DEVELOPMENT OF BIODEGRADABLE FILMS FROM ENZYMATICALLY MODIFIED CASSAVA STARCH

by

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(2010-09-111)

THESIS

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KERALA, INDIA

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DECLARATION

I hereby declare that this thesis entitled **"DEVELOPMENT OF BIODEGRADABLE FILMS FROM ENZYMATICALLY MODIFIED CASSAVA STARCH"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associate ship, fellowship or other similar title, of any other university or society

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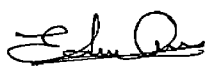

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LIST OF ABBREVIATIONS AND SYMBOLS USED

DE	Dextrose equivalent
HPLC	High pressure liquid chromatography
Fw	Fresh weight
Dw	Dry weight.
%	Per cent
µm	Micro meter
µl	Micro litre
@	At the rate of
°C	Degree Celsius
cm	Centimeter
et al.	And other co workers
Fig.	Figure
g	Gram
g^{-1}	Per gram
mg	Milli gram
ml	Millilitre
sec	Seconds
min	Minutes
R_f	Retention factor
η	viscosity
RB	Rose Bengal Agar medium
KK	Ken Knight medium
NA	Nutrient Agar medium

INTRODUCTION

1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz), popularly known as tapioca is the richest source of carbohydrates among the major root and tuber crops and forms a primary and staple food in many parts of tropical and subtropical countries Being a crop with adaptability to wide range of soil, climate and environment, requiring minimum input and care for its growth, cassava can be very well fitted into the different cropping systems prevailing in India In 2013, it was estimated that cassava covered in an area of 20 39 million hectare with a total harvest of 276 76 million tonnes globally. India has a leading position in global cassava scenario due to high productivity level of 34 96 tonnes per hectare from an area of 0 207 million hectares compared to world average of only about 13 57 tonnes per hectare (FAOSTAT, 2015) In India, Kerala, Tamil Nadu and Andhra Pradesh lead in the production of cassava

Cassava tubers are highly perishable and hence cannot be stored for more than 2-3 days after harvest Hence to avoid heavy post harvest losses, it is necessary to process them immediately The cassava tubers are generally used as food in the form of fresh vegetables after boiling and by producing value added products from its flours and starch It has a high amount of starch varying from 20-40 percent and with its unique physico-chemical and functional properties, cassava starch finds wide applications in food, paper, textile, adhesives etc (Moorthy, 2001) It forms an important ingredient in formulated food system functioning as a gelling and thickening agent, stabilizer and texture modifier Cassava starch mainly consist of two components amylose and amylopectin where amylose is a linear polymer and amylopectin is a branched polymer Starch when heated in the presence of excess water undergoes gelatinization which involves granules swelling, amylose leaching and amylopectin fusion and upon cooling undergoes retrogradation and forms paste or gel

There is a growing concern on the environmental issues over the waste disposal linked with petroleum based polymeric packaging materials. The wide application of such packaging material in food packaging sector is attributed to their low cost, high strength and elongation, gas barrier properties, light in weight and water resistance, convenience in handling, strong and economical, but they are biologically not degradable The excessive amount of disposable used in modern society has led to serious demand for biodegradable plastic materials made from renewable sources Protein, carbohydrate and lipids in different combinations and compositions are an attractive alternative for synthetic packaging material with no contribution to the environmental pollution while being obtained from renewable sources with low cost (Bengtsson *et al*, 2003, Lu *et al*, 2005, Tharanathan, 2003)

Starch and starch derivatives are considered to be promising candidates for the development of biopolymer based environment friendly packaging materials mainly due to their renewability, abundance, low cost, film forming properties, bland taste and colour, low solubility, biodegradability etc (Guohua *et al*, 2006, Tang *et al*, 2008, Yun *et al*, 2008) In recent years, there is a growing interest in developing starch based biodegradable polymers to replace synthetic non-degradable materials. Research has been performed concerning the use of these films as a way of improving shelf life of food However, starch has severe limitations because of its solubility and poor water-resistance, making starch products very sensitive to the relative humidity at which they are stored and used.

Cassava starch when heated in excess water forms a gel or paste depending on the water addition and they form a better candidate for forming biodegradable films But pure starch film by itself is brittle due to strong intermolecular and intra molecular hydrogen bonding in amylose and amylopectin chains and needs plasticization to make it flexible (Averous and Boquillon, 2004) Plasticizers are added along with the cassava starch film solution because it increases the flexibility

due to their ability to reduce internal hydrogen bond Generally used plasticizers are glycerol, sorbitol and polyol.

Several studies have been carried out on film preparation from tropical tuber starches (Chang *et al*, 2000, Mali *et al*, 2002, Mali *et al*, 2005, Bangyekan *et al*, 2006) The studies conducted by different authors on the biodegradable films made from cassava starch added with different plasticisers, hydrocolloids, cross linking agents etc showed improved physico-mechanical and functional properties (Cereda *et al*, 1995, Mali *et al*, 2005b, Alves *et al*, 2007, Veiga-Santos *et al*, 2007 and Muller *et al*., 2008) Starch films can be made from the native starch or its components viz, amylose and amylopectin by various techniques such as thermoplastic processing and solution casting (Rindlav-Westling *et al*, 2002) Preponderance of amylose in starches gives stronger films Branched structure of amylopectin generally leads to films with different mechanical properties, such as decreased tensile stress (Tharanathan, 2003)

Extensive works have been conducted to improve the mechanical and hydrophobic properties of the native starch based biodegradable films The addition of modified starches in the preparation of bio composites will definitely improve the physico-mechanical and water resistance of the biodegradable packaging material On modification, cassava starch can undergo a change in its mechanical, physical, functional and chemical properties and thereby is one of the best source of starch to produce biodegradable films Starches can be modified by physical, chemical and enzymatic methods to customise the starch properties Physical methods are expensive and whereas chemical methods are highly toxic to the environment and to the person handling whereas enzymatic methods are ecofriendly The main enzymes used in starch industries are α-amylase, isoamylase, pullulanase, cyclodextrin glycosyl transferase and isomerases α- amylase catalyze the cleavage of α-1,4-glycosidic linkages without affecting α- 1,6 linkages whereas pullulanase which is a

debranching enzyme hydrolyzes α-1,6 glycosidic bonds to yields mainly maltose and maltotrioses

Extensive works had been carried out on the use of modified starches for the preparation of biodegradable films (Ikawa *et al*, 1978, Ghosh *et al*, 2010, Lomako *et al*, 1993, Chen *et al*,1996, Maarel *et al*, 2002, Fang *et al*, 2005, Akoh *et al*, 2007) Films made from chemically modified starch *viz*, etherification, esterification and cross linking have improved physico-mechanical and reduced sorption properties and the properties depends on the type of modified starch, extent of their incorporation and the glycerol content (Sajeev *et al*, 2013a) Anjana *et al* (2013) developed biodegradable films from hydroxypropylated cassava starch and nanokaoline clay composites and analyzed their physico-mechanical and hygroscopic properties

Enzymatic modification, brings about changes in viscosity and gel strength of the gelatinised starch suspension. The selective enzymatic hydrolysis of starch produces range of products with varying chain length and dextrose equivalent enabling the production of a variety of end use specific products Hence, enzymatically modified starches offer better scope for the production of biodegradable films with desirable properties Very little information is available on the rheology of the enzymatically modified cassava starch and the development and physico-mechanical properties of the enzymatically modified cassava starch based biodegradable films Hence this study was undertaken with the following objectives

1 Rheological characterization of the cassava starch modified with liquefying and debranching enzyme
2 Development of biodegradable film from enzymatically modified cassava starch
3. Physico-mechanical, hygroscopic and functional properties of films made from enzymatically modified cassava starch
4 Assessment of biodegradability of the films made from enzymatically modified cassava starch

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

This chapter broadly divided into different sections viz, starch and its industrial applications, cassava starch and its production process, rheological properties, modification of starch, enzymatic modification of starch, biodegradable film form starch and biodegradability studies

2 1 STARCH AND ITS INDUSTRIAL APPLICATIONS

Starch is one of the most abundant constituents of the world's crops and the majority of starch produced is consumed directly as food or feed, but a significant proportion is also directed into industrial applications (Jobling, 2004, Marz, 2006) Starch-containing crops form an important constituent of the human diet and a large proportion of the food consumed by the world's population originates from them (Maarel *et al*, 2002) The major industrial sources of starch are maize, tapioca, potato and wheat Starch is one of the most abundant, inexpensive and commonly used natural polysaccharides and in both its native and modified forms, has been playing important roles in the food industry (Narayanan and Shanmugasundaram, 1967, Guilbert and Gontard, 2005, Akhila *et al*, 2008)

Starch is consisted of two fractions amylose which is made up of essentially α-(1-4) D-glucopyranosyl units and amylopectin which is made up of a large number of short chains linked together at their reducing end side by α-(1- 6) linkage (Arvanitoyannis and Biliaderis, 1998). Amylopectin is the major component of most starches, and consists of a large number of shorter chains that are bound together at their reducing end side by a α-1, 6 linkage (Hoseney, 1986) Normal starch contains about 25-30 percent amylose, waxy starch 0-13 percent and high amylose starch more than 35 percent (Mac Gregor and Fincher, 1993) Depending on the nature of the substituents and on the degree of substitution, the properties of modified starch can be varied in an extensive way (Light, 1990)

Starch shows various characteristics like solubility, stability of paste, moisture, emulsifiability, viscosity, water retention, and film property after modifying (or processing) (Akhila *et al* , 2008) Native starches produce weak-bodied, cohesive, rubbery pastes when heated and undesirable gels when the pastes are cooled (Adzahan, 2002) The activity of the enzymes involved in starch biosynthesis may be responsible for the variation in amylose content among the various starches (Krossmann and Lloyd, 2000) The crystalline composition of starch consists of around 15-45% of the starch granules and the crystallinity is exclusively associated with the amylopectin component, while the amorphous regions mainly represent amylose (Zobel, 1988a, 1988b) Gelatinization in the narrowest sense is the thermal disordering of crystalline structures in native starch granules, but in the broader sense it includes related events such as swelling of the granules and leaching of soluble polysaccharides (Atwell *et al* , 1988)

2 2 INDUSTRIAL APPLICATIONS OF STARCH

Starches and their derivatives have been used to modify physical properties of food products which include texture, viscosity, gel formation, adhesion, binding, moisture retention, product homogeneity and film formation (Thomas and Atwell, 1997, Liu, 2005) Starch is used mainly in soups, sauces and gravies, bakery products, dairy confectionery, snacks, batters and coatings and meat products (Davies, 1995) Non-food applications of starch include in the field of pharmaceuticals, textiles, alcohol based fuels and adhesives New uses of starch include low-calorie substitutes, biodegradable packaging materials, thin films and thermoplastic materials with improved thermal and mechanical properties (Arvanitoyannis and Biliaderis, 1998) Starch films and coatings have been used for various food and pharmaceutical applications Starch can be modified chemically, physically, or enzymatically to suit various needs (Bemiller, 1997) Several studies reported the use of starches from different sources to prepare films and coatings with different properties, and have indicated that these carbohydrates are promising

materials in this regard (Averous *et al*, 2001, Larotonda *et al*, 2005, Mali *et al*, 2005b)

In addition to being a major constituent of human diet, starch also functions as an excellent raw material for modifying the texture and consistency of foods owing to its ability to form visco-elastic gels when heated in water Starch derived products have considerable applications in non food areas and are detailed in Table 1 (Ellis *et al*, 1998). Koch *et al*, (1993) have remarked that it is possible to produce new generation detergents in which surfactant builders, co builders and bleaching activators could all be derived from starch. Starch could be an alternate material to meet the high demand for petroleum based chemicals in the non food industries like polymer synthesis Polyamides are one of the most important synthetic polymers and methods had already been developed to synthesis carbohydrate or starch derived polymers (Thiem and Bachmann, 1994) Emerging uses of starch included as low fat calorie substitute, biodegradable plastic (Wool, 1994, Arvanitoyannis *et al*, 1994, Griffin, 1994, Arvanitoyannis *et al*, 1997) and edible films (Goheen and Wool, 1991, Arvanitoyannis *et al*, 1996, Psomiadou *et al*, 1996)

2 3 WET MILLING PROCESS FOR CASSAVA STARCH PRODUCTION

Cassava is a tropical crop grown for its starch containing tuberous roots and is also known commonly as tapioca, continues to be a crop of food security for the millions of people especially in the developing countries of the globe (Edison, 2006). Cassava is considered as a subsistence food for over 500 million people in tropical areas, and its world production in 2013 was 276 76 million tonnes from 20.39 million hectares and India has a leading position in global cassava scenario due to high productivity level of 34 96 tonnes per hectare compared to world average of only about 13 57 tonnes per hectares (FAOSTAT,2015) Indonesia, Thailand, Vietnam and India are the major countries growing cassava in Asia and India acquires

significance in the global cassava scenario due to its highest productivity in the world

The starch granules are usually locked up in cells together with other constituents and have to be separated from all other constituents to get the pure form of starch. Manufacture of cassava starch is carried out in three types of establishments *viz*, cottage industries (50-60 kg/day/man), small scale industries (40-50 t/day) and large scale industries (100 t/day and above) (Balagopalan *et al*, 1987) Processing of tubers by wet milling is chiefly employed for the extraction of starch in all types of cassava industries irrespective of their production capacity.

