist viz., RS1, RS2, RS3 and RS4. At present, these are mostly defined according to physical and chemical characteristics (Nugent, 2005). Resistant starches are also referred to as dietary starches because they escape enzymatic digestion in the small intestine and are fermented by colonic microflora in the large intestine to short chain fatty acids (Wasserman *et al.*, 2007). The effect on the body of resistant starch is similar to dietary fiber (Berry, 1986). Resistant starch is used as a functional food ingredient for human nutrition because it can reduce caloric content and has physiological effects similar to dietary fiber (Wasserman *et al.*, 2007). Englyst *et al.* (1992) determined the percentage of undigested raw potato starch and total digested starch after 120 min of alpha-amylase hydrolysis. Results showed that boiled potato, corn and wheat starches were completely digested after two hours of hydrolysis and the native uncooked corn and wheat starch was 30-40 per cent undigested which demonstrates the amount of type II resistant starches in the native starches. The formation of RS is affected by several properties of starch, such as granular structure, crystallinity, amylose and amylopectin ratio, and chain length. Resistant starches have got much attention for both its prospective health benefits and useful properties (Sajilata *et al.*, 2006).

RS has a small particle size, white appearance, bland flavor and low water-holding capacity. It has desirable physico-chemical properties such as swelling, viscosity increase, gel formation, and water- binding capacity, making it useful in a variety of foods (Fausto *et al.*, 1997). RS not only fortifies fiber but also imparts

special characteristics not otherwise attainable in high-fiber foods (Tharanathan and Mahadevamma, 2003).

Amylose and amylopectin ratio affects the formation of RS based on a synergistic effect between the starch molecules that affects starch hydrolysis. RS formation usually increases as amylose content increases. Retrograding amylose was the main factor influencing RS (Leeman *et al.*, 2006). The higher the amylose content the lower the digestibility of the starch or the larger the RS yield (Escarpa *et al.*, 1996). Once gelatinization is complete the starch is easily digestible. When the gel is cooled or dried the starch begins to recrystallize (retrogradation). Ishiguro *et al.* (2000) observed retrogradation of sweet potato starch gels by examining ten different sweet potato varieties. Cooking can also affect the RS content in food, with high moisture and temperature processes disturbing the crystalline structure of RS, thus lowering the content (Sajilata *et al.*, 2006). Higher levels of resistant starch are found in starches containing higher levels of amylose (Unlu and Faller, 1998). It is also found that high amylose starch is more resistant to digestion than amylopectin due to its compact linear structure (Rashmi and Urooj, 2003).

RS can be prepared by using heat, enzyme and chemical treatments. The possibility of preparing a RS concentrate by using enzyme treatment from isolated pea starch was investigated, and sorption of hydrophobic substances (indicative of health-benefiting properties) by such a concentrate was studied by Soral and Wronkowska (2000). A method has been discovered to produce an RS product that retains the same cooking quality as found in untreated rice starch or flour, but has a higher percentage of starch resistant to α-amylase digestion (King and Tan, 2005). This method uses a debranching enzyme, pullulanase, to digest the starch, but does not require pretreating the starch source before enzymatic treatment.

The main step to determine RS *in vitro* in foods must first remove all of the digestible starch from the product using thermostable α-amylases (McCleary and Rossiter, 2004). At present, the method of McCleary and Monaghan (2002) is considered the most reproducible and repeatable measurement of RS in starch and

plant materials, but it has not been shown to analyze all RS as defined (Champ *et al.*, 2003). Advantages of the method are the use of small amount of sample, less reagents and elimination of drying.

2.4.1 Beneficial physiological effects of RS

A number of physiological effects have been ascribed to RS (Nugent, 2005) which have been proved to be beneficial for health. RS appears to be highly resistant to mammalian enzyme and may be classified as a component of fiber on the basis of the recent definitions of dietary fiber given by AACC (2000). Replacement of 5.4 per cent of dietary carbohydrates with RS in a meal could significantly increase postprandial lipid oxidation suggesting reduction in fat accumulation in the long term (Higgings *et al.*, 2004). A body of evidence is also developing for RS2 High Amylose Maize Starch (HAMS) to improve insulin sensitivity (Robertson *et al.*, 2005; Johnston *et al.*, 2010; Maki *et al.*, 2012) with the most recent study indicating a 72 per cent improvement in insulin sensitivity when 30 g of RS2 HAMS was added to the diets of men at risk of pre-diabetes. Recent studies indicate resistant starch may have a role in enhancing both short term (Willis *et al.*, 2009; Anderson *et al.*, 2010) and long-term satiety (Nilsson *et al.*, 2008; Bodinham *et al.*, 2010). Research with dietary components, such as resistant starch, supports a direct link between diet, colonic bacteria and colon cancer, with recent animal studies indicating high resistant starch diets may prevent colon carcinogenesis (Ridlon and Hylemon, 2006). The most novel and relevant recent data describe a role for resistant starch in ameliorating inflammation; the use of resistant starch for optimal bowel health and prevention of colorectal cancer (CRC); and further the systemic effects of resistant starch may be important for the treatment of other forms of cancer, such as breast cancer (Higgins and Brown, 2013). Emerging areas of interest for resistant starch and the product of resistant starch fermentation, short chain fatty acids, include inflammatory conditions and immune function (Maslowski and Mackay, 2011). The potential for resistant starch to also play a role in enhancing the effectiveness of oral rehydration therapy is attracting scientific interest.

2.4.2 Resistant starch as a functional ingredient

Resistant starch which is a natural component that is present in many foods has a role to play in the nutritional benefits of fiber fortification. Although there are several definitions for resistant starch, it consists of starch and its degradation product that are resistant to digestion in the stomach and small intestine of healthy individuals. Resistant starch offers advantages over cellulosic sources of fiber such as bran. It enhances the organoleptic qualities of food as a replacement for or complement to natural fiber and it can be labeled as 'dietary fiber'.

Legumes are rich in resistant starch, and as much as 35 per cent of their starch could escape digestion (Marlett and Longacre, 1997). Green bananas and uncooked potato are also relatively rich in resistant starch. Resistant starch plays important physiological roles and has the potential to improve human health and lower the risk of many diet-related diseases and extensive studies have shown them to have physiological functions similar to those of dietary fiber (Brighenti *et al.*, 1998; Haralampu, 2000; Thompson, 2000; McCleary and Monaghan, 2002; Sajilata *et al.*, 2006).

2.5 Enzymatic extraction of starch

The starch granules present in the roots and tubers are embedded in cellulosic fibers and held together by pectin substrates (Rahman and Rakshit, 2004). In industrial processes potato starch is mainly extracted by mechanical disintegration of the cell wall and washing out of the starch granules by water (Joshi and Kulkarni, 1993). Nandan *et al.* (2011) reported that use of commercial cellulase could enhance the release of starch granules from cell wall components. The most distinctive feature of enzymes is that they can effectively operate at mild physiological conditions, at atmospheric pressure, temperatures up to 100°C and pH of 3-10 (Klacik, 1988). An overall enzymatic milling process for extraction of starch from corn was optimized by Johnston and Singh (2004). After soaking and first grind optimization, enzyme concentration and pH determinations were evaluated using bromelain (protease obtained from pineapple plant). Results

showed that the minimum addition of bromelain to reach starch yields equivalent to conventional yields were 0.4g protein/kg of corn.

Gayal and Hadge (2003) investigated the effect of using cellulase from *Penicillium funiculosum* on isolation of starch from potato. Potato cubes were subjected to cellulase treatment. Hydrolysis of cellulose and release of starch were assessed at various enzyme concentrations, with different incubation periods. About 68 per cent starch was recovered in 6 hours and the recovery was increased to 90 per cent in 2 hours by supplementing with pectinase.

Addition of commercial enzyme increases the extraction of starch from tuber crops considerably even at lower levels of rasping (George *et al.*, 1991; Padmanabhan *et al.*, 1993; Kallabinski and Balagopalan, 1994; Rahman and Rakshit, 2004).

Commercial pectin enzymes have been employed in starch extraction from sweet potato (Rahman and Rakshit, 2004), yam (Daiuto *et al.*, 2005) and cassava (Sriroth *et al.*, 2000). Enzymatic treatment process for starch extraction from potato was investigated using cellulase enzyme and compared with conventional process (Nandan *et al.*, 2014). The increase in starch yield ranged from 1.9% at low enzyme concentration and incubation time and high addition of water to a maximum of 70% increase from conventional process in starch yield was achieved when enzyme concentration and incubation time were high and addition of water was low.

In cassava starch extraction, the combination of mechanical rasping and use of hydrolytic enzymes such as pectinases to disintegrate the pulp and cause better release of starch is possible. This has been found to be cost effective as it saves on energy cost due to increased rasping (Rahman and Rakshit, 2003). The pectolytic enzymes break down the pectate network of the cell wall leading to release of the starch granules (Herron *et al.*, 2000; Rahman and Rakshit, 2003). Daiuto *et al.* (2005), studied on various extraction methods for yam starch, noted treatments which reduced viscosity made the separation of the starch slurry from the residual

mass easier. Serna-Saldivar and Mezo- Villanueva (2003) studied the effect of cell wall degrading enzyme complex in enhancing starch recovery from sorghum and maize. The results indicated that enzyme concentration and incubation time had a positive effect on the increase in starch yield while addition of water had a negative effect. Enzymatic modification of native starch may be considered as one of the techniques to modify native starch by decreasing the molecular weight. The enzyme conversion of starch is used to produce derivatives with varying viscosity, gel strength, thermo reversibility and sweetness. In enzymatic modification techniques, the gelatinised starch is subjected to degradation by enzymes resulting in various products (Alexander, 1992). In order to solve the environmental problem associated with disposal of the kernels rich in starch, extraction using microbial enzymes could yield starch from kernel (Sunita *et al.*, 2008). It was found that enzyme addition could serve as a good alternative to increased rasping, which could break down the starch granules and fiber. With the drop in price of the enzymes this could lead to cost saving over the amount spent in rasping. Thus enzyme assisted extraction can perform a major role in getting better yields and quality of starch in an environmentally friendly way. Commercial pectic enzymes have been used to enhance cassava starch extraction (Sriroth *et al.*, 2000; Dauito *et al.*, 2005). The combined application of 15 Novo cellulase units of cellulase and 122.5 polygalacturonase units of pectinase per g dry pulp for 60 min resulted in 40 per cent starch recovery (Sriroth *et al.*, 2000). Attipoe (1999), in a preliminary study using this crude pectin enzyme extract, observed that the addition of 72 mg total protein extract to 200 mg cassava mash (0.036 %) for 30 min reaction time produced about 61 per cent increase in extraction rate over the controls. Pectin enzymes produced by culturing *Saccharomyces cerevisiae* ATCC 52712 in an appropriate medium for 6 days was used as a crude enzyme extract and tested for its ability to enhance the extractability of cassava starch. An enzyme dosage of 0.02 per cent with a reaction time of 30 min gave the optimum increase in rate of starch extraction (60 %) and increase in yield of starch extracted (53 %) (Dzogbefia *et al.*, 2008).