Table 1. Industrial applications of starch

Industry	Applications
Adhesives	Adhesive products
Agrochemical	Mulches, pesticide delivery, seed coating
Cosmetics	Face and talcum powders
Detergents	Surfactants, bleaching agents and bleaching activators
Food	Viscosity modifier and glazing agents
Medical	Plasma extender/replacer, transplant organ preservation, absorbant sanitary products
Oil drilling	Viscosity modifier
Paper and Board	Binding, sizing, coating
Pharmaceuticals	Diluent, binder, drug deliver
Plastics	Biodegradable filler
Purification	Flocculant
Textile	Sizing, finishing, printing and fire resistance

The various unit operations involved in the wet milling process for extraction of starch is given in Fig 1

a. Washing

It is done to remove and separate all adhering soil as well as protective epidermis to get colourless (white) pure starch Roots are washed manually in tanks with water or by using mechanical washers worked on the principle of mechanical scrubbing

b. Peeling

It is carried out with the help of special knives designed for peeling to minimize the loss of edible fleshy part of the tubers To get a good quality product, washing is done before and after peeling of tubers

c. Rasping

The tubers are turned into pulp or mash using a rasper which destroys the cellular structure, rupture the cell walls and release the starch as discrete, undamaged granules from other insoluble matters Rasper consists of a solid wooden roller around which a punched metal sheet with its protrusions facing outside is nailed The drum rotates inside a housing with a hopper at the top for feeding the tubers and with a perforated metallic plate underneath, through which the rasped pulp passes into the sump below Water is continuously added during rasping In this method, 70-90 percent rasping effect is obtained during first rasping operation itself

d. Sieving or Screening

It is done by rinsing the pulp mass on screens by sprinkling of water, continuously to it The pulp is pumped into a series of diminishing mesh sizes The sieving is completed when the water running out of the screen is partially clear The starch milk obtained after screening is collected in tanks and from where it is channelled for sedimentation. Residual pulp remaining on the screen after second pass is taken for drying in the sun and is used as an ingredient in cattle feed

e. Settling or sedimentation

It includes a series of operations performed to separate the pure starch from other contaminants Settling process should be completed as quickly as possible to prevent chemical, enzymatic and microbial reactions Settling tanks or tables are used for this purpose Starch milk is allowed to settle for a period of about 8-12 hours in the settling tanks whose capacity varies with the processing capacity of the factory Starch settles at the bottom of the tank and the supernatant fruit water is let off through the outlets provided at different depths of the tank The upper layer of settled starch contains many impurities and is scrapped off and rejected

f. Tabling

It is a semi continuous settling process followed to reduce the time of contact between the starch and fruit water. The settling table consists of successive sets of slightly inclined channels or troughs The starch milk is allowed to flow along the trough and when sufficient starch settles at the base of the channel, the flow of starch milk is temporarily stopped and the starch is removed manually

g. Drying

Starch cake so settled contains 35-40 percent moisture It is scooped out and broken into small lumps and spread in thin layer on a large clean open area for sun drying to reduce the moisture content to 15-20 percent

h. Bolting

Dried cassava starch consisting of hard agglomerates is pulverized or milled into powder form, screened to remove the foreign particles and ensure lump free uniform product The starch powder so obtained through the bolting process is stored in a dry place and packed in polyethylene or gunny bags for marketing storage

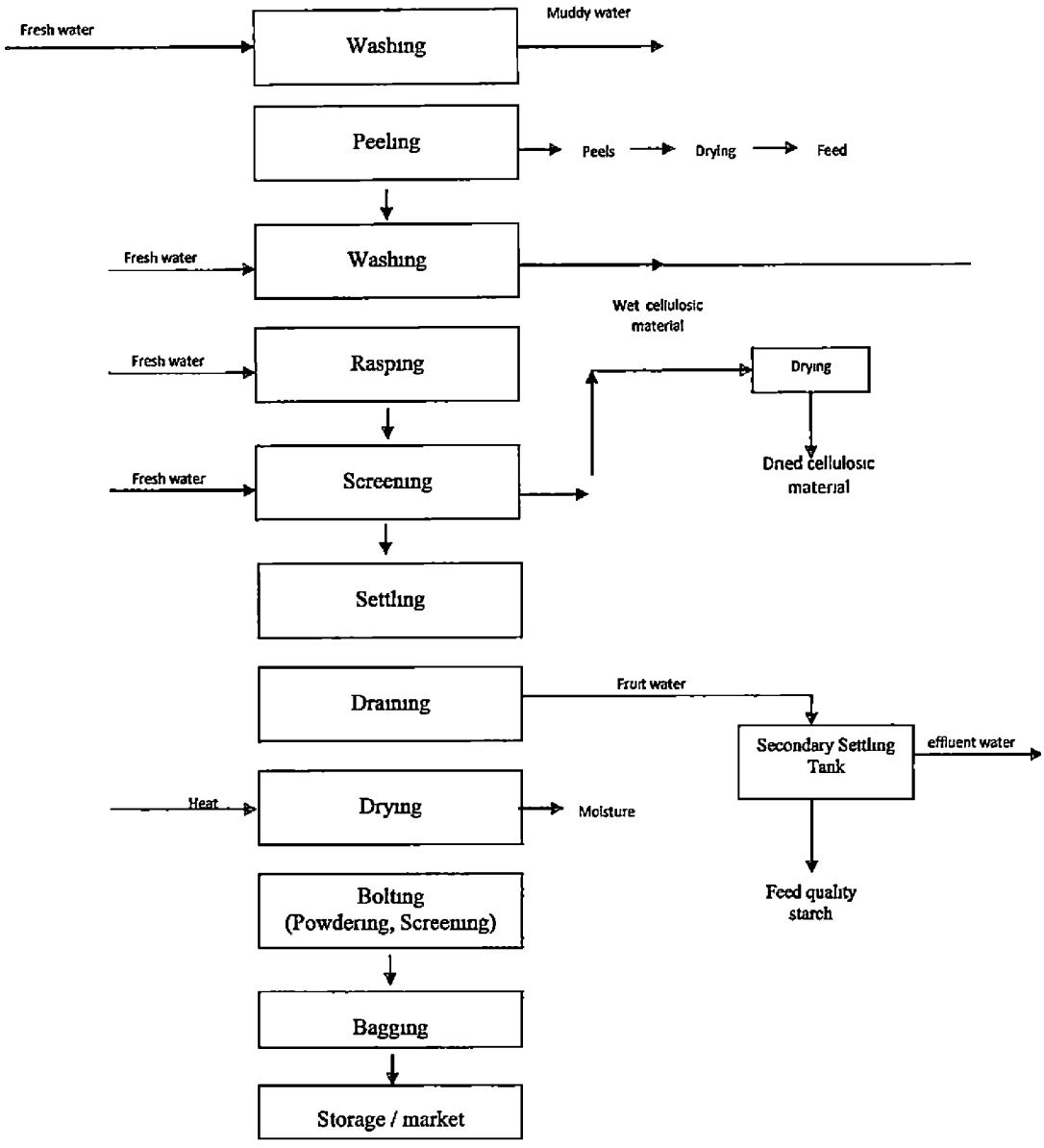


Fig.1. Wet milling process for the extraction of starch from cassava tubers

2 4 STRUCTURE OF CASSAVA STARCH

Cassava starch consists of two types of molecules amylose a substantially linear polymer and amylopectin a highly branched polymer with very high molecular weight. Cassava's role as a traditional human food is changing to an efficient industrial crop in some parts of Africa, for instance in Nigeria (Nweke, 2004) and many parts in Asia and Latin America. Cassava starch can be used for the production of sweeteners which include maltose, glucose syrup, glucose and fructose, which can be further processed into various oligosaccharides (Shuren, 2001). The starch content of cassava roots ranges from 65-91 percent of its total root dry weight depending on the cultivar (Sanchez *et al* , 2008) The high starch content makes cassava a desirable energy source both for human consumption and industrial biofuel applications (Balat and Balat, 2009, Schmitz and Kavallari, 2009) Cassava root is very rich in starch and contains considerable amounts of calcium, dietary fiber, iron, manganese, phosphorous, potassium, vitamin B6 and vitamin C Cassava flour does not contain gluten, an allergenic protein found in wheat, barley, oats and rye It is also known as tapioca flour which can be used by gluten intolerant people to replace wheat flour (Sirituna *et al* , 2004) Cassava is used to manufacture many chemical products such as citric acid and is also used in papermaking, food processing, lubricants, adhesives and textiles (Nweke *et al* , 2002)

2 5 RHEOLOGICAL PROPERTIES

Cassava starch forms an important ingredient in formulated food system functioning as a gelling and thickening agent, stabilizer and texture modifier Starch when heated in the presence of excess water undergoes gelatinization which involves granule swelling, amylose leaching and amylopectin fusion and upon cooling due to retrogradation, solubilized starch forms a viscous dispersion or paste or gel depending on the temperature of processing and concentration of dispersion, varieties, harvesting age and growth season (Rickard *et al* , 1991; Asaoka *et al* , 1991; 1992, Defloor *et al* , 1998, Lagarigue and Alvarez, 2001, Moorthy, 2001, Fourmann *et al* ,

2003) The viscoelastic behavior of starchy food systems is characterized by dynamic rheological tests and can be evaluated by different models (Montalvo *et al*, 1996, Steffe, 1996, Subramanian and Gunasekharan, 1997, Cortes *et al*, 1999, Rao, 1999, Ak and Gunesekharan, 2001, Acharya *et al*, 2004, Cuppola *et al*, 2004, Ortega-Ojeda *et al*, 2004a, b, 2005, Nishinari, 2007)

Starch when heated in presence of excess water resulted in granule swelling and disruption as well as leaching of soluble amylase component called as gelatinization The viscous mass obtained by the process of gelatinization consists of a continuous phase of basically solubilized amylose and discontinuous phase of remnant granules of amylopectin (Zobel and Stephen, 1995) The cooling of such hot paste results in a viscoelastic gel Dynamic rheometric experiments are often used in food process engineering to evaluate the viscoelastic behavior of food materials (Wu *et al*, 2001, Lindahl and Ehasson, 1986, Chen *et al*, 1996, Rao, 1999)

A gel like material shows distinct behavior that is different from liquid or concentrated solutions when subject to a frequency sweep in a rheometer. With a dilute solution, the loss modulus (G") is larger than the storage modulus (G') over the entire frequency range, but approach each other at higher frequencies For a gel, G' is significantly larger than G" throughout the frequency range, for a true gel G' is almost independent of frequency, while higher dependency of both modulus on frequency exists for a weak gel (Steffe, 1996) For a concentrated solution G" is larger than G' at lower frequencies until a cross over (or critical) frequency is reached At frequencies higher than the cross over frequency, G' is higher than G" (Rao, 1999).

The rheological behaviour of starch in its native or modified form is of special importance when they are used to modify textural attributes It is also well recognized that rheological properties play a role in process design, evaluation and modeling These properties are sometimes measured as an indicator of product quality (eg indication of total solids or change in molecular size) Rheological data

are required for calculation in any process involving fluid flow and play an important role in the analyses of flow conditions in food processes such as pasteurization, evaporation, drying and aseptic processing

Dynamic rheological data over a frequency range of 0 63-62 9 s showed that 10% corn starch and soybean protein isolate dispersions behaved like a weak gel and the storage modulus of the corn starch dispersion decreased with increase in proportion of protein.