2.6 Physico-chemical properties of Starch

2.6.1 Viscosity

Continued heating of starch in excess water with stirring causes the granules to further swell, the amylose to leach more, and the granules to disintegrate, forming a viscous material called paste (BeMiller, 2007). Pasting occurs after or simultaneously with gelatinization. In the dilute regime, the viscosity is governed by the volume fraction of swollen granules (Steeneken, 1989). Pasting properties of starch are important indicators of how the starch will behave during processing and are commonly measured using various viscometers like Rapid Visco Analyzer (RVA). Initially heating starch suspension results in swelling of starch granules. As heating continues, an increase in viscosity can be observed, which reflects the process of pasting. The temperature at the onset of viscosity increase is termed pasting temperature. Viscosity increases with continued heating, until the rate of granule swelling equals the rate of granule collapse, which is referred to as the peak viscosity (PV). Viscosity normally stabilizes at a final viscosity or cold paste viscosity, which is related to the capacity of starch to form viscous paste or gel after cooking and cooling (Batey, 2007).

2.6.2 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) is a thermal technique that measures the amount of heat required to increase the temperature of a sample. DSC measures both temperature and enthalpies of gelatinization. DSC records phase transitions such as melting, glass transitions or exothermic decompositions which involve a change in energy or heat capacity changes (Fennema *et al.*, 1996). Data collected from the DSC analysis is presented in joules/gram (J/g °C). The collected data from the starch gelatinization parameters are recorded as peak onset, peak temperature, end of peak and gelatinization enthalpy information. Different varieties of the same species can have variation in gelatinization temperatures. Lockwood and King (2008) found apparent differences in gelatinization characteristics between white fleshed and orange-fleshed sweet

potato starches. The orange-fleshed sweet potato starch granules gelatinized at a lower temperature than those of the white-fleshed sweet potato starch. The DSC results obtained from starches are useful for the development of an enzyme resistant starch (RS) in processed foods due to its possible nutritional significance for dietary fiber purposes (Sievert and Pomeranz, 1990; Chuchajowsk *et al.*, 1991; Gruchala and Pomeranz, 1993). The level of RS in foods, such as pastas and breads, is dependent upon the type of starch and the particular processing or cooking conditions (Szczodrak *et al.*, 1992).

2.6.3 Swelling volume and Solubility

The swelling volume (SV) is the ratio of the wet weight of the sedimented gel to its dry weight of starch (Crosbie, 1991). The granule swelling ability is usually quantified by swelling power (the weight of sedimented swollen granules per gram of dry starch) or swelling volume (the volume of sedimented swollen granules per gram of dry starch) at the corresponding temperature (Konik *et al.*, 1993; Pinnavaia and Pizzirani, 1998; Konik *et al.*, 2001). Starch swelling behaviour not only depends on starch origin but also depends on amylose content. Starch granules are hygroscopic and exhibit limited swelling in the presence of water (Jane, 1999). High proportions of long chain (degree of polymerization >35) molecules in amylopectin contributed to the increase in swelling (Sasaki and Matsuki, 1998). 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 (Tester and Morrison, 1993). The swelling volume and solubility provide evidence of the magnitude of interaction between starch chains within the amorphous and crystalline domains. The extent of this interaction is influenced by the amylose to amylopectin ratio, and by the characteristics of amylose and amylopectin in terms of molecular weight/distribution, degree and length of branching and conformation (Hoover, 2001).

2.6.4 Gelatinization

Gelatinization is a major step which exhibits featured characteristics of starch. Gelatinization improves the availability of starch for amylose. The gelatinization temperature of most starches is between 60°C - 80°C. Differential Scanning Calorimetry (DSC) is the most common technique used to study the thermal properties of starches. In general, there is a negative relationship between the amylose content of starch and the gelatinization temperature. Starch transition temperatures and gelatinization enthalpies by DSC may be related to characteristics of the starch granule such as degree of crystallinity. Heating in excess water disrupts the granule crystalline structure and is known as gelatinization (Jane, 1999). Energy required to break noncovalent interactions in starch crystallites is quantified using enthalpy change of gelatinization (ΔH). Both ΔH and range of gelatinization temperature are generally increased in high-amylose starches (Kibar *et al.*, 2010). Gelatinization of the starch eliminates the crystalline structure allowing for enzyme hydrolysis and reduces the RS content of the starch. Recrystallization and chemical modification will increase the RS content (Zhang *et al.*, 2006). The temperature range over which gelatinization occurred, from onset to completion measured with DSC was from 69.9 to 92.9°C with a peak temperature ranging from 80.4 to 83.6°C. Tester and Morrison (1993) has postulated that the extent of crystalline perfection is reflected in the gelatinization temperatures. Enthalpy of gelatinization ($\Delta Hgel$) gives an overall measure of crystallinity (quality and quantity) and is an indicator of the loss of molecular order within the granule.

2.7 Ultrastructural studies on starch

Starch granules from different sources showed distinct shape and size. Cassava starch granules displayed round shapes, whereas the sweet potato starch showed granules with round and polygonal shapes, and potato starch granules were elliptical (Rocha *et al.*, 2010). According to Oates (1997), enzymes cause alteration on the granule surface and degrade the external part by exocorrosion.

2.8 Pasta as a novel food with functional attributes

Pasta is a convenient and nutritionally palatable, low glycemic food which has its origin in Italy (Petitot *et al.*, 2009). Pasta, as a food rich in complex carbohydrates with low glycemic index is gaining wide acceptance in the recent years (Sadehi and Bhagya, 2008). Pasta products, largely consumed all over the world are traditionally manufactured from durum wheat semolina, known to be the best raw material suitable for pasta production due to its unique colour, flavor and cooking quality. The intrinsic quality attributed to the pasta are influenced primarily by the properties of the protein and the starch fraction and by many other factors such as the origin of the semolina (Wood *et al.*, 2001) and the pasta production process (mixing, extrusion and drying conditions). Starch and proteins undergo successive structural changes during the pasta making process. Knowledge must therefore be gathered about pasta processing, structuring and nutritional characteristics so as to be able to develop food products with both high sensorial and nutritional qualities (Petitot *et al.*, 2009). Sweet potato flour is reported to contain high amounts of dietary fiber ($\simeq$ 17%) (Yadav *et al.*, 2006). Various workers have attempted the use of sweet potato for making noodles and pasta (Collado and Corke, 1996; Pangloli *et al.*, 2000; Chen *et al.*, 2003; Limroongreungrat and Huang, 2007).

Several studies have been carried out to improve the nutritional properties of pasta, using protein and dietary fibre sources, isoflavones, ω-fatty acid, β-glucans, carotene sources, resistant starches, etc. (Mestres *et al.*, 1988; Collado and Corke, 1996; Anthony *et al.*, 1998; Messina, 1999; Manthey *et al.*, 2004; Limroongreungrat and Huang, 2007; Petitot, 2010). Fortification of pasta with various protein sources such as legume flours, cheese, soy proteins, mustard protein isolate and gluten meal has been attempted by several workers, with a view to enhancing the nutritional value of pasta (Sadehi and Bhagya, 2008; Petitot, 2010). The possibility of using sweet potato for noodle and pasta manufacture has also been explored by different researchers (Collado and Corke, 1996; Limroongreungrat and Huang, 2007).

Cereal bran sources have been used to fortify wheat pasta to enhance their dietary fiber content (Collado and Corke, 1996; Anthony and Clarkson, 1998; Manthey *et al.*, 2004). The type and amount of added fiber have been reported to influence the cooking and starch degradation in durum wheat semolina pasta (Messina, 1999; Manthey *et al.*, 2004). Generally, pasta is consumed within a short period after cooking. Good quality pasta is defined as having high degree of firmness and elasticity, which is mainly, termed as *'al dente'* (Lamberti *et al.*, 2004). The structural and textural properties of pasta are influenced by several factors, the most important being the properties of the raw material and the drying conditions (Zweifel *et al.*, 2003). Starch gelatinization and protein coagulation cause the major structural changes during cooking.

Most important factor determining the glycaemic impact of starch is its rate of digestion, because this determines the rate at which free glucose and maltose are released the glycaemic index (GI) has been introduced in order to estimate the blood glucose response of foods after ingestion by humans. It is measured by the postprandial glycaemic area of a test meal, expressed as the percentage of the corresponding area of the reference food glucose or white bread (Petitot *et al.*, 2009) into the gut for absorption (Mishra and Monro, 2009). Starch properties also contribute to pasta cooking quality (Sajilata *et al.*, 2006). Among cereal products, pasta appears to possess unique nutritional features in that the starch is slowly digested and absorbed in the small intestine, hence promoting a low plasma glucose response (Björck *et al.*, 2000).

Materials and Methods

3. MATERIALS AND METHODS

The study entitled "Enhancing sweet potato (*Ipomoea batatas* Lam.) starch extractability using enzymes" was conducted at the Division of Crop Utilization, Central Tuber Crops Research Institute (CTCRI), Sreekariyam, Thiruvananthapuram during 2013-2014. Details regarding the raw materials used and methodology adopted for various experiments are presented in this chapter.

3.1 Raw material collection

White fleshed local cultivar (Plate 3) collected from farmers field in Palakkad were used for the whole study.

Starch extractions were carried out in both fresh tuber slurry as well as dry flour slurry treated with enzymes, in three different ratios 1:2, 1:4 and 1:6. Therefore, the study comprised 6 enzyme treatments, 2 samples, 3 slurry concentrations and 2 replications. Hence, altogether of 72 starch samples were used for the study.

3.2 Methods used for starch extraction:

Starch extractions from white fleshed sweet potato tuber were performed by two methods, conventional and enzyme assisted extraction method. In enzyme assisted extraction of starch, the effects of three enzymes as single/cocktail were studied.