Detailed studies had been carried out for analyzing and modeling the dynamic rheological properties of starches from selected cassava varieties by Sajeev *et al.*, (2009) The dynamic spectra of the gelated starch showed the characteristics of concentrated biopolymer dispersion and described using Maxwell and power law model and the results showed that rheological properties varied considerably among the varieties and besides the physico-chemical properties, interaction between them and structural make up of the tuber parenchyma had a great influence on rheological properties

Sajeev *et al.*, (2010) studied the effect of incorporating hydrocolloids and plasticizer on the viscometric, rheological and bio film forming properties of sweet potato starch The storage modulus increased and loss modulus decreased by hydrocolloids and glycerol, whereas phase angle decreased, maximum influence by carraggeenan. Min and Oh, (2009) studied the mechanical and rheological properties of films fabricated with sweet potato starch and catfish gelatin. Addition of catfish gelatin extract reduced the viscosity and dynamic rheological properties

Fasina *et al*, (2003) studied the viscoelastic properties of restructured sweet potato puree with the aim to investigate the influence of alginate on the gel-like behavior of sweet potato puree at different temperatures The addition of a calcium/alginate complex changed the viscoelastic properties and increased the firmness of sweet potato puree, as evidenced from the higher values of the G' for restructured sweet potato puree in comparison with non restructured sweet potato puree Temperature had significant effect on the dynamic rheological properties of both restructured and non restructured sweet potato puree Means and Schmidt, (1986) showed that when alginate/calcium gel was added to structured meat, the binding force at the cooked state was higher than the binding force at the precooked state

Rheological properties of selected hydrocolloids as a function of concentration and temperature were studied by Marcotte *et al*, (2001) Higher gum concentrations resulted in an increase of both Newtonian and apparent viscosities Xanthan was found to be the most pseudoplastic and the least temperature dependent of all hydrocolloids studied Gelatin viscosities were independent of shear rate (Newtonian) and slightly affected by concentration Carrageenan was mostly affected by temperature and exhibited an enormous yield stress at low temperature Starch and pectin showed an intermediate behaviour as compared to carrageenan and xanthan with respect to concentration and temperature dependency of rheological properties

Mitchell and Blanshard, (1976) studied the rheological properties of pectate gels by creep compliance tests on calcium pectate gels using a parallel plate viscoelastometer The rheological behaviour of pectate gels had many similarities to gels prepared from alginates containing a high proportion of guluronic acid residues However, pectate gels were more sensitive to calcium ions than alginate gels and at high levels, the Newtonian viscosity of the pectate gel was much higher than that found for the alginates

Some rheological properties of galactomannan based gels were investigated as a means of obtaining temporary plugging agents for petroleum engineering. Polysaccharide based gels are commonly used in this type of application because of their unique properties and among the polysaccharides that provide a three dimensional network, guar gum and its derivatives are the most frequently used (Horner and Walker, 1965; Black and Melton, 1966).

2.6. MODIFICATION OF STARCH

Modified starches have been developed for a very long time and it applications in food industry are really significant (Abbas *et al.*, 2010). The purposes of modification are to enhance its properties particularly in specific applications such as to improve the increase in water holding capacity, heat resistant behavior, reinforce its binding, minimized syneresis of starch and improved thickening (Adzahan, 2002; Miyazaki *et al.*, 2006). The market-related properties are product properties such as the structure, aesthetics, organoleptic consideration and shelf stability (Sajilata and Singhal, 2004).

Physical modification involves pre-gelatinization heat-treatment of starch, etc (Miyazaki and Morita, 2006). Pre-gelatinized starches are pre-cooked starches that can be used as thickener in cold water. While the heat-treatment processes include heat–moisture and annealing treatments, both of which cause a physical modification of starch without any gelatinization, damage to granular integrity, or loss of birefringence (Abbas *et al.*, 2010). Physical modification of starch is mainly applied to change granular structure and convert native starch into cold water soluble starch or small crystalline starch and a large number of physical methods are available including heat moisture treatment, annealing, retrogradation, freezing, ultra high pressure treatment, osmotic pressure treatment, glow discharge plasma treatment etc. (Neelam *et al.*, 2012).

Pregelatinized starches or flours are paste-forming products in the presence of cold water or (partially or totally) soluble products in cold water and present the following characteristics: they disperse more easily and absorb more water than their untreated matches, they form gel at room temperature and are less prone to deposit (Powell, 1965; Colonna *et al.*,1984). The use of gelatinized starch in food products affects their characteristics and qualities, such as bread volume and crumb (Williams and Lesselleur, 1970).

Reactions used to produce most commercially modified starches have been reviewed by Wurzburg, 1986. Chemical modification of starch generally involves esterification, etherification or oxidation of the available hydroxyl groups on the B-D-glucopyranosyl units that make up the starch polymers (Roberts, 1965). The classic model of obtaining gelatinized starches, where starch granules are slowly heated in a medium with little agitation and much water, which promotes imbibition, swelling and polymer release (Leach, 1965).

Chemical modification involves introduction of functional groups into starch molecule, resulting in markedly altered physicochemical properties and such modification of native granular starches profoundly alter their gelatinization, pasting and retrogradation behavior (Neelam *et al.*, 2012). The chemical and functional properties achieved when modifying starch by chemical substitution depends on starch source and reaction condition such as reactant concentration, reaction time, pH and presence of catalyst, type of substituent, extent of substitution and the distribution of substituents in the starch molecule (Hirsch and Kokini, 2002). Cross-linking of starch molecules can potentially reinforce the intermolecular binding by introducing covalent bonds and supplements natural intermolecular hydrogen bonds which in turn improves the mechanical properties and water resistibility of the resulted films (Yu *et al.*, 1998).

Jyothi and Moorthy, (2012) discussed about the various modified tuber starches in industry. They explained about the pre gelatinised starch, starch esters, cross linked starch, oxidized starch, cationic starch, hydroxyalkylated starch used in various food and non food industries

Enzymatic modification involves the exposure of starch suspension to a number of enzymes primarily including hydrolyzing enzymes that tend to produce highly functional derivatives. Enzymatic modification of starch is hydrolysis of some part of starch into a low molecular weight of starch called maltodextrin, or dextrin using amylolytic enzymes (Miyazaki *et al.*, 2006). The enzymatically modified starches are widely used for food and pharmaceutical industries (Ogura, 2004).

Genetic modification of starch is the latest method of modification of starch to achieve desirable characteristics. Over expression of a plastidial envelope adenylatetranslocator from *Arabidopsis* in potato increased ADP-glucose levels two fold and increased starch content by 16-36 percent compared with control tubers (Geigenberger *et al.*, 2001). The down regulation of a plastidialadenylate kinase, an enzyme that inter converts two molecules of ADP into ATP and AMP, resulted in a ten fold increase in ADP-glucose levels and a doubling of the starch content in potato tubers, in both green house and field trials (Regierer *et al.*, 2002).

Most attempts to enhance starch accumulation have focused on engineering ADP-glucose pyrophosphorylase (AGPase) activity in plants and when *glgC16* was targeted to plastids in transgenic potatoes, some of the lines had up to 60 percent more starch in tubers on a fresh-weight basis than control plants (Stark, 1992). The down regulation of GWD led to substantial increases of the starch content in leaves, which ultimately should lead to enhanced digestibility of the fodder crops (Frohberg and Baeuerlein, 2006). Down regulating GWD in potato tubers inhibits cold sweetening and reduces acrylamide production during frying (Rommens *et al.*, 2007).

The first transgenic potato and cassava plants with modified starch were those in which amylose was eliminated via the down regulation of GBSS (Pilling and Smith, 2003).

2.7. BIODEGRADABLE FILMS FROM STARCH

The production and consumption of plastics continue to increase and most of these plastics are crude oil based, and an increase in their production results in an increase of oil use and causes serious environmental pollution, due to wasted and undegraded polymers (Parra *et al.*, 2004). One of the matters of great concern nowadays is the environmental impact caused by the excessive quantity of non-degradable waste materials discarded every day. This reality has been stimulating research to develop new biodegradable packaging materials that could be considered environmentally friendly raw materials (Averous *et al.*, 2001).

Recently, environmentally friendly materials from natural and renewable resources have received much attention. Products designated, as eco efficient products are the new generation of bio based products made from sustainable materials that conform to ecological and economic requirements (Narayan, 1994, 1998). The development of innovative materials as substitutes for synthetic polymers has become an important challenge. Among these materials are biopolymers from vegetable or animal proteins (Arvanitoyannis *et al.*, 2006; Xiong *et al.*, 2008). The production of biodegradable and edible films from carbohydrates and proteins adds value to low-cost raw materials and can play an important role in food preservation (Averous *et al.*, 2001; Krochta and Miller, 1997).

Biodegradable polymers based on natural polysaccharides, particularly starch, can be produced at low cost and at large scale (Parra *et al.*, 2004). Starch based materials reduce nonrenewable resources use and environmental impact associated with increasing emissions as CO_2 and other products (Narayan, 2001). The interest in combining polysaccharides, proteins, and lipids is due to the advantages and

disadvantages of these components (Baldwin *et al.*, 1995). The use of natural blends of protein, polysaccharides and lipids directly obtained from agricultural sources takes advantage of each component in the original system and appears to be a new opportunity for material in the area of edible films (Tapia-Bla´cido *et al.*, 2005).

According to Ellis *et al.* (1998), when a starch suspension in excess water is heated at or above its gelatinization temperature, starch granules become disordered and swell to many times their original size. It is at this point, amylose molecules diffuse more easily than amylopectin molecules, which remain by hydrogen unions or crystallized inside the granule (Noel *et al.*, 1992) thus improving the mechanical properties of starch. Branched structure of amylopectin generally leads to films with different mechanical properties, such as decreased tensile stress (Tharanathan, 2003). Amylose is responsible for the film-forming capacity of starch (Romero-Bastida *et al.*, 2005). Biodegradable polymers from renewable resources have their importance based on the control or reduction of CO_2 emissions and sustainable development of carbon based polymeric materials (Parra *et al.*, 2004).

Mark *et al.*, (1966) and Diaz-Sobac *et al.*, (2002) observed that starch films and coatings are odourless, tasteless, colourless, nontoxic, biologically absorbable, semi-permeable to carbondioxide, good oxygen barriers. But they are tacky at high relative humidity and rather brittle at low humidity (Jokay *et al.*, 1967). Edible films had been prepared from wheat flour (Rayas and Hernandez,1997), soy flour and apple pectin (Mariniello *et al.*, 2003) and amaranthus flour (Tapia-Bla´cido *et al.*,2005 and Colla *et al.*, 2006). As polysaccharides themselves do not have plasticity, they are often used after chemical, physical or enzymatic modifications and/or a blend with a biodegradable synthetic polymer (Okada, 2002).

Starch films are usually modified by the addition of plasticizers. Polyols (glycerol, sorbitol and polyethylene glycol) are commonly used as plasticizers (Goutard *et al.*, 1993). These additives decrease the intermolecular attraction between

adjacent polymeric chains, resulting in film flexibility and decrease in film strength (Donhowe and Fennema, 1993; 1994; Laohakunjit and Noomhorm, 2004). Fanta *et al.,* (2002) applied starch coatings to polyethylene films by immersing it in starch solutions to impart hydrophilic properties and thus modifying it. Cationic starches made from cassava starch are particularly suitable for the sizing and coating of paper in high-speed paper making machines (Shuren, 2001).

Plasticizers generally decrease gas, water vapor and solute permeability of the film and can decrease elasticity and cohesion (Gontard *et al.*, 1993; Sobral *et al.*, 2001). Many researchers have studied thermal and mechanical properties of edible films as a function of plasticizing agent (Arvanitoyannis *et al.*, 1997). The reinvention of 'edible films' was due mainly to their numerous applications, such as sausage coatings, chocolate coatings for nuts, fruits and vegetables (Arvanitoyannis *et al.*, 1996).

Several materials from agricultural sources have been used to produce renewable biodegradable and edible packaging, frequently called agricultural or agro packaging materials, such as protein and polysaccharides (Cuq *et al.*, 1998). They produce films with good mechanical properties and coverings that are efficient barriers against low polarity compounds. However, they do not offer good barrier against humidity (Azeredo *et al.*, 2000; Kester and Fennema, 1986).

Cassava starch is able to form transparent coatings and flexible films without any previous chemical treatment, neither plasticizer addition (Bergo and Sobral, 2007). Barrier and mechanical properties of starch based films are affected by factors such as amylose content, molecular weight of starch and type of plasticizer (Lloyd and Kirst, 1963).

Effect of glycerol and amylose enrichment on cassava starch film was studied by Alves *et al.*, 2005 and found that mechanical and barrier properties of cassava starch films were influenced by glycerol and amylose contents. Glycerol acted as a

typical plasticizer in starch films; with increasing glycerol concentration, water vapor permeability, and strain at break and puncture deformation increased, while stress at break, Young's modulus and puncture strength decreased. Physical characterization of cassava starch biofilms with special reference to dynamic mechanical properties at low temperatures was studied by Fama *et al.*, 2006.

Evaluation of the effects of glycerol and sorbitol concentration and water activity on the water barrier properties of cassava starch films was studied by Muller *et al.*, 2007 through a solubility approach. In all cases, an increase in water permeability values were observed with increasing plasticizer concentration and water content.

Water sorption and mechanical properties of cassava starch films and their relation to plasticizing effect was studied by Mali *et al.*, 2004. Films plasticized with glycerol, under all RH conditions, adsorbed more moisture with higher initial adsorption rate, and films with higher plasticizers contents exhibited higher equilibrium moisture contents. Mechanical properties were affected by plasticizing effect, including the water adsorbed, resulting in higher strain and Young's modulus values for starch films and, in all cases, glycerol exerted a more effective plasticization.

Influence of gelatinization process on functional properties of cassava starch films was studied by Paes *et al.*, 2008. Films were prepared by casting starch pastes gelatinized under eight different conditions: 70, 80, 90 or 100°C at 18,000 rpm and low shear rate 150 rpm. It was found that the use of a high shear affected all mechanical properties determined by tensile tests, which were lower for all temperatures compared to the films prepared using low shear. Furthermore, films prepared at different temperatures and high shear showed a high scattering of data for all mechanical parameters viz. elasticity modulus, strain at break and maximum stress. The films prepared with low shear also showed high scattering for the strain at

break at low moisture contents. It was shown that different methods of paste preparation resulted in different film structures.

Effect of preservatives on the functional properties of tapioca starch was studied by Ofman *et al.*, 2004. It was observed that the addition of potassium sorbate to starch before the gelatinization process increased the amount of water sorbed by the system, but moisture content of the mixture was lower than the one predicted by a mass balance, revealing the existence of an interaction between starch and the preservatives. Mechanical properties of the powder obtained after freeze drying of gels were also influenced by the addition of the preservatives: an increase in bulk density, higher cohesiveness and initial Young modulus was observed after antimicrobial addition.

Effects of controlled storage on thermal, mechanical and barrier properties of plasticized films from corn, cassava and yam starch was studied by Mali *et al.*, 2006. Crystallinity was affected by plasticizer concentration and storage time and unplasticized films showed higher water vapor permeability values than that of added with plasticizers.

Effect of various polyols and polyol contents on physical and mechanical properties of potato starch-based films was studied by Talja *et al.*, 2007. Water content and water permeability of films increased with increasing relative humidity and plasticizer content. Young's modulus decreased with increasing polyol content with a parallel increase in elasticity of films. Both increased polyol and water content increased elongation at break with a decreased tensile strength.

Effects of plasticizers on thermal properties on heat sealability of sago starch films was studied by Abdorreza *et al.*, 2011. They plasticized sago starch films with sorbitol, glycerol and a combination of sorbitol and glycerol in ratio of 1:1, 1:3 and 3:1. Films were then sealed with an impulse heat sealer dwell time of 1 s and temperature of 110±10°C. Thermal properties of the film show that at the onset of

temperature of the sorbitol plasticized films were significantly lower than those of glycerol plasticized film.

Influence of storage time on the physico-chemical properties of cassava starch films was studied by Fama *et al.*, 2007. The effect of the pH of the film forming systems was also evaluated. It was observed that, for storage times of 4 weeks or longer, tan d curves shifted to higher temperatures independently of the pH. Films obtained from systems of pH 5 showed a decrease in the intensity of the loss tangent-peak observed between 30 and 10°C, fact that is in accordance with a slight increase in the crystalline fraction. Colour parameters and sorbate content did not change significantly along 8 week-storage, showing that sorbate was not destroyed along the period studied.

Edible films of blended cassava starch and rice flour with sorbitol and their mechanical properties were studied by Rachtanapun *et al.*, 2012. The addition of a plasticizer agent up to 30% w/w of blending compositions improved the mechanical properties of the generated films. The mechanical properties of the edible blended films with 30% plasticizer were strongly dependent on the blending compositions. Their findings pointed out that the cassava starch and rice flour films at a ratio of 70:30 with sorbitol 30% (w/w) had the highest tensile strength which related to folding endurance of the films.

Preparation and properties evaluation of chitosan-coated cassava starch films was reported by Bangyekan *et al.*, 2006. The results of mechanical properties evaluation showed that an increase in chitosan coating concentration resulted in a significant increase in tensile stress at maximum load and tensile modulus, and a decrease in percent elongation at break, a remarkable decrease in water uptake was observed due to the contribution of hydrophobicity of chitosan coating layer, the hydrophobic acetyl groups of chitosan caused a notable reduction of wettability as

well as water vapour permeability which are preferable for packaging film application.

Sajeev *et al.*, (2013a) developed biodegradable films from cassava starch clay nanocomposites using different clays viz., Nanocalibre 100 SD, Nanocalibre 100A and supershine 90 hydrated for 24 and 48h at different clay proportions (0.1-0.6%) by casting. Tensile force of the clay based films were however comparatively higher than that of native starch based films, whereas solubility was higher than that of native starch film. There is not much variation in the thickness and whiteness index of the films. Hygroscopic or sorption studies at different relative humidity levels showed lower values of moisture absorption for starch clay composite films than native starch films.

Films made from chemically modified starch *viz.*, etherification, esterification and cross linking have improved physico-mechanical and reduced sorption properties and the properties depends on the type of modified starch, extent of their incorporation and the glycerol content (Sajeev *et al.*, 2013b). Anjana *et al.*, (2013) developed biodegradable films from hydroxypropylated cassava starch and nanokaoline clay composites and analyzed their physico-mechanical and hygroscopic properties.

2.8. ACTIVITY OF ENZYMES ON STARCH

In recent years bio catalysis has emerged as an active area of research and development (Loos, 2011; Drauz and Waldmann, 1995). One successful application of bio catalysis has been the use of enzymes to modify the structures of polysaccharides (Cheng and Gu, 2012). Enzymes are often chemo specific, region specific and/or enantio specific, enabling the synthesis of products with well-defined or stereospecific structures (Gu and Cheng, 2005). Another advantage is the mild conditions under which many enzymatic reactions can be done, often leading to products with less color or odor, and reduced levels of undesirable by-products

(Cheng and Gu, 2012). Enzymes such as soluble starch synthase and branching enzyme synthesize the amylopectin and amylose molecules (Smith, 1999)

A variety of different enzymes are involved in the synthesis of starch and sucrose is the starting point of starch synthesis (Maarel *et al.*, 2002). Starch degrading enzymes have been used to modify the physico-chemical properties of polysaccharides to achieve the desired functional properties (Khatoon *et al.*, 2009). Essentially five groups of enzymes are involved in the hydrolysis of starch namely endo and exo amylases that have activity primarily on the α-amylase, 4 linkages (MacAllister, 1979), whereas debranching enzymes act exclusively on the α-amylase, 6 linkages (Norman, 1982). A fourth group of enzyme, the isomerases, act on glucose to transform it into fructose (Guilbot and Mercier, 1985; Maldonado and Lopez, 1995). Finally the cyclodextrin glycosyl transferase group degrades starch by catalyzing cyclization and propotionation reactions (Nelson and Fennema, 1991).

In the course of conventional enzymatic liquefaction, slurry containing 15-35 percent starch is gelatinized, where it is heated to 105°C to physically disrupt the granule and open the crystalline structure for the enzyme action (Singh *et al.*, 2001). This increases the viscosity of the slurry by 20 fold (Robertson *et al.*, 2006). In recent years, the importance of the enzymatic liquefaction of raw starch without heating has been well recognized, mainly due to energy savings and the effective utilization of biomass, which reduces the overall cost of starch processing (Robertson *et al.*, 2006).

Differences in the *in vitro* digestibility of native starches, among and within species have been attributed to the interplay of many factors, such as starch source (Ring *et al.*, 1988), granule size (Snow and O'Dea, 1981), extent of molecular association between starch components (Dreher *et al.*, 1984), amylose/amylopectin ratio (Hoover and Sosulski, 1985), degree of crystallinity (Hoover and Sosulski, 1985), type of crystalline polymorphic (A, B or C) form (Jane *et al.*, 1997),

distribution of B type crystallites in the granule (Gerard *et al.*, 2001), amylose–lipid complexes (Guraya *et al.*, 2001), physical distribution of starch in relation to dietary fiber components (Dreher *et al.*, 1984), anti nutrients (Thompson and Gabon, 1987), α-amylase inhibitors (Lajolo *et al.*,1991), physical insulation of starch by thick walled cells (Wursch *et al.*,1986), porosity (Colonna *et al.*, 1988) and the influence of drying and storage conditions (Kayisu and Hood, 1979).

2.8.1. α- Amylase

This group comprises those enzymes that have the following features ie., they act on glycosidic bonds and hydrolyze this bond to produce anomeric mono or oligosaccharides (hydrolysis) form, 1-4 or 1-6 glycosidic linkages (transglycosylation), or a combination of both activities; they possess a TIM barrel structure containing the catalytic site residues; they have four highly conserved regions in their primary sequence which contain the amino acids that form the catalytic site, as well as some amino acids that are essential for the stability of the conserved TIM barrel topology (Kuriki and Imanaka, 1999).

Most of the enzymes that convert starch belong to one family based on the amino acid sequence homology: the amylase family or family 13 glycosyl hydrolases according to the classification of Henrissat, (1991). The amylase catalysed hydrolysis of starch is among the most important industrially applied enzyme reactions (Gupta *et al.*, 2003).

It is difficult for amylases to act on raw starch granules than on gelatinized starch. Studies by Iefuji *et al.*, (1996) indicated that the saccharification of raw starch by amylolytic enzymes might be related to the extent of adsorption of enzyme to the starch granules. According to Leloup *et al.*, (1990) there are several steps involved in the enzymatic reaction which comprises the diffusion to the solid surface, the adsorption of the enzyme and finally the occurrence of the catalysis. The penetration

of hydrolyzing enzymes and other large molecules, however, is restricted and only possible through pores or channels (Oates, 1997).

Amylases also must functionally bind glucan chains through several glucose units to their sub sites (Oates, 1997). The number and the position of the sub sites in the active center are unique for each type of amylase (Meagher *et al.*, 1989). The hydrolysis occurs layer by layer with an attacked layer of granule being completely hydrolyzed (Wang *et al.*, 1995). The susceptibility of starch to amylase attack depends on the properties of the specific starch, such as degree of gelatinization, and the characteristics of the specific amylase (Bijttebier *et al.*, 2008). In addition, amylases showed widely different activities on various kinds of solubilised starches (Mukerjea *et al.*, 2006). Amylases are, hence, often defined by their starch degrading characteristics and less by their action on the individual starch polymers (Bijttebier *et al.*, 2008).

Hydrolysis of granular starch at sub-gelatinization temperature using a mixture of amylolytic enzymes was done by Uthumporn *et al.*, (2010). Native granular starches -corn, cassava, mung bean, and sago were hydrolyzed using a mixture of alpha-amylase and glucoamylase at 35°C for 24h. Hydrolyzed starches were analyzed for the degree of hydrolysis and for physicochemical and functional properties. Corn starch showed the highest degree of hydrolysis and amylose content was significantly lower in all starches except for sago starch. The action of enzymes caused significant changes in pasting properties and in the swelling/solubility of starches. They concluded that amylolytic enzymes were capable of hydrolyzing granular starches at sub gelatinization temperature (35°C) and found out that the relative order in the susceptibility of different types of starches after 24h of enzyme hydrolysis at sub gelatinization temperature was as follows: corn >mung bean > cassava > sago.

Properties of enzyme modified (with heat stable α-amylase) corn, rice and tapioca starches were studied by Khatoon *et al.*, (2009). Dextrose equivalent (DE) of 8–12 was achieved by hydrolyzing the starch samples (10- 20% w/v) for 30 min at 90 ± 2°C. Scanning electron micrographs showed that starch granules had broken down to smaller particles. High performance liquid chromatography with refractive index detection reflected that oligosaccharides with broad molecular weight distributions were present in the reaction products and hydrolyzed starch dispersions were analyzed for their rheological properties.

Comparative studies of starch susceptibilities to α-amylase degradation of different cereal and root crops of Nigeria was studied by Adejumo *et al.*, 2013. Amylose/amylopectin content of each starch samples was determined and their susceptibilities to α-amylase were studied. Large differences in enzymes susceptibilities were observed when studied within 4h with white maize having the highest value of dextrose equivalent of 42 percent, followed by yellow maize 37.5 percent, and cassava varieties, okoyawo 27.3 percent and odongbo 24.75 percent.

Alcoholysis reactions from starch with α-amylases was studied by Santamara *et al.*, 1999. The ability of α-amylases from different sources to carry out reactions of alcoholysis was studied using methanol as substrate. It was found that while the enzymes from *Aspergillus niger* and *Aspergillus oryzae*, were capable of alcoholysis reactions, the classical bacterial liquefying α-amylases from *Bacillus licheniformis* and *Bacillus stearothermophilus* are not.

Hydrolysis of native starches with amylases was studied by Tester *et al.*, 2006. Native starch granules are semi-crystalline and resist hydrolysis by amylases. When gelatinised, however, they are readily hydrolysed and converted to sugars and dextrins. Factors that control the rate and profile of hydrolysis by amylase *in vitro* and *in vivo* are interconnected.