3.3 Enzymes tried for the extraction:

1. Accellerase 1000 (Ms. Genencor, USA)
2. Cellic C Tec 2 (Ms. Novozymes, Denmark)
3. NS 22119 (Ms. Novozymes, Denmark)
4. Combination of Accellerase and Cellic (C+A)
5. Combination of Accellerase and NS (A + NS)
6. Combination of Cellic and NS (C+NS)

Plate 3. White fleshed sweet potato tuber

3.4 Starch extraction from sweet potato

Starch was extracted by the method described by Kallabinski and Balagopalan (1994) with some modifications. The tuber were washed under tap water so that any dirt adhered to it may be removed. After washing, the skin was peeled off and chopped using a chipping machine in order to facilitate grinding. For the extraction of starch from the flour, chopped tuber were sun dried. The grinding was done in a kitchen blender. The ground sweet potato meal was then transferred to a large beaker and appropriate amount of water was added to it. The enzyme solution was added to the slurry using a pipette. After 18 h of incubation the slurry was filtered through a starch sieve of mesh size 250 μm and allowed it to settle. The settled starch was washed with water and again kept for sedimentation for 1 h. After sedimentation the supernatant was discarded. The separated starch was sun dried for 48 h. Major procedures followed for extracting starch from sweet potato tuber are presented below (Fig.1).

Fresh sweet potato tuber
↓
Peel and chip the tuber
↓
Blend with sufficient quantity of water
↓
Add enzyme to the slurry
↓ ◄— Incubate for 18 h
Wash out the starch with adequate quantity of
water through a starch sieve (250 μm)
↓
Allow starch to settle
↓
Solar dry moist starch – 48 h
↓
Sweet potato starch
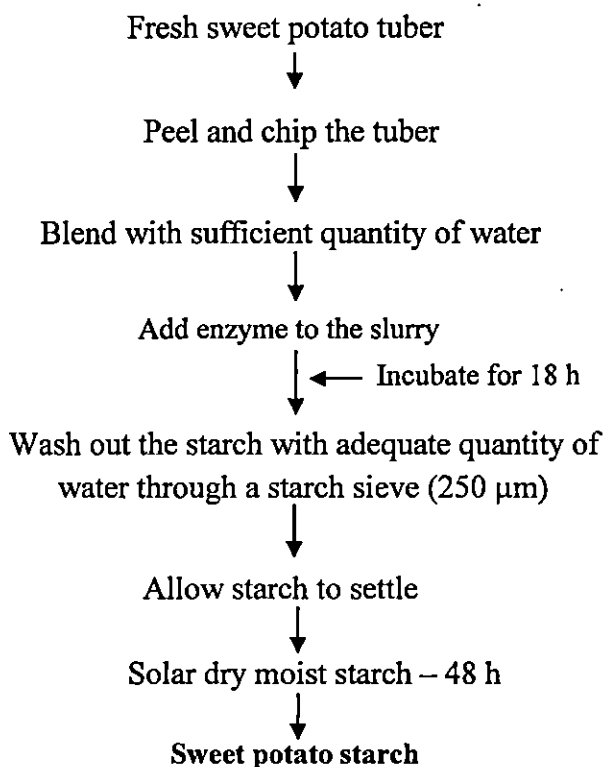
Figure 1. Flow chart for enzyme assisted extraction of sweet potato starch

Each determination was replicated. Control reactions contained distilled water in place of enzyme extract. Sun dried chips were ground in a blender as above and starch was extracted. The starch separated after enzyme treatment as well as the residue were dried and kept for further studies.

3.5 Computation of starch yield and starch recovery

Starch yield and recovery were computed using the formula:

$$\text{Starch yield (SY)} = \frac{\text{Extracted starch (g)}}{\text{Sweet potato tubers used (g)}} \times 100$$

$$\text{Starch recovery (SR)} = \frac{\text{Yield of starch (SY)}}{\text{Starch content in the tubers}} \times 100$$

3.6 Titrimetric Assay of Starch content

3.6.1 Preparation of samples

One gram of extracted starch was weighed into 100 ml Erlenmeyer flasks. To each flask, 20 ml ethanol (80%) was added and left overnight to extract the sugars.

The extracted sugars were separated from the residue, by filtration through Whatman No.1 filter paper. The filtrates were collected separately for sugar estimation. Residue on the filter paper was washed with 20 ml of distilled water to remove adhering sugar particles and the filtrate added to original filtrate.

The residue was transferred back into the conical flask using 20.0 ml of 2 N HCl. The starch in the residue was then hydrolysed by leaving the flask on a hot plate at 100°C for 30 minutes. The hydrolysates were cooled to room temperature and their volume increased to 100 ml using distilled water. This supernatant was then directly used for titration for starch ditermination. The alcoholic sugar filtrate was treated with 1.0 ml concentrated HCl and heated for 30 minutes on a hot plate at 100°C. This would convert the non-reducing sugars also to reducing sugars.

The volume of sugar extract was raised to 50 ml and used for titration for sugar determination.

3.6.2 Titrimetric Assay

Ten milliliters (10 ml) of Pottassium ferricyanide reagent (1%) were pipetted out into 100 ml Erlenmeyer flasks. To this, 5.0 ml NaOH (2.5 N) was added. The contents were mixed thoroughly. The flasks were then kept over the flame for boiling. When the reagent began to boil 3 drops of dilute methylene blue indicator were added. The solution immediately turned blue-green. The starch hydrolysate was taken in a 2.0 ml blow pipette and added drop by drop to the boiling reagent. The nearing of end point was indicated by rapid disappearance of the violet colour. The titre values were noted.

The starch content in the sample was calculated from the formula:

$$\text{Starch (g/100 g)} = \frac{10^{\,a} \times 100^{b} \times 0.9^{c} \times 100}{T \times 2^{d} \times 1000}$$

a = titre obtained for ferricyanide reagent while calibrating against standard glucose (ie. 10)

b= Total volume of starch hydrolysate (ml)

c = 0.9 is Morris factor for converting sugar to starch

d = Weight of sample (g)

T= Titre value of starch hydrolysate (ml)

3.7 Determination of Swelling Volume and Solubility of Starch

Starch (400 mg) was weighed into 100 ml conical flask, 40 ml of distilled water was added with vigorous swirling and the flask was kept in a water bath for boiling. The flask was continuously swirled so that the starch completely gelatinizes. If lumping occurs, the sample was discarded. After complete gelatinization of the starch, the flask was kept in the water bath for 15 min with occasional swirling. After 15 min, the flask was taken out and allowed to cool to room temperature. The contents were transformed carefully into a graduated 50

ml centrifuge tube and centrifuged at 2000 rpm for 15 min. The volume of the gelatinous precipitate at the bottom of the centrifuge was noted for Swelling Volume.

For determination of solubility, 10 ml of the supernatant of the centrifuged material was transformed into pre-weighed petri-dish. The petri-dish was kept at 100°C for 3–6 h and weight of the soluble material was obtained by subtracting the weight of empty petri dish.

3.8 Viscosity studies on extracted starch samples

The viscosity behaviour was studied using Rapid Visco Analyser (M/s New Port, Australia). The starch samples were placed in special canisters. A paddle was placed in the canister and the top of the paddle inserted into the paddle coupling. The paddle coupling was driven by the stirring motor located in the RVA's tower. Viscosity of the sample was detected through continuous monitoring of torque on the paddle sensor throughout the test. Viscosity and temperature data were recorded over time.

3.9 *In vitro* digestibility Test

In vitro Starch digestibility of Starch samples was determined by the procedure modified from the original methods of Englyst *et al.* (1996), Mc Cleary and Monaghan (2002) and Kim *et al.* (2008). The cooked pasta samples were surface dried on Whatman No. 1 filter paper.

Native starch, enzyme treated starch and pasta were used for the test. Cooked pasta (5.0 g) was cut into small pieces of 1.0 mm length, and in the case of native starch and enzyme treated starch they were used in the dried form.

Samples were mixed with 10 ml HCl-KCl buffer (Appendix I) and kept in a water bath (SW 21; M/s Julabo Industries) at 37° C for 10 min. Pepsin (EC 3.4.23.1; M/s SIGMA, USA) was added to initiate proteolysis (0.5 ml per sample from an enzyme solution containing 1.0 g pepsin/10 ml HCl-KCl buffer). Samples were incubated at 37 °C for one hour, after which 50 ml sodium phosphate buffer

(Appendix II) was added. One tablet of Panzynorm- N (manufactured by M/s German Remedies India Ltd., Mumbai, India) containing 10,000 unit of lipase, 9000 units of amylase and 500 unit of protease was dissolved in 5.0 ml sodium phosphate buffer (0.1 M; pH 8.0) and 0.5 ml each was added to the samples and incubation continued for 120 min. Enzyme and substrate blanks were maintained for each sample. Sample aliquots (1.0 ml) were drawn from the assay system after every 20 min interval upto 120 min, added to 3.0 ml sodium acetate buffer (Appendix III) and incubated at 60°C for further 10 min with 0.25 ml dextrozyme GA (M/s Novo Industries, Denmark) Glucose released in each system was quantified by the Glucose oxidase (EC 1.1.3.4) – PAP method (M/s Beacon Diagnostics Pvt. Ltd., Gujarat, India). Glucose released at each time from 20 to 120 min was expressed on 100g starch basis for each sample to nullify the difference in starch content between the formulations.

Starch fractions such as rapidly digested starch (RDS), slowly digested starch (SDS) and resistant starch (RS) were computed from the data on the in vitro kinetics of starch hydrolysis in different samples as given.

RDS = Glucose released at 20 min × 0.9/100g starch in sample

Where 0.9 is the Morris factor, for converting the glucose value to starch.

SDS = (Glucose released at 120 min – Glucose released at 20 min) × 0.9

RS = 100 - (RDS + SDS)

3.10 Colour indices of Starch samples

The starch samples were filled in a petri plate of size 60 mm and height 17 mm. The primary colour coordinates 'L', 'a' and 'b' values were measured by Minolta Spectrophotometer C2600 by keeping the aperture opening in the medium position (8/11 mm) and by covering with granular material cover. From the above primary parameters, the total colour difference (ΔE) and whiteness index of the powdered samples were measured using standard equation as follows (Mohammadi et al., 2008; Saricoban and Yilmaz, 2010).

Total colour difference, $\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2}$

Whiteness index $= 100 - \sqrt{(100 - L)^2 + a^2 + b^2}$

Where L = degree of lightness /darkness

a = degree of greenness/ redness

b = degree of blueness/ yellowness

L_0 = degree of lightness /darkness of the standard = 99.34

a_0 = degree of greenness / redness of the standard = -0.03

b_0 = degree of blueness / yellowness of the standard = -0.1

3.11 Thermal characterisation by differential scanning calorimetry

Gelatinization (Onset and peak) temperature and enthalpies were determined by DSC (Mettler- Toledo, Switzerland).

Approximately 5 mg of individual starch samples were weighed in aluminium coated pans and 10 µl of distilled water was added. The pans were hermetically sealed. An empty double pan was used as reference. Scan's were performed 25-120 °C at a controlled constant rate of 10 °C min^{-1}. Onset (T_o) and peak (T_p) gelatinization temperatures were measured and gelatinization enthalpy (ΔH) was calculated from the area of the peak endotherm using the Analysis Software. Runs were done in duplicate.