Vasanthan and Bhatty, (1996) fractionated starch granules extracted from waxy, normal and high amylose barley into small and large granules. They found two points of interest for these starch fractions (which had similar compositions and properties) ie., the native small granules were hydrolysed more rapidly than large by α-amylase and the high amylose genotypes were resistant to amylase hydrolysis. These data confirm the relevance with respect to granule size controlling (at least in part) the amylase digestibility of native starch granules.

The amount of native starch hydrolysis by amylases is reported to be inversely related to the amylose content (Cone and Wolters, 1990), where high amylose starches are especially resistant (Gallant *et al.*, 1992) which fits with the generally held view that amylose represents amorphous starch. Lauro *et al.*, (1999) however, reported that at the early stages of barley starch granule hydrolysis both amorphous *and* crystalline parts of barley starch granules are equally solubilised by α-amylase.

Amylose exist in the free form or complexed with lipid in native starch granules (Cheetham and Tao,1998). Whilst amorphous starch is readily hydrolysed by α-amylase, the presence of lipid and in particular lipid-complexed amylose does provide some resistance to hydrolysis and digestion (Karkalas *et al.*, 1992). The lipid inclusion complex makes the amylose chains much less readily accessible to the active site of the α-amylase enzyme although it does not confer complete resistance to hydrolysis, just a prolonged hydrolysis profile. Although native cereal starches contain endogenous amylose lipid complexes, these are also formed during processing of starch in the presence of lipids although the free fatty acid or mono-glyceride (Tester and Karkalas, 2002).

2.8.2. Pullulanase

Pullulanases (E.C. 3.2.1.41, α-dextrin 6-glucano-hydrolase) are endo-acting debranching enzyme capable of hydrolysing α-(1,6)-*D*-glycosidic bonds in pullulan, β-limit dextran and amylo pectin, forming maltotriose (Abdullah and French, 1966).

Debranching using pullulanase has been used to produce a glucan with linear, low molecular weight and recrystallization polymer chains (Guraya *et al.*, 2001; Yin *et al.*, 2007). This releases a mixture of varied length unit chains from the parent amylopectin molecule that induce retrogradation (Pongjanta *et al.*, 2009).

Pullulan is produced from starch by the fungus *Aureobasidium pullulans* (Bataille *et al.*, 1997; Glinel *et al.*, 1999). Pullulanases type I, which are able to hydrolyse efficiently the α-(1,6) glucosidic bonds in pullulan and branched polysaccharides, have been extensively studied (Rudiger *et al.*, 1995 and Kim *et al.*, 1996). During starch conversion process, starch is first gelatinised and solubilised at high temperatures and the long-chain molecules broken down into smaller units (malto dextrins) which can be either in the form of branch or linear (Ling *et al.*, 2012).

Pullulanase genes from *Anaerobranca gottschalkii*, *Desulfurococcus mucosus*, and *Klebsiella aerogenes* have also been reported; *Escherichia coli* and *Bacillus subtilis* were the two commonly used host strains for the expression of pullulanase gene (Nair *et al.*, 2007). Five groups of pullulan-hydrolyzing enzyme have been reported such as pullulanase type-1, amylo pullulanase, neo pullulanase, iso pullulanase and pullulan hydrolase type-111 (Ling *et al.*, 2012).

Pullulanases are used in detergent industry as effective additives in dish washing and laundry detergents for the removal of starches under alkaline conditions (Van and Willem, 1990); in the starch processing industry for the production of maltose syrups and high purity glucose and fructose and in the manufacturing of low caloric beer (Olsen *et al.*, 2000). It is also possible to use pullulanase as a dental plaque control agent (Marotta *et al.*, 2002). Pullulanase is used in saccharification of starch to glucose since 1960 (Olsen *et al.*, 2000).

Much of the starch hydrolysates available in the market are enzyme converted products of higher DE (Ling *et al.*, 2012). It is well suited for numerous applications

in food processing such as in the manufacturing of high-quality candy and ice cream (Shaw and Sheu, 1992). High-glucose syrup is used as a carbon source in fermentation and feed for making high-fructose syrups and crystalline glucose (Olsen *et al.*, 2000). Pullulanase has also been used to prepare high-amylose starches, which have huge market demand (Vorwerg *et al.*, 2002). Pullulanase has also been used to enhance the yield of cyclodextrins by the reaction of CGTase with gelatinized starches and maltodextrin syrups in the presence of cyclodextrins (CDs) complexing agents (Rendleman,1997). Pullulanase also finds minor application in the manufacturing of low calorie beer (Olsen *et al.*, 2000) and in baking industry as the anti-staling agent to improve texture, volume and flavor of bakery products (Maarel *et al.*, 2002).

Several studies have been done on the aspect of modification of starch through pullulanase to achieve different desirable products. Properties of pullulanase debranched cassava starch and type-III resistant starch was studied by Vatanasuchart *et al.*, (2010) with the objective to produce type-III resistant starch (RS-III) by the pullulanase reaction. A 10% (dry weight) cassava starch suspension with pullulanase of 3%, 5% and 10% (v/w) of starch weight was used for the study. The results showed that the reducing sugars obtained with any treatments tended to increase with the length of the reaction time. Thus, conditions of pH 5.0, hydrolysis of 10% pullulanase for 24 h and hot air drying were suitable for partially debranching amylopectin of the cassava starch as it resulted in a higher amylose content, consequently providing small linear fragments and small clusters of the amylopectin for recrystallization and formation of the RS-III.

Effects of preheat treatments on physic chemical properties of resistant starch Type III from pullulanase hydrolysis of high amylose rice starch was studied by Pongjanta *et al.*, 2009. A debranching enzyme was introduced to modify the amylopectin molecules which had been preheated at 95°C for 30 min. As a result, retrogradation gels of debranched starches with different degrees of hydrolysis were

then induced at 4°C for 16 h followed by freeze thaw process. Results showed that pullulanase hydrolysis enhanced the degree of syneresis for non-debranched and debranched starches which had been preheated. The debranched starches with higher degree of hydrolysis provided products with higher resistant starch contents.

Extraction of starch and enzymatic production of high amylose starch from sweet potato (*Ipomea batatas*) var. Telong was studied by Madzlan *et al.*, 2012. A high amylose starch was produced by debranching the amylopectin of the sweet potato starch using 0.5 percent pullulanase (Promozyme D2) at 60°C for 24 h. The optimum conditions for the production of high amylose starch were at pH 5.0, 5.0 percent (w/v) starch concentration and incubated at 60°C for 8 h. The amylose content increased from 21-84 percent after 8 h of incubation.

Effects of autoclaving and pullulanase debranching on the resistant starch (RS) yield of normal maize starch was studied by Milasinovic, (2010). Autoclaving at 120°C (30 min) increased the RS content of all samples. The highest RS yield in the autoclaved starch samples was 7.0 percent. After pullulanase debranching at 50°C and retrogradation at 4°C, the RS yields ranged from 10.2 to 25.5 percent in all samples. Debranched starch samples with a maximum RS yield of 25.5 percent were obtained after a debranching time of 24 h. This study showed that starch from the ZP maize genotype is suitable for pullulanase treatment.

2.9. SOIL BURIAL TEST AND MICROBIAL ANALYSIS

According to Bastioli, (1995) starch promotes the biodegradability of a non biodegradable plastic and it can also be used together with fully biodegradable synthetic plastic producing biodegradable blends of low costs. Starch blended biodegradable polyethylene (PE) films grafted with vinyl acetate showed better printability performance without affecting the biodegradation properties (Ghosh *et al.*, 2002). Thakore *et al.*, (1999) tested the biodegradability of low-density

polyethylene (LDPE)/starch and LDPE/ starch/ starch acetate blends and observed the biodegradability on the starch acetate content.

Krupp and Jewell, (1992) studied biodegradability of plastic films in controlled biological environments. PE with 6 percent starch in PE matrix was reported to degrade in landfills. Water absorption was dropped as burial time is increased, owing to the fact that some starch particle was leached away from the specimen (Ke and Sun, 2003). Maharana and Singh, (2006) carried out graft copolymerization of LDPE onto starch with glucose-cerium (IV) redox initiator in aqueous sulfuric acid medium under nitrogen atmosphere and tested the biodegradability of starch grafted PE by applying soil burial test.

LDPE films containing starch were tested for biodegradation by Dave *et al.*, (1997) and it was found that films containing 30 percent starch showed maximum biodegradation leading to a loss of 6.3 percent in weight and 84.5 percent starch upon burial in a soil compost mixture for 48 weeks. Ratanakamnuan and Aht-Ong, (2006) exposed binary polymer films containing different percentages of corn starch and LDPE to soils over a period of 8 months and monitored for starch removal and chemical changes of the matrix using Fourier transform infrared spectroscopy (FTIR). The matrix did show evidence of swelling, an increase in surface area, and removal of low molecular weight components. Griffin, (1974) determined the rate and extent of deterioration of starch-plastic composites over a 2-year period for samples buried in a muncipal solid waste landfill.

Soil burial biodegradation studies of starch grafted polyethylene and identification of *Rhizobium meliloti* was studied by Gautam and Kaur, (2013). Biodegradable behavior of the grafted PE was determined by soil burial test. Percent weight loss was measured as a function of number of days and they found out that it increased with increasing number of days. Microanalysis of the soil containing samples was carried out which substantiated the degradation. The biochemical tests

was performed on the microorganism isolated from the soil in micro analysis, identified the organism as *Rhizobium meliloti* which helped in biodegradation of PE-starch samples. The degradation was further confirmed by carrying out the physical characterization of the original samples and the degraded samples by scanning electron microscope (SEM) and thermogravimetric analysis. Hydrolysis of grafted samples, taken out from the soil after a specified number of days also corroborated in the findings, revealing continuous loss of weight with increasing number of days.

Effect of soil burial on properties of polypropylene (pp)/ plasticized potato starch (pps) blends was done by Onuoha *et al.*, 2013. Polypropylene and plasticized potato starch with and without compatibilizer were produced through melt blending for soil burial that lasted for 90 days. The results showed that tensile properties of the various PP blends decreased progressively with the increase in starch content and burial time for PP/PPS blends. Similarly, tensile properties of PP/PCPS blends followed the same trend but with less decrease in tensile properties than PP/PPS blend due to compatibilizing effect of maleic anhydride-graft polypropylene which offered an improved interfacial adhesion between starch and matrix. The tensile properties however, for both PP/PPS and PP/PCPS decreased with increased in starch content and burial period.

Biodegradation of synthetic polyesters (BTA and PCL) with natural flora in soil burial and pure cultures under ambient temperature was studied by Gouda *et al.*, 2012. The results showed that the BTA films buried in canal shore and garden soil were degraded faster than that in the other soils. It was seen that after six weeks about 90, 88 and 80 percent were degraded in garden, canal shore soil and compost respectively, whereas only 52 percent were degraded in Peat moss. On the other hand, 95 and 93 percent weight loss was obtained for PCL films buried for three weeks in canal shore and garden soil respectively. The scanning electron microscope photos confirm the results of weight loss and revealed the presence of cracks and fungal growth on films buried in different soils. The results with pure cultures, especially

with *Fusarium solani*, also confirmed the biodegradability of two polyesters under ambient temperature. Finally, it was concluded that both synthetic polyester are degradable under ambient conditions.

Degradation behavior of bio composites based on cassava starch buried under indoor soil conditions was studied by Maran *et al.*, 2013. From the results, it was observed that, increased water sorption promotes the entry of soil microorganism and it utilizes the starch films as a source of energy for their growth. The reduction in weight and mechanical property was associated with preferential loss of matrix components of the films. The microorganisms associated with the degradation of films were quantified and identified. The predominant bacterial species identified from the samples were *Pseudomonas* sp., *Streptococcus* sp., *Staphylococcus* sp., *Bacillus* sp. and *Moraxella* sp. Among the fungal species identified was *Aspergillus* sp and *Penicillin sp*. Scanning electron microscopy (SEM) analysis showed the formation of patterns and cracks on the surface of the materials aged in the soils. From the results, second order polynomial models were developed for the responses.

Impact of soil composting using municipal solid waste on biodegradation of plastics was studied by Vijaya *et al.*, 2008. Loss of weight and reduction in tensile strength of polythene films were taken as the criteria indicating biodegradation of these materials. Composting of polythene films for 4 months did not show any degradation. After 4 months of composting, the loss in weight was 2.9-4.5 percent for HDPE films and 10.5-11.6 percent for LDPE films. Similarly, the reduction in tensile strength ranges from 16-20 percent for HDPE and 12-13 percent for LDPE films. The study indicates that the biodegradation of polythene films occur in natural environment at a very slower rate.

This showed that biodegradation of plastic waste takes a plenty of time to degrade whereas biodegradation of biopolymer films degrades in faster rate giving promises of a less toxic environment and a more green future.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study was conducted during the period 2014-2015 at Crop Utilization Division, ICAR-Central Tuber Crop Research Institute (ICAR-CTCRI), Sreekariyam, Thiruvananthapuram, Kerala and Department of Plant Biotechnology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala. This chapter deals with the different materials used for the study, rheological characterization of the enzymatically modified starches, development and characterization of the biodegradable films form the enzymatically modified starches.

3.1 MATERIALS

Cassava (variety:CMR 256), mobile starch extraction plant, cassava starch, 80 mesh sieve, tray, grinder, alpha amylase (SIGMA Life Science), pullulanase (SIGMA Life Science), weighing balance (SARTORIUS), shaking water bath (JULABO SW22), teflon coated plates, biofilm dryer, screw guage (Mitutoya), colourimeter (Konica Minolta) , desiccators, food texture analyzer (Stable Micro Sytem), rheometer (Anton Paar), viscometer (Brookfield), hot air oven (BESTON), laminar air flow (Toshibha), NA medium (HIMEDIA), RB medium (HIMEDIA), KK medium (HIMEDIA), autoclave, refrigerator (LG), UV-VIS spectrophotometer (Perkin Elmer), HPLC (SHIMADZU), glass wares (RIVIERA and ASGI).

3.2 METHODOLOGY

3.2.1. Extraction of cassava starch

Cassava starch was extracted by employing wet processing method. Fifty kilogram of the cassava tubers from the variety : CMR 256 was collected from the

ICAR-CTCRI farm and they were peeled and washed thoroughly with clean water. The samples were then sliced into small pieces (3-5cm) and it was crushed using a mobile starch extraction plant (Plate 1) after adding enough volume of water. The mash obtained after crushing was passed through an 80 mesh sieve and water was added on the sieve to release all starch particle from the mash through the sieve. The starch suspension obtained was collected in buckets and allowed for gravity settling for overnight. The wet starch, after decanting the supernatant water was scooped out and sun dried. The dried starch was again ground in a mixer-grinder and packed in polythene bag for further uses.

Recovery of starch was calculated by taking the weight of the dry starch obtained from 50 kg of the tubers and expressed as percentage.

3.2.2. Properties of starch

3.2.2.1. Colour

The colour of films was analysed using a spectrophotometer (CM 2600d, Konica Minolta, Japan). The primary colour parameters 'L' , 'a' and 'b' were measured in UV excluded (spin) calibration mode with lens position kept at MAV. 'L' , 'a', 'b' values describe the precise location of a colour in a 3D visible colour space where, 'L' parameter represent light-dark spectrum with a range from 0 (black) to 100 (white), 'a' represents the green-red spectrum ranging from -60 (green) to +60 (red) and 'b' represents the blue-yellow spectrum with a range from -60 (blue) to +60 (yellow) dimensions respectively. From these primary colour coordinates, total colour difference and whiteness index were calculated using standard equations.

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

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

where, $L_0 = 99.34$, $a_0 = -0.03$, $b_0 = -0.1$

3.2.2.2. Rheology

Native starch solution (5%) was taken and allowed to gelatinize at 90°C in shaking water bath for 20 min to ensure the starch was gelatinized fully to obtain a viscous transparent solution. It was cooled to ambient temperature and the gelated starch so obtained after cooling was subjected to dynamic viscoelastic tests by frequency sweep at 0.1 to 10 Hz (0.623 to 62.3 sec^{-1}) at 0.1% strain (with in the linear viscoelastic range) using a rheometer with parallel plate (20 mm diameter) geometry (PP20 SN 5912) with a gap of 1 mm using Rheoplus MCR 51 Rheometer (Anton Paar, Germany) (Plate 2). The rheogram obtained from the study gives storage modulus (G'), loss modulus (G''), complex viscosity (η) and phase angle (θ).

3.2.2.3. Swelling volume and solubility

Starch (400 mg) was weighed into a 100ml conical flask, 40ml distilled water is added with vigorous swirling and the flask was kept in a boiling water bath. The flask was continuously swirled so that the starch completely gelatinizes. After gelatinization of starch, the flask was kept in a water bath for 15 mins with occasional swirling. After 15 min, the flask was taken out and allowed to cool to room temperature. The contents are transferred into a graduated 50 ml centrifuge tube and centrifuged at 2200 rpm for 15 min. The volume of the gelatinous precipitate at the bottom of the centrifuge was noted.

Swelling volume (ml/g) = volume of the swollen starch (ml) × 2.5

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

$$\text{Solubility (\%)} = \frac{\text{Wt. of soluble starch} \times (40 - \text{swelling volume}) \times 100}{\text{Wt. of dry sample} \times 10}$$

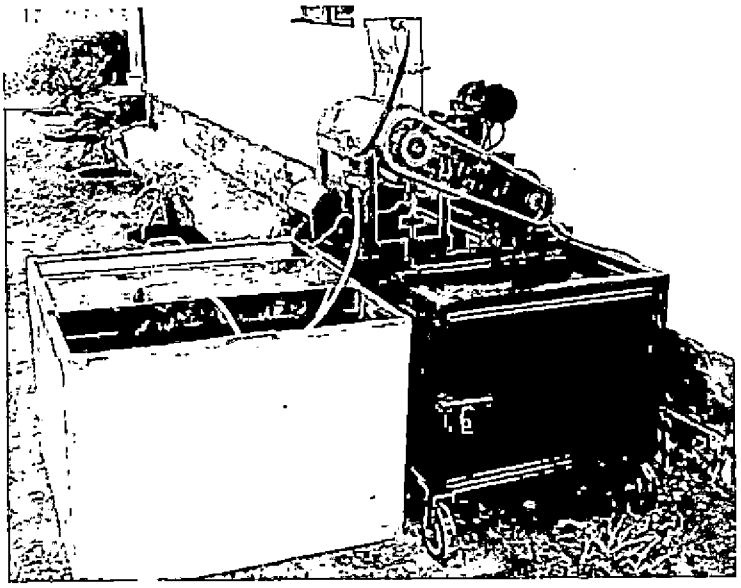


Plate 1. Mobile starch extraction plant for the wet milling of cassava tubers

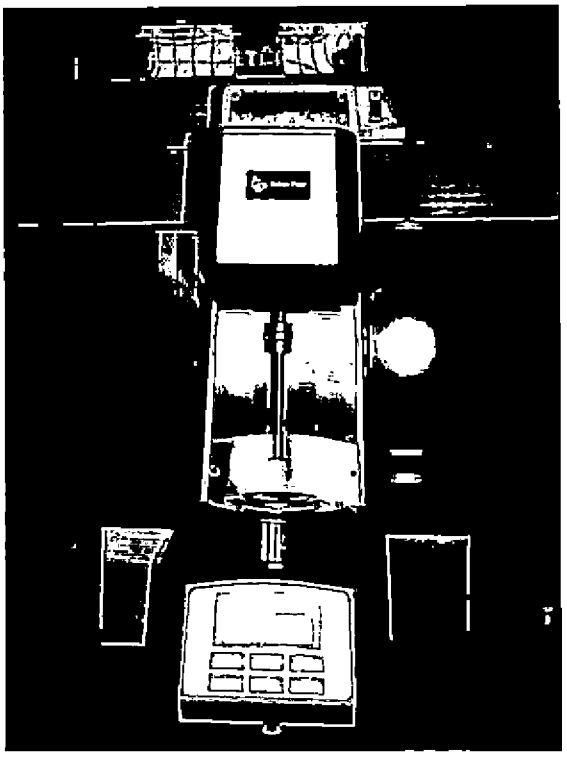


Plate 2. Rheometer

3.2.3. Preparation of starch film by casting method

Filmogenic solution was prepared by dissolving 10g starch in 200 ml distilled water added with 2g glycerol giving rise to 5 percent starch solution and 20 percent glycerol of starch and was gelatinized at 90°C for 20 min in a shaking water bath. After 20 min, the flask containing filmogenic solution was placed in a water bath set at 70°C for 30min for removing the bubbles formed in the solution. Cassava starch based film was prepared by casting method for which the hot suspension at 70°C was immediately transferred to a leveled non stick teflon coated plate (25 x 25 cm) through a cheese cloth to remove the air bubbles, if any. Care is being taken to uniformly distribute the solution on the plate. After drying the films for overnight at 40°C in a film dryer (Plate 3), they were peeled off from the plate and used for characterization

3.2.4. Standardization of biodegradable film production using α-amylase

Preliminary trials were conducted to fix the level of α-amylase to be added in the filmogenic solutions to obtain clear films using casting methods. Filmogenic solution was prepared by dissolving 10g starch and 2g (20% of starch) in 200ml distilled water to get 5 percent starch concentration. Enzyme concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 1.5, 2.0 ml was added to this starch solution and gelatinized as above and films were prepared as above, but it was found that the film was so thin that it could not be peeled off from the casting plates.

Considering the above facts, a stock solution of α-amylase was then prepared by dissolving 1 ml of enzyme to 100 ml distilled water. From that 25, 50, 75, 100, 200, 300, 400, 500, 1000 µl was added to the solution and again films were made. But no thick film was obtained at these concentrations also. So the concentration was lowered again by preparing another stock solution by adding 0.1 ml in 100 ml

distilled water. Again films were prepared by adding 25, 50, 75, 100, 150, 200, 300, 400, 500, 600, 700, 800, 1000 µl and it was found that adding the stock solution upto 600µl, good quality film was obtained and among this, it was found that the concentrations of 100, 200 and 300 µl gave the best films and they were chosen for further experiments. Also the temperature of gelatinization were varied as 80, 85 and 90°C and the time for gelatinization as 20, 30 and 40 min. The experiments were designed using response surface methodology using Box- Behnken design and the experimental design is given Table

Table 2. Experimental design for the development of biodegradable films using α-amylase

Sample no.	Time (min)	Temperature (°C)	Enzyme concentration (µl)
1	20	80	200
2	20	90	200
3	40	80	200
4	40	90	200
5	30	80	100
6	30	80	300
7	30	90	100
8	30	90	300
9	20	85	100
10	40	85	100
11	20	85	300
12	40	85	300
13	30	85	200
14	30	85	200
15	30	85	200

3.2.5. Standardization of biodegradable film production using pullulanase

Preliminary trials were carried out to develop biodegradable films form the filmogenic solution containing pullulanase by varying temperature of incubation as 45, 50 and 55 °C, time of incubation as 8, 16 and 24 h and pullulanase concentrations of 1, 2, 3, 4, 5, 10, 15 units. Out of these concentrations, it was found that at 2, 3 and 4 units gave good films and hence selected for the study by keeping the temperature and time of incubation as above. Box- Behnken design was then employed to obtain 15 different experimental conditions.

Table 3. Experimental design for the development of biodegradable films using pullulanase

Sample no.	Time (h)	Temperature (°C)	Enzyme concentration (unit)
1	8	45	3
2	8	55	3
3	24	45	3
4	24	55	3
5	16	45	2
6	16	55	4
7	16	45	2
8	16	55	4
9	8	50	2
10	24	50	2
11	8	50	4
12	24	50	4
13	16	50	3
14	16	50	3
15	16	50	3

3.2.6. Characteristics of filmogenic solution of enzymatically modified starch

3.2.6.1. Rheology of the filmogenic solutions

Different combinations of alpha amylase/pullulanase treated starch solution as described in Table 2 and 3 were prepared by varying the enzyme concentration, time and temperature of gelatinization using a shaking water bath to get 15 different filmogenic solutions. This gelatinized filmogenic solutions after cooling were taken for the rheological studies as described in Section 3.2.2

3.2.6.2. Dextrose equivalent

Dextrose equivalent of the filmogenic solution was analyzed by Nelson – Somogyi method for the 15 combinations each for α- amylase and pullulanase as described in Table 2 and 3. 0.2 and 0.4 ml of each sample was taken in respective test tubes and the volume was made upto 2 ml. Two milli litre distilled water as taken in another test tube as blank. 1ml alkaline copper tartarate was added to all the tubes. The tubes were then incubated in a boiling water bath for 10 min and was then allowed to cool. One ml arseno molybolic acid reagent as added to this mix and the volume was made up to 10 ml with distilled water. Glucose standard is also done, stock solution is 0.1g/100ml and working standard is 100µg/ml. Glucose solution was taken as standard in the volumes 0.2, 0.4, 0.6, 0.8 and 1.0 respectively. After 10 min incubation, the samples were checked for its absorbance in a Spectrophometer at OD of 620nm. Reducing sugar was obtained by employing this method. Dextrose equivalent (DE) was calculated using the formula,

DE = Reducing sugar content (glucose)/ total solids content * 100

3.2.6.3. Sugar profile

The chromatographic analyses were carried out in a Shimadzu, high performance liquid chromatography equipped with a LC-20AD pump. A Shimadzu wavelength UV/VIS detector SPD-10Avp, a RID-10A RI detector and LC solution, HPLC

System Controller and analysis Software were also used. Filmogenic solutions made from alpha amylase and pullulanase are used to study the sugar profile using HPLC. 0.1ml of filmogenic solution is diluted with 0.9ml of distilled water is used as the HPLC sample.