3.12 Scanning Electron Microscopy (SEM)

Scanning electron microscopy was done to study the surface morphology of the starch granules as well as the residue after the extraction of starch. A gold coating (10-15 nm thick) was then applied using JEOL JFC 1600 magnotron sputtering unit with 10 mA current for 80 s. Bulk samples were examined at 10 kV and 1Pa vacuum using a JEOL JSM6390 LV scanning electron microscope (Oxford, UK).

3.13 Preparation of pasta

Starch samples from the best treatments were used for pasta making i.e., Accellerase extracted and Cellic extracted starch samples, along with control starch. The flour blends were mixed with the requisite quantity of water, which was standardized through repeated trials to obtain the smooth outer finish and unbreakable structure for the extruded pasta. Pasta mix was extruded using two types of die *viz.*, tube die (No.62) and corrugated die (No.98) in a pasta making machine (M/s La Monferrina, Italy, P3 Model), at room temperature (30 ±1°C). Pasta was cut using a cutting device attached with the basic unit, to approximately 3.0 cm length and the fresh pasta was dried in an oven at 50° C for 18 h. The dry pasta samples were stored under dark in airtight bottles till analyses.

3.14 Pasta formulations

Pasta was made using sweet potato control starch as well as the starch extracted from the best treatments as per the formulation given in Tables 2 and 3.

Table 2: Formulation for pasta preparation using control starch

Ingredients	Maida based blend (C1)	Sweet potato-maida blend (C2)
Flour	85 % maida	72 % sweet potato flour + 13 % maida
Sweet potato starch	10 % control starch	10 % control starch
Gelatinized starch	5 %	5 %

Table 3: Formulation for pasta preparation using enzyme extracted starch

Ingredients	Maida based blend (T1)	Sweet potato-maida blend (T2)
Flour	85 % maida	72 % sweet potato flour + 13 % maida
Sweet potato starch	10 % Accellerase extracted starch	10 % Cellic extracted starch

Table 3 continued

Gelatinized starch	5 %	5 %
Ingredients	**Maida based blend (T3)**	**Sweet potato-maida blend (T4)**
Flour	85 % maida	72 % sweet potato flour + 13 % maida
Sweet potato starch	10 % Cellic extracted starch	10 % Accellerase extracted starch
Gelatinized starch	5 %	5 %

3.15 Swelling Index (SI) (Mestres *et al.*, 1988)

Fifty grams (50 g) dried pasta (W1) from control and treatments were kept in an oven at 105° C for 2-3 h. This along with 1.0 g common salt (NaCl) was added to 500 ml boiled water in a beaker. The pasta was cooked for exactly 7 min and the water was drained to a weighed beaker. The cooked pasta was surface dried over a cloth and weight of the cooked pasta (W2) was taken.

$$\text{Swelling Index} = \frac{W2 - W1}{W1}$$

3.16 Cooking loss (Debbouz and Doetkott, 1996)

The water drained from each treatment after cooking was separately dried in Petri dishes (pre-weighed) and kept in an oven at 105° C for overnight. The weight of the dry residue was quantified (W2 g).

$$\text{Cooking loss (\%)} = \frac{W2 \times 100}{W1}$$

where W1 is the initial weight of pasta.

Results

4. RESULTS

The results of the study entitled "Enhancing sweet potato (*Ipomoea batatas* Lam.) starch extractability using enzymes" carried out at the Division of Crop Utilization, CTCRI, Sreekariyam during 2013-2014 are presented in this chapter.

4.1 Yield, recovery and purity of starch obtained from various treatments

It was found from the study that enzyme treatment facilitated enhanced recovery of starch from fresh tubers as well as flour. Whilst the starch yield from the control treatment was only 15.25 per cent, it ranged from 22.37 to 24.99 per cent for the most effective enzyme treatments (Table 4) in the case of fresh tubers. Highest starch yield of approximately 25 per cent was obtained for Cellic + NS (1:2) combination, which was however not significantly different from Accellerase + NS (1:2) and Cellic + Accellerase (1:2). Very low starch recovery percentage of 56.94% was observed for the control treatment. Nevertheless, enzyme treatments either alone or combinations enhanced the starch recovery significantly in most cases, except NS (1:2, 1:4 and 1:6). Highest starch recovery (93.75%) was obtained for Cellic + NS (1:2) which was closely followed by Accellerase + NS (1:2) (91.47%) and Cellic + Accellerase (1:2) (90.55%).

In order to check whether the increased starch yield and recovery consequent to enzyme treatments resulted from the fragmentation of fiber and its appearance as fine powder along with the dry starch, the purity of starch was ascertained through chemical quantification of starch in each sample. It was found that the control starch without any treatment had the highest purity of starch viz., 96.77 per cent. Enzyme treated starch had varying degrees of purity depending on the extent of fiber contamination (Table 4). However, two treatments viz., Accellerase (1:2) and Cellic + Accellerase (1:6) had starch purity of 93.75 per cent. The treatment, Cellic + NS (1:2), which gave the highest starch yield and starch recovery gave starch having purity of 84.9 per cent.

The data on starch yield, recovery and purity of starch extracted from dry sweet potato flour presented in Table 5 indicate that the starch yield was the

highest (80%) for Cellic + Accellerase (1:2), compared to only 50.62 per cent for the control treatment. As in the case of fresh tubers, Cellic + NS (1:2) was not effective for flour. It was found that the starch recovery (%) was the highest (94.36%) for Accellerase (1:2), which was not significantly different from Cellic + Accellerase (1:2). As in the case of fresh tubers, starch purity was the highest (91.83%) for the Cellic + Accellerase (1:4) treatment, which was almost similar to control and NS treatments, as well as the Cellic (1:2 and 1:4) treatments (Table 5).

Table 4: Yield, Recovery and Purity of starch in various treatments on fresh tuber of sweet potato *

Sample	Starch yield (%)	Starch recovery (%)	Starch purity (%)
CONTROL	15.25	56.94	96.77
Accellerase 1:2	23.87	89.68	93.75
Accellerase 1:4	23.75	88.68	87.37
Accellerase 1:6	22.37	83.54	86.53
Cellic 1:2	22.75	84.95	90.00
Cellic 1:4	20.25	75.61	88.20
Cellic 1:6	23.65	88.31	85.71
NS 1:2	14.12	52.73	79.64
NS 1:4	14.90	55.64	79.64
NS 1:6	13.05	48.34	81.80
C+A 1:2	24.25	90.55	85.70
C+A 1:4	22.15	82.71	85.70
C+A 1:6	17.75	66.28	93.75

Table 4 continued

A+NS 1:2	24.50	91.47	81.80
A+NS 1:4	22.69	84.74	81.80
A+NS 1:6	23.23	86.76	78.26
C+NS 1:2	24.99	93.32	84.90
C+NS 1:4	22.59	84.38	84.90
C+NS 1:6	22.60	84.41	84.90

*Mean value from two observations

Starch content in fresh tuber is 26.78 g/100g fresh wt. and 84.93 g/100g starch on dry wt. basis.

Table 5: Yield, Recovery and Purity of starch in various treatments on flour from sweet potato*

Sample	Starch yield (%)	Starch recovery (%)	Starch purity (%)
Control	50.62	67.00	90.00
Accellerase (1:2)	71.25	94.36	86.00
Accellerase (1:4)	66.87	89.07	85.71
Accellerase (1:6)	60.62	80.29	85.71
Cellic (1:2)	71.87	95.24	90.00
Cellic (1:4)	70.00	92.76	90.00
Cellic (1:6)	67.50	89.45	87.00
NS (1:2)	77.50	91.25	90.00

Table 5 continued

NS (1:4)	75.00	88.30	90.00
NS (1:6)	75.00	88.30	90.00
C+A (1:2)	80.00	94.19	86.53
C+A (1:4)	78.75	92.72	91.83
C+A (1:6)	73.75	86.83	86.53
A+NS (1:2)	72.55	85.42	77.81
A+NS (1:4)	65.03	76.56	75.43
A+NS (1:6)	66.22	77.97	78.24
C+NS (1:2)	66.25	78.12	76.27
C+NS (1:4)	61.25	72.11	81.08
C+NS (1:6)	63.75	75.06	78.95

*Mean value from two observations

4.2 Physico-chemical properties of extracted starch:

4.2.1 Viscosity:

Viscosity studies of all starch samples from fresh tuber as well as flour were conducted. The viscosity is related to swelling volume of starch. Brookfield viscosity (cP) of suspension (4 % w/v) of enzyme extracted starch samples and native starch were measured in 120 RPM (Tables 6 and 7). The viscosity of native starch was higher than enzyme extracted starch from fresh tuber as well as flour, 36.35 and 23.95 cP respectively. Viscosity of starch samples from flour was comparatively lower than starch from fresh tuber.

Table 6: Viscosity of starch samples from fresh tuber*

Sample	Viscosity at 120 rpm (cP)
Native starch	36.35
Accellerase extracted starch (1:2)	21.4
Accellerase extracted starch (1:4)	13.07
Accellerase extracted starch (1:6)	10.40
Cellic extracted starch (1:2)	12.64
Cellic extracted starch (1:4)	25.84
Cellic extracted starch (1:6)	8.96
NS extracted starch (1:2)	7.52
NS extracted starch (1:4)	8.00
NS extracted starch (1:6)	4.16
C + A extracted starch (1:2)	20.05
C + A extracted starch (1:4)	9.97
C + A extracted starch (1:6)	17.60
C + NS extracted starch (1:2)	11.05
C+ NS extracted starch (1:4)	11.39
C + NS extracted starch (1:6)	11.58
A + NS extracted starch (1:2)	14.77
A + NS extracted starch (1:4)	11.73
A + NS extracted starch (1:6)	10.24

*Mean value from three observations

Table 7: Viscosity of starch samples from flour*

Sample	Viscosity at 120 rpm (cP)
Native	23.95
Accellerase extracted starch (1:2)	3.8
Accellerase extracted starch (1:4)	16.5
Accellerase extracted starch (1:6)	9.5
Cellic extracted starch (1:2)	10.7
Cellic extracted starch (1:4)	8.27
Cellic extracted starch (1:6)	12.46
NS extracted starch (1:2)	8.21
NS extracted starch (1:4)	5.87
NS extracted starch (1:6)	7.63
C + A extracted starch (1:2)	12.25
C + A extracted starch (1:4)	12.96
C + A extracted starch (1:6)	18.50
A + NS extracted starch (1:2)	12.42
A + NS extracted starch (1:4)	12.23
A + NS extracted starch (1:6)	9.01
C + NS extracted starch (1:2)	11.84
C + NS extracted starch (1:4)	16.05
C + NS extracted starch (1:6)	19.52

*Mean value from three observations

4.2.2 Solubility and swelling volume of extracted starch

The solubility and swelling volume of all starch samples were studied to understand the interactions between the water molecules and starch chain in the crystalline and amorphous regions during heating. The swelling volume and solubility are shown in Tables 8 and 9.

The enzyme extracted starch from various treatments exhibited different swelling volume and solubility when heated in water. Swelling volume ranged from 18.75 ml/g to 25 ml/g. Accellerase extracted and Cellic extracted starch showed higher swelling volume, while the lowest was observed in NS extracted starch.

The solubility of the starch ranged from 8.44 to 21.25 per cent, with the lowest found in Accellerase (1:2) whereas control showed highest solubility.

Table 8: Swelling volume and Solubility of sweet potato starch from fresh tuber*

Sample	S.V (ml/g)	Solubility (%)
Control	18.75	21.25
Accellerase (1:2)	25.00	8.44
Accellerase (1:4)	25.00	10.31
Accellerase (1:6)	25.00	8.62
Cellic (1:2)	25.00	12.56
Cellic (1:4)	18.75	12.22
Cellic (1:6)	18.75	12.22
NS (1:2)	15.00	15.00
NS (1:4)	15.00	13.12

Table 8 continued

NS (1:6)	15.00	17.50
C+A (1:2)	23.75	9.34
C+A (1:4)	25.00	7.87
C+A (1:6)	25.00	7.87
A+NS (1:2)	18.75	14.87
A+NS (1:4)	18.75	11.15
A+NS (1:6)	18.75	13.28
C+NS (1:2)	18.75	13.28
C+NS (1:4)	18.75	13.28
C+NS (1:6)	18.75	13.28

*Mean value from three observations

Table 9: Swelling volume and Solubility of sweet potato starch from flour*

Sample	S.V (ml/g)	Solubility (%)
Control	20.00	17.50
Accellerase (1:2)	22.50	15.31
Accellerase (1:4)	22.50	13.34
Accellerase (1:6)	22.50	13.12
Cellic (1:2)	25.00	10.50
Cellic (1:4)	25.00	10.87
Cellic (1:6)	25.00	11.62

Table 9 continued

NS (1:2)	18.75	14.61
NS (1:4)	18.75	14.61
NS (1:6)	18.75	14.08
C+A (1:2)	22.50	16.62
C+A (1:4)	22.50	15.75
C+A (1:6)	22.50	14.44
A+NS (1:2)	18.75	15.34
A+NS (1:4)	18.75	14.07
A+NS (1:6)	18.75	14.12
C+NS (1:2)	18.75	18.59
C+NS (1:2)	22.50	17.06
C+NS (1:2)	22.50	16.62

*Mean value from three observations

4.3 Thermal properties

The thermal properties of the sweet potato starches are presented in Table 10. Gelatinization temperature is the temperature at which heated starch granules undergo transition from the crystalline state to a gel. Starch gelatinization is an important parameter in starch characterization. These various thermal parameters (onset transition temperature - T_o; peak temperature -T_p; and enthalpy of gelatinization - $\Delta Hgel$) were recorded. T_o and T_p of the enzyme extracted starch samples were 76.04 $^{\circ}$C and 79.16 $^{\circ}$C respectively for Accellerase extracted starch and 76.43 $^{\circ}$C and 79.34 $^{\circ}$C respectively for Cellic extracted starch. These values were significantly higher compared to the control starch (68.5 $^{\circ}$C and 73.27 $^{\circ}$C) for T_o and T_p respectively. DSC graph was obtained as shown in Figure 2.
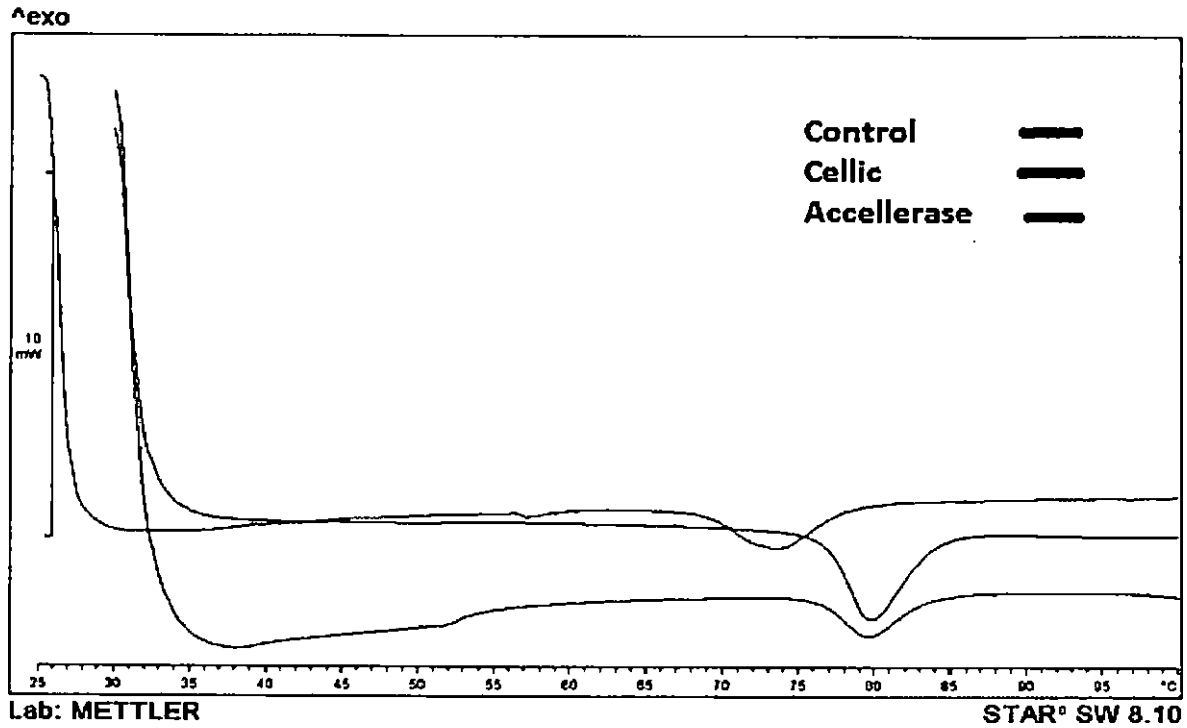
Figure 2. DSC pattern showing gelatinization temperature of starch samples

Table 10: Gelatinization temperatures and enthalpy of extracted starch*

Sample	Onset (^{o}C)	Peak (^{o}C)	Enthalpy (J/g)
Control starch	68.50	73.27	10.27
Cellic extracted starch	76.43	79.34	17.02
Accellerase extracted starch	76.04	79.16	7.44

*Mean value from three observations

4.4 Total Colour Difference and Whiteness index of starch

Colour scores of starch samples were measured using Minolta Spectrophotometer C2600. The total colour difference (ΔE) and whiteness index (WI) of starch samples are shown in Tables 11 and 12.

Colour changes due to incorporation of enzymes in extraction can be fully described using colour functions such as ΔE which indicates the degree of total colour difference (TCD) from the standard colour plate and WI which indicates degree of whiteness.

The addition of enzymes resulted in significant increase in ΔE (decrease in clearness) and decrease in whiteness index when compared with the control.

Table 11: TCD and Whiteness index of starch from fresh tuber*

Sample	TCD	WI
Control	7.967	91.601
Accellerase 1:2	14.886	84.677
Accellerase 1:4	11.543	88.120
Accellerase 1:6	10.903	88.732

Table 11 continued

Cellic 1:2	17.570	82.039
Cellic 1:4	16.472	83.137
Cellic 1:6	22.335	77.149
NS 1:2	22.826	76.783
NS 1:4	24.404	75.183
NS 1:6	25.839	73.779
C+A 1:2	16.369	83.218
C+A 1:4	15.168	84.408
C+A 1:6	19.795	79.796
C+NS 1:2	21.469	78.157
C+NS 1:4	21.857	77.725
C+NS 1:6	18.075	81.521
A+NS 1:2	20.753	78.866
A+NS 1:4	16.907	82.698
A+NS 1:6	18.040	81.552

*Mean value from three observations

Table 12: TCD and Whiteness index of starch from flour*

Sample	TCD	WI
Control	11.108	88.411
Accellerase 1:2	17.619	81.880
Accellerase 1:4	14.432	85.111

Table 12 continued

Accellerase 1:6	14.662	84.830
Cellic 1:2	16.910	82.589
Cellic 1:4	13.396	86.175
Cellic 1:6	13.210	86.278
NS 1:2	14.320	85.305
NS 1:4	15.268	84.343
NS 1:6	13.941	85.649
C+A 1:2	15.766	83.794
C+A 1:4	12.505	87.062
C+A 1:6	13.200	86.363
C+NS 1:2	14.143	85.423
C+NS 1:4	15.121	84.379
C+NS 1:6	12.290	87.278
A+NS 1:2	14.436	85.235
A+NS 1:4	10.832	84.213
A+NS 1:6	13.422	85.349

*Mean values from three observations

4.5 *In vitro* starch digestibility:

On the basis of starch yield and purity, 4 starch samples along with the control were selected and *in vitro* starch digestibility test were performed. There was significant difference in SDS, RDS and RS content as shown in Table 13.

Enzyme digestibility of raw starch is an important factor to be considered when evaluating their usefulness in diverse food applications. Enzyme mediated extraction of sweet potato starch led to increase in the resistant starch content (25-33 %) compared to 15.72 per cent in conventional method. Hence Accellerase extracted and Cellic extracted starch samples were used for pasta preparation.

Table 13 : *In vitro* starch digestibility test results of selected samples*

Sample	RDS (%)	SDS (%)	RS (%)
Control	81.23	3.05	15.72
Accellerase extracted 1:2 (from tuber)	64.35	2.70	32.95
Cellic extracted 1:2 (from tuber)	66.60	7.58	25.82
NS extracted 1:2 (from flour)	76.68	5.41	17.91
C+A extracted 1:2 (from flour)	78.21	4.48	17.31

*Mean value from three observations

RDS – Rapidly digested starch

SDS – Slowly digested starch

RS – Resistant starch

4.6 Ultrastructural studies on starch samples

Two starch samples, Accellerase extracted (1:2) and Cellic extracted (1:2) were selected based on the recovery, purity, colour, resistant starch content and other physio-chemical properties etc. The starch and residues of the selected treatments were further analyzed (Plate 4).