3.2.6.4. Colour

The colour of the filmogenic solution was analyzed by measuring the colour coordinates 'L', 'a' and 'b' using a spectrophotometer (CM 2600 D, Konica Minolta, Japan) as described in Section 3.2.2

3.2.7. Biodegradable film preparation using α-amylase

Filmogenic solution was prepared by mixing starch with glycerol and α-amylase enzyme as follows: cassava starch (10 g) with 2 g glycerol was dispersed in 200ml distilled water. Different enzyme concentration, temperature and time combinations was kept as shown in Table 2 and kept the samples for the experimental conditions in a shaking water bath for gelatinization. Air bubbles formed during boiling were removed by keeping the gelatinized solution in a water bath at 70°C for 30min. Cassava starch based films were prepared by casting method for which the hot suspension at 70°C was immediately transferred to a leveled non-stick teflon coated plate (25x25cm) through a cheese cloth to remove the air bubbles, if any. Care is being taken to uniformly distribute the solution on the plate. After drying the films at 40°C overnight in film dryer, the films were peeled off from the plate and used for characterization of physico-mechanical, functional, biodegradation and packaging properties.

3.2.8. . Biodegradable film preparation using pullulanase

Filmogenic solution was prepared by mixing starch with glycerol and pullulanase enzyme as follows. Cassava starch (10 g) was dispersed in 200ml distilled water. Different enzyme concentrations, temperature and time combinations

as shown in the Table 3. was kept in a shaking water bath for incubation. After incubation for the respective conditions, added with 2 g glycerol, the solution was gelatinized at 90°C for 20 min in a shaking water bath. Air bubbles formed during boiling were removed by keeping the gelatinized solution in a water bath at 70°C for 30min. Cassava starch based films were prepared by casting method for which the hot suspension at 70°C was immediately transferred to a leveled non-stick Teflon coated plate (25x25cm) through a cheese cloth to remove the air bubbles, if any. Care is being taken to uniformly distribute the solution on the plate. After drying the films in a film dryer at 40°C for overnight, they were peeled off from the plate and used for characterization of physico-mechanical, functional, degradable and packaging properties.

3.2.9. Physico-mechanical and functional properties of modified films

3.2.9.1. Moisture content

The film moisture content was determined gravimetrically by drying small strips of film (1*1cm) in an oven with air circulation at 110°c for 6 h till constant weight (Belibi *et al.*, 2013). The moisture content was expressed on wet basis, as the ratio of the moisture evaporated to the initial weight of the sample. Experiments were replicated twice and the average values were reported.

3.2.9.2. Conditioning of films

The films were conditioned at 50% RH for about 1 week by keeping over 45% concentrated sulphuric acid solutions taken in a desiccator. Conditioned films were used for thickness, colour and mechanical strength analysis.

3.2.9.3. Film thickness

The thickness of the film was measured using the screw gauge of 0.001 mm accuracy. It was measured at 10 different locations and average was reported

3.2.9.4. Film colour

The colour of the films were analyzed by measuring the colour coordinates 'L', 'a' and 'b' using a spectrophotometer (CM 2600 D, Konica Minolta, Japan). From this primary colour coordinates, the total colour difference and whiteness index were calculated using standard equations.

3.2.9.5. Mechanical properties

Mechanical properties viz., tensile force and elongation at break was measured using food texture analyzer (TA HDi, Stable microsystems, Surrey, U.K) (Plate 4.) with Texture Expert Software under the following conditions: mode – measure force in tension, pre and post test speed – 10 mm per sec, test speed – 2mm per sec and distance – 200mm using a tensile grip (A/TG). The upper tensile grip was attached to the load cell carrier and the lower grip was secured to the base of the machine. The tensile grip was calibrated to start from a set distance apart for each test of 50mm. Calibration is done by lowering the grips so that they are very close together, click on TA, Then calibrate probe and specify the distance for the grip to start apart from each for each test is 50mm. Clamp the conditioned strip of the film (10mm wide and 100mm long) in a vertical manner and test was started. When the test commenced, the upper grip pull the film upwards till the film breaks into two pieces. From the force- deformation (time) curve, the maximum peak force was noted as tensile force and the distance to which it elongates before it breaks into two pieces is designated as elongation at break.

3.2.9.6. Swelling power and water solubility of films

Solubility is defined as the percentage of film dry mater solubilized after 24h of immersion in distilled water and is calculated by the method described by Gontard *et al.*, 1992. The films were cut into pieces and about 1g of film strips was weighed in a pre weighed conical flask. The initial percentage of dry matter was determined by drying the film in an oven set at 110^{0} for 6 h. After 6 h, the flask was re weighed.

Plate 3. Film dryer

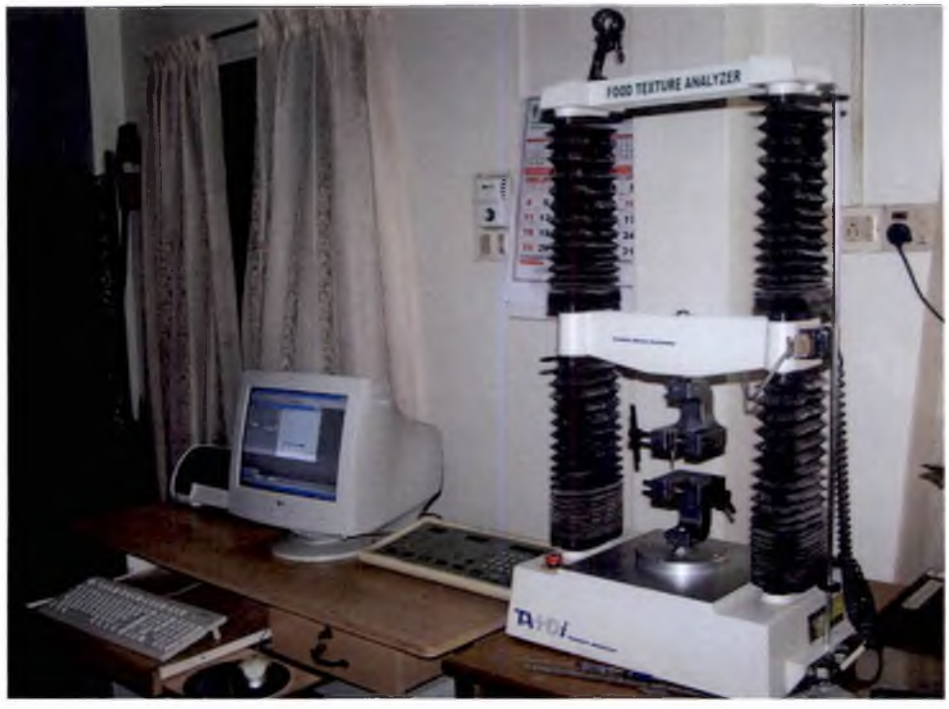


Plate 4. Food texture analyser

Then add 20ml distilled water to the flask with periodic stirring kept for 24h at room temperature. Then it was filtered to obtained undissolved film, weighed to find out the weight of the swollen film. The weight of the water absorbed during swelling of the films (swollen film) with respect to initial weight of the film is termed as swelling power. Final dry weight of undissolved film was determined by drying again in an oven at 110°C for 6h. The percentage of total soluble matter (% solubility) was determined as ratio of the difference between initial and final dry weight of the films to initial dry weight.

3.2.9.7. Hygroscopic properties

Hygroscopic or sorbtion isotherms properties of the films developed with α-amylase and pullulanase enzymes were studied by placing the film pieces in different humidity environments in desiccators containing saturated salt solutions of LiCl (11%), CH_3COOK (22.5%), K_2CO_3(43.16%), $Mg(NO)_3$ (53%), $NaNO_2$ (65%), NaCl (75%), KCl (85%) and Na_2SO_4 (95%). The amount of water absorbed was calculated on each day and the hygroscopic properties as percentage water absorbed were measured when there is no difference between consecutive readings.

3.2.10. Soil degradation study and Microbial population analysis

Degradability of the films in soil was studied by employing soil burial method. The soil samples were collected from the field and they are conditioned by adjusting the moisture level by to about 40-45%. The films from each enzyme combination of α-amylase and pullulanase as derived by response surface Box-Behnken design was used for the study. The films were cut into small pieces (1×1") and they were tied with a plastic thread and their weight was taken. A depth of 3 inches was pondered in the soil and the films were then buried in it. The pot was then covered with a foil paper to maintain its moisture level. Weight of the samples were then periodically measured in one week interval and the degradability of the film was

studied. Also films were visually observed to see the degradability of the films in soil.

The soil which was employed in the degradability study was taken for the test along with two controls. The soil sample after sun drying and sieving was used for was used for the study. NA medium for bacteria, RB for fungi and KK for actinomycetes were prepared. The medium and other glasswares used in the study were autoclaved at 121°C and 15 psi for 15 min. 10 g soil samples were then serially diluted and the samples were then plated into NA, RB and KK plates by pour plate technique. 10^{-6}, 10^{-5}, 10^{-4} dilution was plated in NA, KK and RB respectively. Triplicates were kept of each sample. The plates were then incubated at room temperature. After 48, 96 and 120 h, bacterial, fungal and actinomycetes colonies in each plate was counted respectively. Using this data microbial flora was calculated.

3.2.11. Packaging properties

Both α-amylase and pullulanase treated starch films were sealed to understand the sealability of the films by using hand sealer for different time intervals. After placing cashew nuts in the films, they were sealed at proper temperature using hand sealer. The weight of this packed film was measured and kept at room temperature. Weights were then taken after a time interval of one week. The difference between the initial and final weights was used to study the packaging quality of the films.

RESULTS

4. RESULTS

This chapter deals with the results obtained on the properties of the native starch, rheological properties of the enzymatically modified starch, physico-mechanical, functional and hygroscopic properties of the biodegradable films prepared from the modified starch. Biodegradability as judged by the soil burial test and storage studies are also included in this chapter.

4.1. PROPERTIES OF NATIVE STARCH

The starch obtained from 50 kg of tubers extracted by using the mobile starch extraction plant employing wet processing method was about 13.75 kg giving rise to a recovery of 27.5 percent. The properties of the freshly extracted starch were analyzed for their colour, rheology and functional properties. It was observed that the starch had a whiteness index of 98.04 and total colour difference of 1.43. Rheological properties showed the storage modulus as 1910Pa, loss modulus as 1845Pa, phase angle as 44° and complex viscosity as 42.25Pas. These values showed that the paste obtained from the starch exhibited visco-elastic nature. The swelling volume of starch was 53.5ml/g and solubility was 15.5 percent.

4.2. COLOUR OF FILMOGENIC SOLUTION

4.2.1. α-amylase treated starch solutions

The colour of the filmogenic solution calculated as the total colour difference and whiteness index is shown in Table 4. For the filmogenic solutions containing α-amylase, the total colour difference was found to be highest (77.59) for the solution having 100 µl enzymes prepared at 90°C for 30 min and lowest (72.21) for the filmogenic solution with 200 µl enzyme prepared at 85°C for 30 min. The highest value of whiteness index was found to be 27.13 for the filmogenic solution prepared

with 200 µl enzyme for 30 min at 85°C and minimum of 21.75 for the solution having 100 µl enzyme for 30 min at 90°C.

Table.4. Colour parameters of the filmogenic solutions containing α-amylase treated starch

Sample	Time, min	Temperature, °C	Enzyme concentration, µl	Total colour difference	Whiteness index
1	20	80	200	73.34	26
2	20	90	200	74.41	24.93
3	40	80	200	73.22	26.12
4	40	90	200	75.08	24.26
5	30	80	100	75.66	23.68
6	30	80	300	76.43	22.91
7	30	90	100	77.59	21.75
8	30	90	300	76.03	23.31
9	20	85	100	74.52	24.82
10	40	85	100	75.61	23.73
11	20	85	300	75.67	23.67
12	40	85	300	74.43	24.91
13	30	85	200	72.21	27.13
14	30	85	200	74.28	25.06
15	30	85	200	74.55	24.79

When comparing the colour parameters of the filmogenic solutions made with different enzyme concentration, for 100µl enzyme, the total colour difference was found to be highest (77.59) for 30 min at 90°C and lowest (74.52) for 20 min at 85°C whereas the whiteness index was highest (24.82) for 20 min at 85°C and lowest (21.75) for 30 min at 90°C. In the case of film solutions containing 200µl enzyme, the

total colour difference value was highest (75.08) for 40 min at 90°C and lowest (72.21) for 30 min at 85°C and the whiteness index was highest (27.13) for 30 min at 85°C and lowest (24.26) for 40 min at 90°C. For the film solution prepared with 300µl enzyme, the total colour difference value was found to be highest (76.43) for 30 min at 80°C and lowest (74.43) for 40 min at 85°C whereas the whiteness index was highest (24.91) for 40 min at 85°C and lowest (22.91) for 30 min at 80°C.