The micrographs of the selected starch samples and their respective residues after enzymatic extraction are shown in Plates 5 and 6. Intact as well as
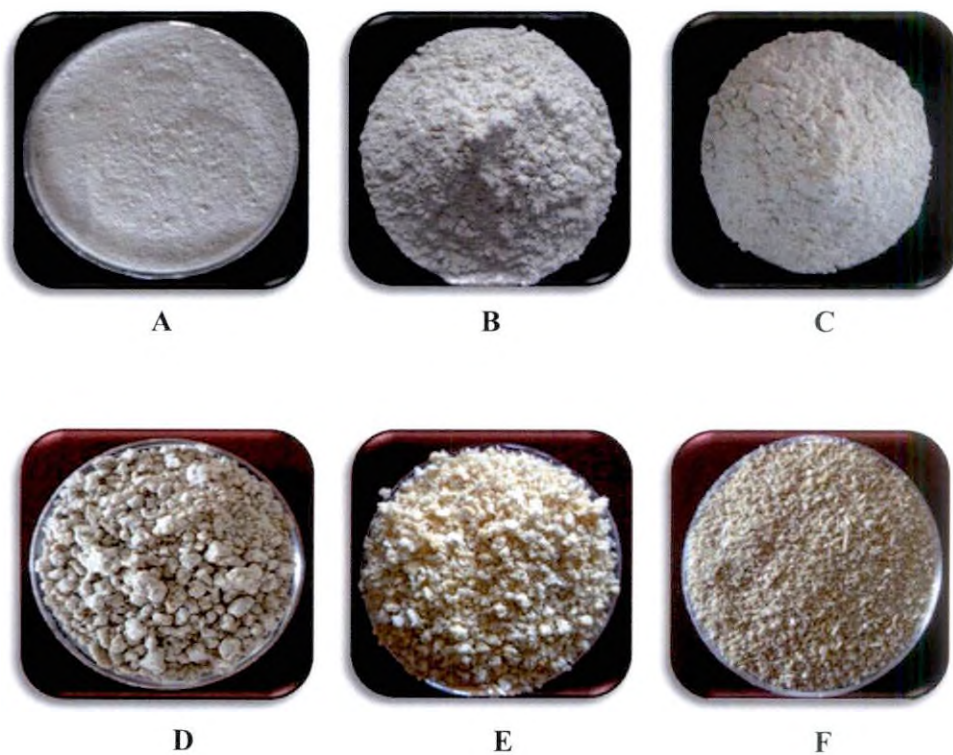
Plate 4. Starch and residue obtained from best treatments. A, Starch from conventional extraction; B, Starch from Accellerase extraction; C, Starch from Cellic extraction; D, Residue after conventional extraction of starch; E, Residue after Accellerase extraction of starch; F, Residue after Cellic extraction of starch.
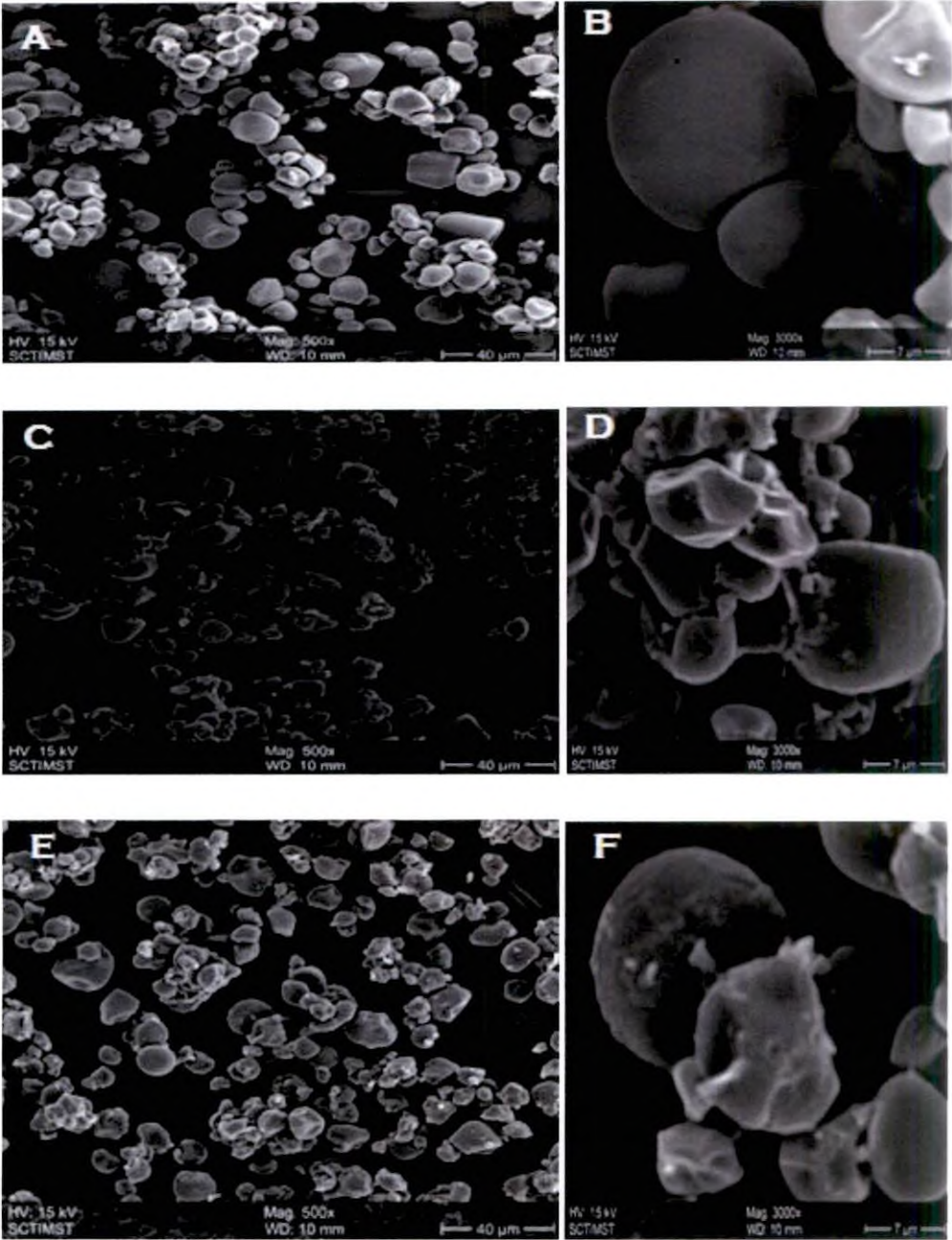
Plate 5. Scanning electron micrographs of starch granules after extraction. Figures in parentheses denote the degree of magnification. A, Native starch (× 500); B, Native starch (× 3000); C, Accellerase extracted starch (× 500); D, Accellerase extracted starch (×3000); E, Cellic extracted starch (× 500); F, Cellic extracted starch (× 3000).

Plate 6. Scanning electron micrographs of residues after the extraction of starch. Figures in parentheses denote the degree of magnification. A, Residue from control (× 500); B, Residue from control (× 100); C, Residue after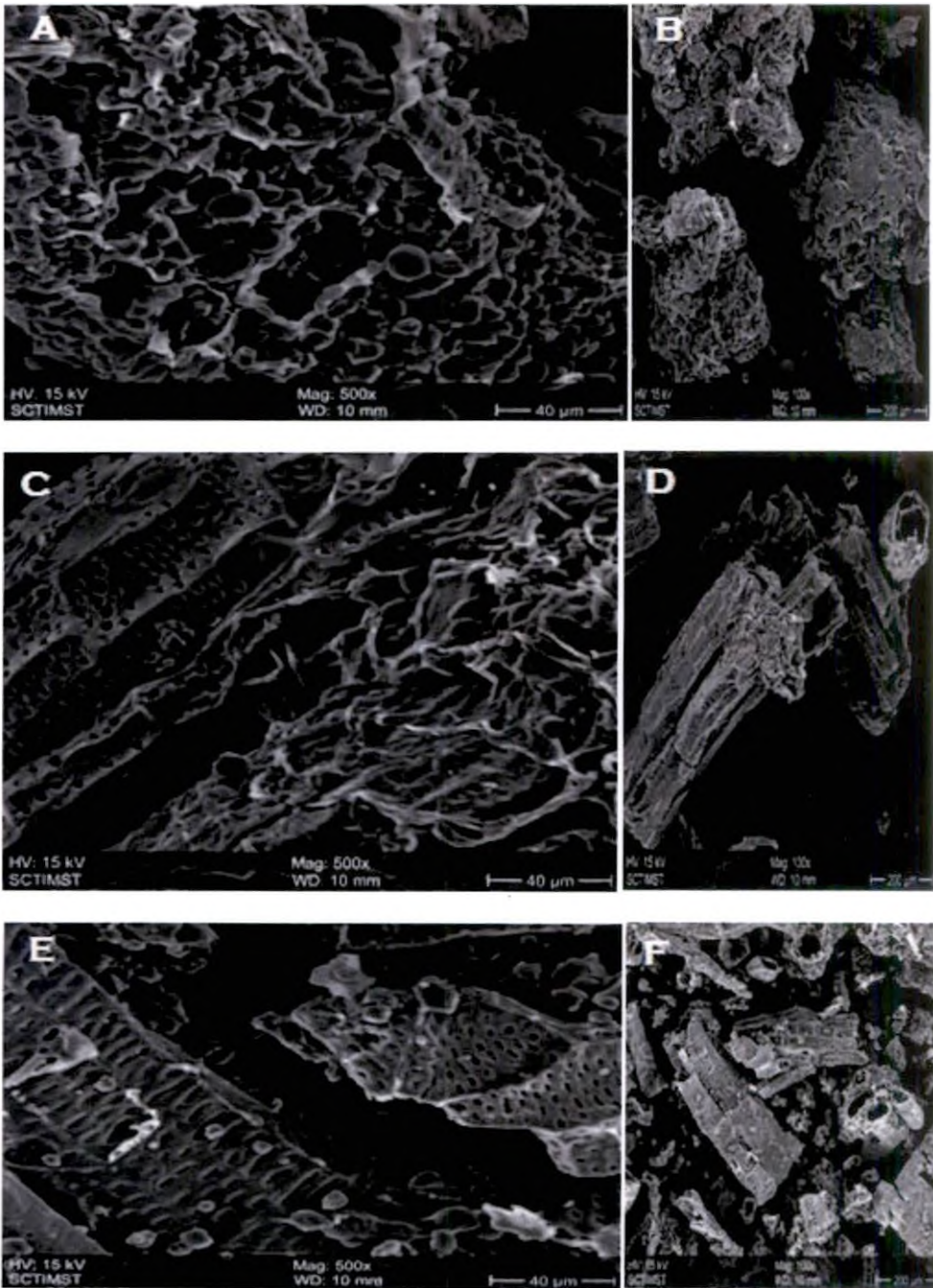 Accellerase treatment (× 500); D, Residue after Accellerase treatment (×100); E, Residue after Cellic treatment (× 500); F, Residue after Cellic treatment (× 100).

agglomerated granules of starch were visible in the SEM images of control and Accellerase or Cellic extracted starch from sweet potatoes (Plate 5). This indicated that the enzyme treatments to facilitate enhanced extraction of starch did not alter the surface morphology of the starch granules.

SEM images of the residue left after the extraction of starch by conventional method indicate the presence of lot of starch granules embedded in the cellulose fibril matrix. The low recovery of starch from sweet potatoes could be thus attributed to this entrapment. The residues from the Accellerase and Cellic treated sample (Plate 6), shows clearly the fibrillar honey comb structure, typical of celllulose and most of the starch granules had disappeared.

4.7 Cooking loss and Swelling index

The quantity of solids going into water during cooking of pasta is a determinant of pasta quality and compact textured pasta leads to lower cooking loss (Del Nobile *et al.*, 2005). The optimal cooking time (OCT) determined as per the AACC method was 2-4 min for the various pastas. Cooking loss (CL) was quantified at the OCT and it was found that most of the pasta samples had higher cooking loss (Table 14) when compared to control except for T3, containing Cellic extracted starch (Plate 7).

Table 14: Cooking loss and swelling index of pasta samples*

Sample	Cooking loss	Swelling Index
C1A	6.045	1.688
C1B	4.334	1.572
C2A	11.941	1.738
C2B	7.332	2.799

Plate 7. Pasta products developed by using starch from the best treatments and control

C1- Maida + SPS (native) + Gelatinized starch

C2- Maida + SPF + SPS (native) + Gelatinized starch

T1- Maida + SPS (Accellerase) + Gelatinized starch

T2- Maida+ SPF + SPS (Cellic) + Gelatinized starch

T3- Maida + SPS (Cellic) + Gelatinized starch

T4- Maida + SPF + SPS (Accellerase) + Gelatinized starch

Table 14 continued

T1A	8.195	1.926
T1B	7.162	3.017
T1B	7.162	3.017
T2A	13.503	1.688
T2B	16.140	2.489
T3A	4.380	1.757
T3B	8.010	2.976
T4A	13.456	1.535
T4B	17.169	2.341

*Mean value from three observations

4.8 *In vitro* starch digestibility test of cooked pasta samples

The enzyme digestibility of pasta samples prepared by using starch extracted from the treatment of Accellerase and Cellic enzyme in different formulations were measured by *in vitro* starch digestibility method as shown in Table 15. Pasta from Accellerase extracted starch had a very high resistant starch retention (73.21 %) after cooking.

Table 15: RDS, SDS and RS content in cooked Pasta samples*

SAMPLES	RDS (%)	SDS (%)	Resistant starch (%)
C1A	26.117	10.447	63.436
C1B	28.947	4.788	66.265

Table 15 continued

C2A	26.651	9.743	63.606
C2B	32.955	4.012	63.033
T1A	22.519	4.267	73.215
T1B	25.837	4.741	69.422
T2A	31.586	10.144	58.270
T2B	24.439	8.531	67.031
T3A	25.126	2.607	72.267
T3B	32.237	4.267	63.496
T4A	26.514	3.228	70.258
T4B	22.825	13.142	64.033

*Mean value from three observations

Discussion

5. DISCUSSION

Sweet potato (*Ipomoea batatas* Lam.) is cultivated throughout the tropics and warm temperate regions of the world for its starchy roots which provide nutrition, besides energy. Sweet potato roots with 18-30 per cent starch are one of the major sources for the commercial extraction of starch, but the poor extractability of starch as well as the discolouration limits the use of sweet potato starch. The starch granules present in sweet potato are embedded in cellulosic fibers and held together by pectic substances (Rahman and Rakshit, 2004). The conventional method of starch extraction is often energy intensive and requires large quantities of water. These methods results in low starch recovery from sweet potatoes and also alter the integrity of starch granules. Hence the present study was undertaken to lead to a technology to maximize the extraction of starch from sweet potatoes and to enhance the utilization prospects of sweet potato starch for food product development.

Enzyme assisted as well as conventional method of starch extraction was done and comparative studies on the properties of extracted starch were performed. Out of the six enzyme combinations, the use of Accellerase 1000 alone and Cellic C Tec 2 alone were found to be the effective enzyme treatments for the maximum extraction of starch from sweet potatoes.

Out of the three ratios *viz.,* 1:2, 1:4 and 1:6, 1:2 was found to be the effective solid to water ratio on starch extraction. The extraction was high at low addition of water since the enzyme concentration available for cellulolysis may be higher in low addition of water where as the enzymes gets diluted at higher slurry volumes and also there is chance for sedimentation of the substrate particles at higher slurry volumes due to a longer settling column available and hence the contact between the suspended enzyme and sediment substrate could be reduced, leading to poor hydrolysis of cellulose and hence reduced starch recovery (Spanheimer *et al.,* 1972; Nandan *et al.,* 2014).

The primary reason for the poor extractability of starch from sweet potatoes is due to its entrapment in the cellulose matrix, which could be concluded through SEM studies in the project. The cellulolytic enzymes *viz.,* Accellerase and Cellic were used to break the cellulose-hemicellulose matrix and deconstruct the cellulose, releasing the trapped starch. Enhanced starch recovery of 89.68 per cent and 84.95 per cent was obtained in the Accellerase and Cellic treatments, in comparison to only 56.94 per cent in the conventional method. The extracted starch had purity in the range of 90 – 93 per cent compared to 96 per cent in the conventional method (Figure 3), indicating the presence of only very small quantities of impurities especially the fragmented fiber particles. Considering the food use of the extracted starch, this will only add value to the product due to the enhancement in the dietary fiber fraction of the treated starches.
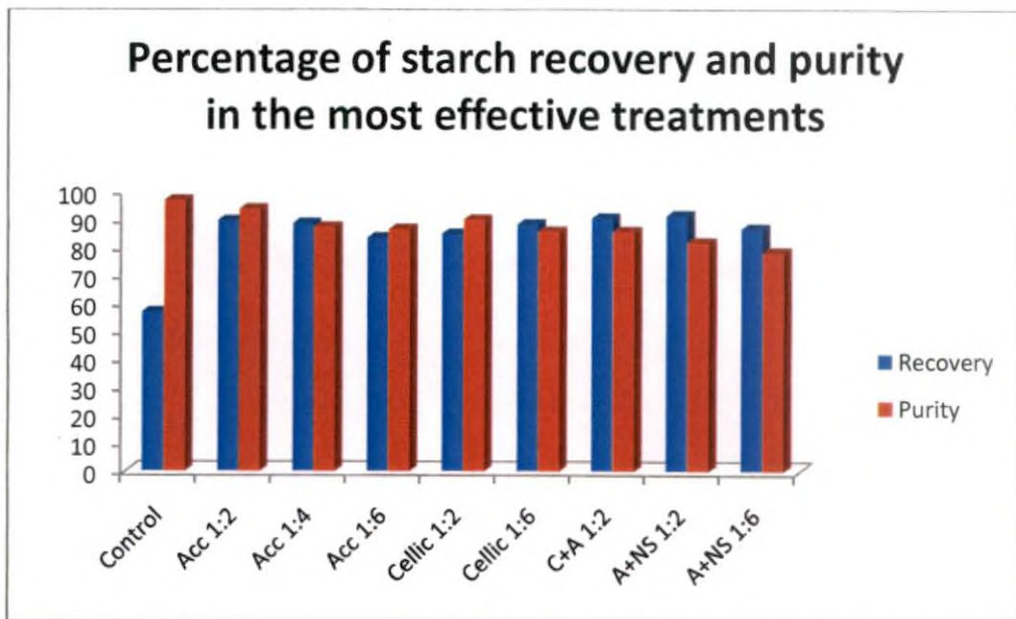
Figure 3. Percentage of starch recovery and purity in the most effective treatments

The physico-chemical properties of extracted starch samples were studied such as viscosity, swelling volume and solubility. The viscosity is much related to swelling volume of starch. Plant source, starch purity and the interaction among the starch component strongly influence the pasting properties of starches, which is easily observed from the viscosity readings.

Swelling and solubility pattern of the starches were studied to understand the interactions between the water molecules and the starch chains in the crystalline and amorphous regions during heating. The swelling volume and solubility of starches are shown in Tables 5 and 6. The starch samples exhibited different swelling volume and solubility when heated in water at 90^0 C. The swelling volume of starches from the various treatments ranged from 15 to 25 ml/g. Swelling and solubility of sweet potato starch are less than those of cassava (Moorthy, 2002). Hydrogen bonds stabilising the structure of the double helices in crystalline region of starch are broken during gelatinization and are replaced by the hydrogen bonds with water and swelling is regulated by the crystallinity of the starch (Tester and Karkalas, 1996). The conformational changes associated with complex formation lead to increased internal bonding and retarded swelling of granules. Swelling of starch is determined by the strength of associative force between starch molecules and also contributes to viscosity of pastes. The swelling of starch granule due to the breakage of intermolecular hydrogen bonds in the amorphous regions of the granules that allow irreversible and progressive water absorption (Bello-Perez *et al.*, 1999).

The colour of the starch obtained from various enzyme treatments were measured using colorimeter and it was observed that there was slight difference in whiteness of starch in comparison to control, which may be due to action of enzyme used for the extraction.

Thermal properties of starch samples from the best treatments were studied along with the control starch. Starch gelatinization plays an important role during the processing of starch based system and it considerably affects their physiochemical and functional properties. Gelatinization describes the irreversible collapse or disruption of molecular order within a starch granule when heated in excess water. The addition of enzyme retarded the onset of gelatinization relative to native starch (Table 7). Gelatinization is an energy absorbing process that can be followed by Differential Scanning Calorimetry (DSC). The decrease in gelatinization enthalpy for Accellerase extracted starch (7.44 J/g) in comparison

with the control starch (10.27 J/g) is interpreted as resulting from an exothermic effect due to the complextion of enzyme with starch molecules.

Gelatinization is primarily a property of amylopectin (Tester and Morrison, 1990) in which gelatinization temperature reflects crystalline perfection (Tester, 1997) and gelatinization enthalpy is a measure of the overall crystallinity of amylopectin (i.e. the quantity and quality of crystallites) mainly reflecting the loss of double-helical order (Cooke and Gidley, 1992). The high gelatinization temperature and enthalpy are closely related with a high degree of crystallinity caused by high amylopectin content (Gernat et $al.$, 1993; Cheetham and Tao,1998; Fujita et $al.$, 1998;, Matveev et $al.$, 2001) which was observed in Cellic extracted starch (Table 7).

The variation in the T_{peak} among the DSC results indicated that 73.27 $^\circ$C (control) had lowest value and highest in Cellic extracted starch (79.34 $^\circ$C). The DSC results also pointed out that there is considerable difference in the structure of the starch granules.

Slow gelatinization indicates much more time required to enter the granules and swell the starch granules. T_{onset} and T_{peak} had similar trends. Gelatinization enthalpy (ΔH) which indicates the crystallinity of the amylopectin fractions was highest for Cellic extracted starch (17.02 J/g) and lowest for Accellerse extracted starch (7.44 J/g), showing that big values had very strong crystalline structure compared to others. This is clear from the graph (Figure 1).

According to Vasanthan et $al.$ (1999), starches with higher gelatinization transition temperatures (T_o and T_p) and enthalpy would require higher heat of solubilization, as in Cellic extracted starch. ΔH_{gel} mainly reflects the loss of molecular order within the internal structure (Cooke and Gidley, 1992). Furthermore, variation in the gelatinization properties of the starches could be attributed to various factors including; mineral composition, proportion of large and small granules and the molecular architecture of the crystalline region of starches (Kaur et $al.$, 2007).

Enzyme mediated extraction of sweet potato starch led to increase in the resistant starch content (25- 33 %) compared to 15.72 per cent in conventional method (Figure 4), which is probably due to fragmented fibers.
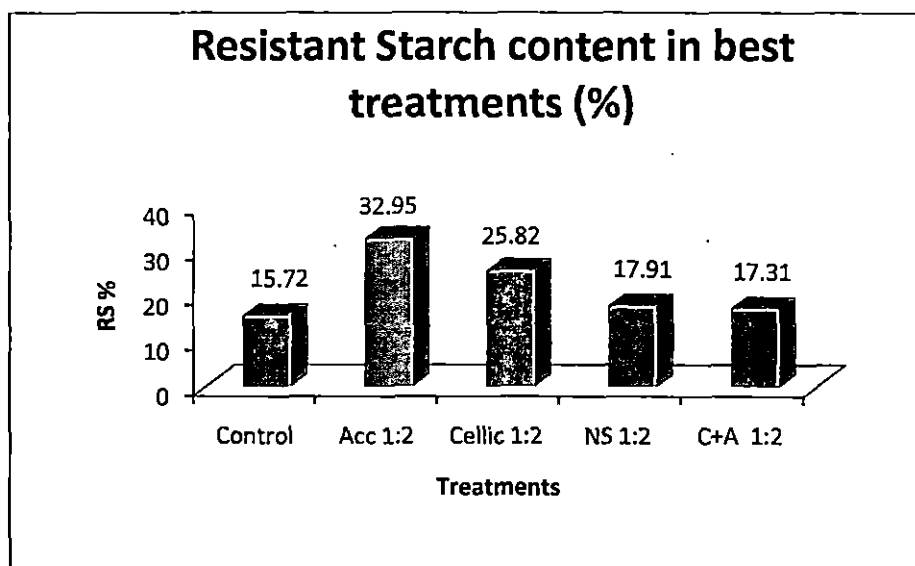

Figure 4. Resistant starch content in best treatments

In the Ultrastructural studies conducted for the best starch samples, intact as well as agglomerated granules of starch were visible in the SEM pictures of native as well as Accellerase/Cellic extracted starch from sweet potatoes. This indicated that the enzyme treatments to facilitate enhanced extraction of starch did not alter the surface morphology of most of the starch granules.

SEM pictures of the residue left after the extraction of starch by conventional method, indicated the presence of lot of starch granules, embedded in the cellulose fibril matrix. The low recovery of starch from sweet potatoes could be thus attributed to this entrapment.

The residues from the Accellerase and Cellic treated sample showed clearly the fibrillar honey comb structure, typical of cellulose and most of the starch
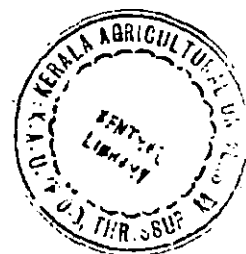
granules had disappeared. Out of the two enzymes, Accellersae was performing the best and this was evident from the SEM pictures also.

Excellent pasta products with good cooking characteristics and appearance could be prepared with the extracted starch. Pasta from the Accellerase extracted starch had a very high RS content (73.20 %) in cooked product and hence has high value as a food for diabetic/obese people.

Summary

6. SUMMARY

The study entitled "Enhancing sweet potato (*Ipomoea batatas* Lam.) starch extractability using enzymes" was conducted at the Division of Crop Utilization, CTCRI during 2013-2014. The objective of the study was to maximize the extraction of starch from sweet potatoes and also to enhance the utilization prospects of sweet potato starch for food product development. The salient findings of the study are summarized below.

Out of the six enzyme combinations, the use of Accellerase 1000 alone and Cellic C Tec 2 alone were found to be the effective enzyme treatments for the maximum extraction of starch from sweet potatoes. The extracted starch had purity in the range of 90 – 93 per cent compared to 96 per cent in the conventional method. Among the three ratios *viz.,* 1:2, 1:4 and 1:6, 1:2 was found to be the effective solid to water ratio on starch extraction.

Gelatinization enthalpy (ΔH) which indicates the crystallinity of the amylopectin fractions was highest for Cellic extracted starch (17.02 J/g) and lowest for Accellerse extracted starch (7.44 J/g), showing that big values had very strong crystalline structure compared to others.

Enzyme mediated extraction of sweet potato starch led to increase in the resistant starch content (25- 33 %) compared to 15.72 per cent in conventional method of extraction, which is probably due to fragmented fibers.

The ultrastructural studies indicated that the enzyme treatments to facilitate enhanced extraction of starch did not alter the surface morphology of most of the starch granules. The residues from the Accellerase and Cellic treated sample showed clearly the fibrillar honey comb structure.

Excellent pasta products with good cooking characteristics and appearance could be prepared with the extracted starch. Pasta from the Accellerase extracted

starch had a very high RS content (73.20 %) in cooked product and hence has high value as a food for diabetic/obese people.

The technology seems to be cost effective as only small quantities of enzymes were used and there is a significantly high increase in the yield of starch. The entrapment of starch in the cellulose-hemicellulose matrix being the major impediment in the use of sweet potatoes as raw material for starch extraction, enzyme mediated release of the trapped starch is only alternative. There are also added advantages that enzyme treatment is relatively safe from the consumption point of view, as no acids, bases or hazardous chemicals are used and also that the extracted starch had more functional value as a food additive.

Since the enzyme extracted starch from sweet potato had high resistant starch content compared to the native starch and hence this could have potential applications in the development of low calorie food products for targeted groups of population. Possibility exists to expand the study to more varieties and to explore the commercial possibility for industrial extraction of starch from sweet potatoes, which hitherto does not exists in India.

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Appendices

APPENDIX I

HCl- KCl buffer

Solution A – 0.2 M KCl

Solution B – 0.2 M HCl

50 ml Solution A + 33.3 ml Solution B diluted to 200 ml

pH – 1.5

APPENDIX II

Sodium phosphate buffer

Solution A – 0.2 M $NaH_2PO_4.2H_2O$

Solution B – 0.2 M $Na_2HPO_4.2H_2O$

45 ml Solution A + 55 ml Solution B, made up to 1 litre, add 17.85 g NaCl.

pH – 6.9

APPENDIX III

Sodium Acetate buffer

Solution A – 0.2 M Acetic acid

Solution B - 0.2 M Sodium acetate

20 ml Solution A + 30 ml Solution B, made up to 100 ml.

pH – 4.8

Abstract

ENHANCING SWEET POTATO (*Ipomoea batatas* Lam.) STARCH EXTRACTABILITY USING ENZYMES

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Abstract of the
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ABSTRACT

The study entitled "Enhancing sweet potato (*Ipomoea batatas* Lam.) starch extractability using enzymes" was conducted at the Division of Crop Utilization, CTCRI during 2013-2014, with an objective to lead to a technology to maximize the extraction of starch from sweet potatoes and also to enhance the utilization prospects of sweet potato starch for food product development.

Sweet potato (*Ipomoea batatas* Lam.) is cultivated throughout the tropics and warm temperate regions of the world for its starchy roots which can provide nutrition, besides energy. The edible tuberous root is either long and tapered, ovoid or round with a skin colour ranging from white, brown, purple or red and the flesh colour ranging from white, pale cream, orange or purple. Sweet potato starch finds commercial application in the production of noodles, sugar syrups, thickener etc. However, poor extractability of starch as well as the discolouration limits the use of sweet potato starch.

Enzyme assisted as well as conventional method of starch extraction was done and comparative studies on the properties of extracted starch were performed. The distinctive feature of enzymes is that they can operate at mild conditions, at atmospheric pressure and at reasonably low or ambient temperatures. Mainly six enzyme combinations were tried for the extraction of the starch. Out of the six enzyme combinations, the use of Accellerase 1000 alone and Cellic C Tec 2 alone were found to be the effective enzyme treatment for the maximum extraction of starch from sweet potatoes. Enhanced starch recovery of 89.68 % and 84.95 % was obtained in the Accellerase and Cellic treatments, in comparison to only 56.94 % in the conventional method. The extracted starch had purity in the range of 90 – 93 % compared to 96 % in the conventional method.

The physico-chemical properties of extracted starch samples were studied such as viscosity, swelling volume and solubility. The viscosity is much related to swelling volume of starch. The colour of the starch obtained from various treatments were measured using colorimeter and it was observed that there was

slight difference in whiteness of starch, which may be due to action of enzyme used for the extraction.

Thermal properties of starch samples from the best treatments were also studied along with the control starch. Starch gelatinization plays an important role during the processing of starch based system and it considerably affects their physiochemical and functional properties. The addition of enzyme retarded the onset of gelatinization relative to native starch.

Ultrastructural studies were conducted for starch samples as well as their residues. This indicated that enzyme treatments to facilitate enhanced extraction of starch did not alter the surface morphology of most of the starch granules and the residues from the best treatment clearly displayed the fibrillar honey comb structure, typical of cellulose.

Enzyme mediated extraction of sweet potato starch led to increase in the resistant starch content (25- 33 %) compared to 15.72 % in conventional method, which is probably due to fragmented fibers. Excellent pasta products with good cooking characteristics and appearance could be prepared with the extracted starch. Pasta from the Accellerase extracted starch had a very high RS content (73.20 %) in cooked product and hence has high value as a food for diabetic/obese people.