4.2.2. Pullulanase treated starch solution

The total colour difference and whiteness index value of the filmogenic solution containing pullulanase treated starch is shown in Table 5. In the case of filmogenic solutions containing pullulanase treated starch, total colour difference value was found to be highest 77.55 for the film solution made with 3U enzyme incubated at 55°C for 24h and lowest of 71.91 for the solution with 2U enzyme incubated at 45°C for 16h. The highest value for the whiteness index was found to be 27.43 for the filmogenic solution prepared with 2U enzyme incubated for 16h at 45°C and lowest 21.79 for 3U enzyme for 24h at 55°C.

When comparing the filmogenic solutions with different enzyme concentration, for 2U enzyme, the total colour difference of the film solutions was found to be highest (76.69) for 16h incubated at 55°C and lowest (71.91) for 16h incubated at 45°C. The whiteness index was found to be highest (27.43) for the filmogenic solution prepared with the starch incubated for 16h at 45°C and lowest (22.65) for 16h at 55°C. In the case of film solution with 3U enzyme, the total colour difference value of the film was found to be highest for 24h at 55°C (77.55) and lowest (74.12) for 16h at 50°C. The whiteness index of the film was found to be highest (25.22) for 16h at 50°C and lowest (21.79) for 24h at 55°C. For the filmogenic solution prepared with 4U enzyme, the total colour difference value of the film solution was found to be highest (76.43) for 16h at 45°C and lowest (74.78) for

24h at 50°C whereas the whiteness index was highest (24.56) for 24h at 50°C and lowest (22.91) for 16h at 45°C.

Table.5. Colour parameters of the filmogenic solutions containing pullulanase treated starch

Sample	Time, min	Temperature, °C	Enzyme concentration, (unit)	Total colour difference	Whiteness index
1	8	45	3	74.76	24.58
2	8	55	3	75.39	23.95
3	24	45	3	74.15	25.19
4	24	55	3	77.55	21.79
5	16	45	2	71.91	27.43
6	16	45	4	76.43	22.91
7	16	55	2	76.69	22.65
8	16	55	4	76.00	23.34
9	8	50	2	74.02	25.33
10	24	50	2	75.62	23.72
11	8	50	4	75.44	23.91
12	24	50	4	74.78	24.56
13	16	50	3	75.42	23.92
14	116	50	3	74.12	25.22
15	16	50	3	76.65	22.69

4.3. DEXTROSE EQUIVALENT OF FILMOGENIC SOLUTIONS

The dextrose equivalent (DE) of the filmogenic solutions prepared with of α-amylase and pullulanase treated starch are shown in Table 6 and 7. Factor 'F' was obtained as 87.8.

In the case of filmogenic solutions having α-amylase treated starch (Table 6), the DE was found to be highest (8.4) for the filmogenic solution made with 300µl enzyme prepared at 85°C for 40min and lowest of (1.6) was for the filmogenic solution with 100µl enzyme prepared at 90°C for 30min.When comparing the filmogenic solutions made with different enzyme concentration, for 100µl enzyme, the DE was found to be highest (2.6) for 40min at 85°C and lowest (1.6) for 30min at 90°C. In the case of film solutions containing 200µl enzyme, the DE value was highest (6) for 30 min at 85°C and lowest (2.2) for 20 min at 90°C. For the film solution prepared with 300µl enzyme, the DE value of the filmogenic solution was found to be highest (8.4) for 40 min at 85°C and lowest (4.5) for 30 min at 90°C.

Table 6. Dextrose equivalent of filmogenic solutions containing α-amylase

Sample	Time, min	Temperature, °C	Enzyme concentration, µl	Dextrose equivalent
1	20	80	200	4.2
2	20	90	200	2.2
3	40	80	200	5
4	40	90	200	4.2
5	30	80	100	2.1
6	30	80	300	6.5
7	30	90	100	1.6
8	30	90	300	4.5
9	20	85	100	2.4
10	40	85	100	2.6
11	20	85	300	8.1
12	40	85	300	8.4
13	30	85	200	5.1
14	30	85	200	6
15	30	85	200	5.5

In the case of filmogenic solutions containing pullulanase treated starch (Table 7), the DE was found to be highest (16) for the solution made with 4U enzyme prepared at 50°C for 8h and lowest of (2.2) was for the filmogenic solution with 2 enzyme prepared at 50°C for 8h. When comparing the filmogenic solutions made with different enzyme concentration, with 2U enzyme treated starch, the DE of the filmogenic solution was found to be highest (4.8) for 24h at 50°C and lowest (2.2) for 8h at 50°C. In the case of film solutions containing 3U enzyme, the DE value of the film solution was found to be highest (9.7) for 24h at 45°C and lowest (4.6) for 8h at 45°C. For the film solution prepared with 4U enzyme, the DE value of the filmogenic solution was found to be highest (16) for 8h at 50°C and lowest (13) for 16h at 55°C.

Table 7. Dextrose equivalent of filmogenic solutions containing pullulanase treated starch

Sample	Time, min	Temperature, °C	Enzyme concentration, µl	Dextrose equivalent
1	8	45	3	4.6
2	8	55	3	5.6
3	24	45	3	9.7
4	24	55	3	8.9
5	16	45	2	2.7
6	16	45	4	14
7	16	55	2	2.5
8	16	55	4	13
9	8	50	2	2.2
10	24	50	2	4.8
11	8	50	4	16
12	24	50	4	14
13	16	50	3	7.1
14	116	50	3	6.3
15	16	50	3	6.9

4.4.SUGAR PROFILE OF ENZYME TREATED STARCH.

The retention time of different sugars (standards) and those same sugars formed from enzyme treated cassava starch is given in Fig. 2-4. Retention time for standards of fructose, glucose, sucrose and maltose were 6.09, 6.77, 8.40, 9.49 min respectively. The retention times of sugars in various samples were different from each other.

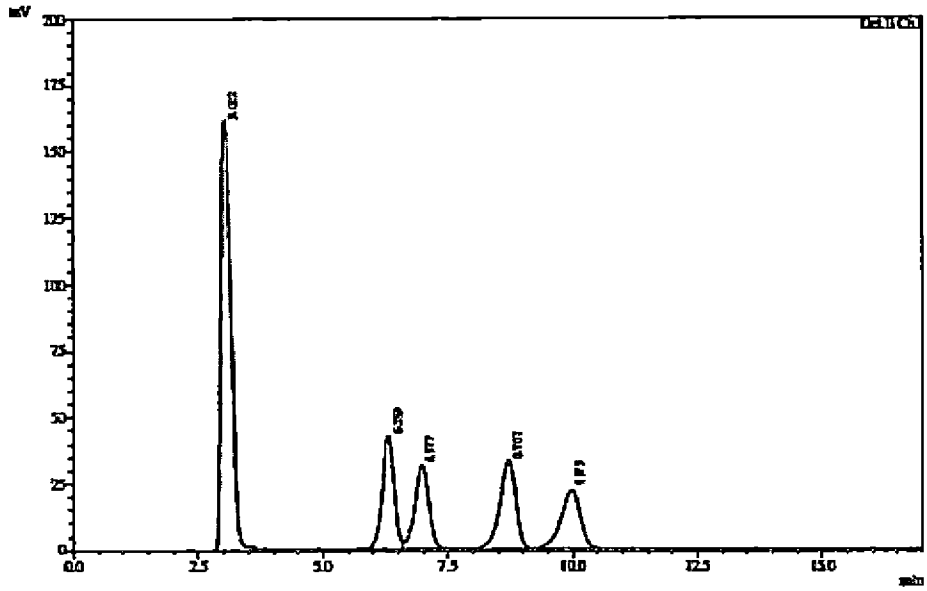


Fig.2. HPLC profile of oligosaccharide standards

Comparing with the standard's retention time almost all the sample possess the same range as that of standard. Retention time of standard glucose is 6.77 min, sucrose 8.40 min, maltose 9.49 min and for samples , glucose 6.72 min and maltose 9.32min. In all the samples, Maltose have the highest retention time.

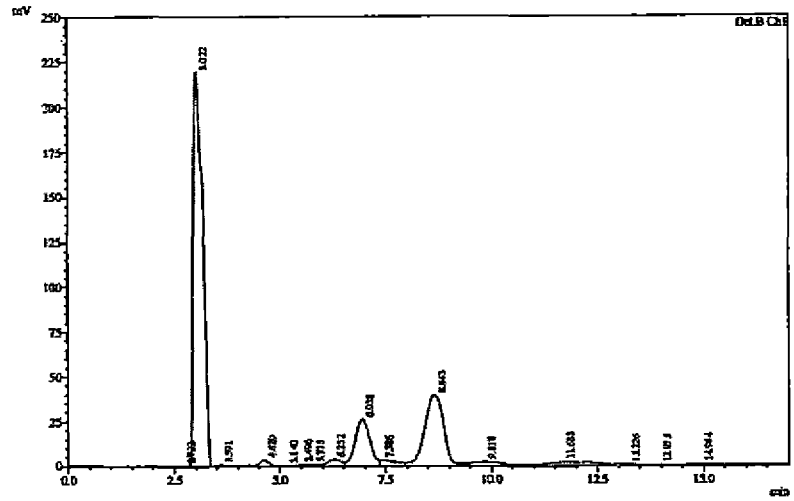


Fig.3. HPLC profile of various sugars formed from α- amylase treated starch

Comparing with the standard's retention time almost all the sample possesses the same range as that of standard. Retention time of standard glucose is 6.77 min, sucrose is 8.40min, maltose is 9.49 min and for the samples, glucose 6.56 min, maltose 9.01min. In all the samples, maltose have the highest retention time.

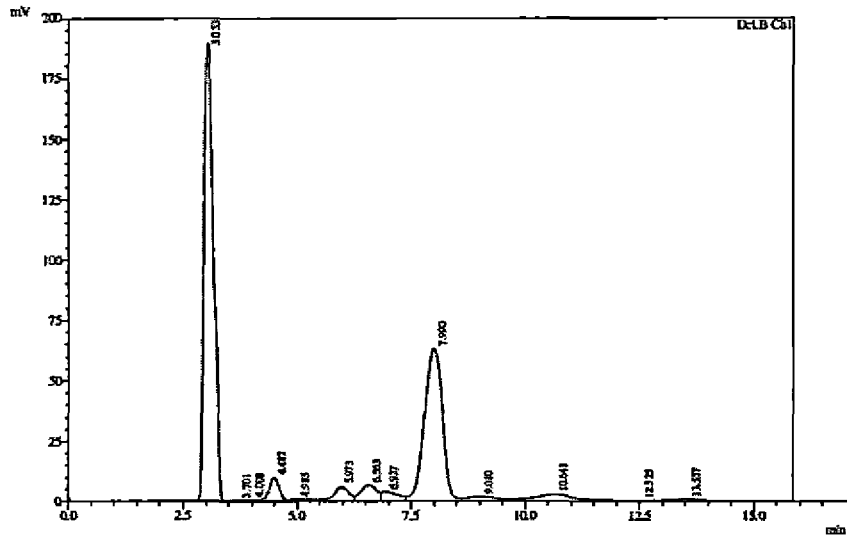


Fig. 4. HPLC profile of various sugars formed from pullulanase treated starch

4.5. RHEOLOGICAL PROPERTIES OF ENZYMATICALLY MODIFIED STARCH

4.5.1. α-Amylase modified starch

The variation of rheological parameters viz., storage modulus (G′), loss modulus (G″), phase angle (θ) and complex viscosity (η) with respect to enzyme concentration (E), time (T) and temperature (H) as plotted by response surface prediction profiles are represented in Fig. 5.

The enzyme concentration and temperature had both negative and positive quadratic effect on the storage modulus of the films whereas time had negative quadratic effect as is seen from the Fig.. Variation of the storage modulus (G′) of the films with respect to time and temperature is depicted by the equation:

$$G' = 1310.67 - 36.25T + 22.5H - 7.5E + 26.54T^2 - 183.75T*H + 6.25T*E + 46.54H^2 + 43.75T*E + 34.04*E^2$$

The loss modulus were found to have negative quadratic effect with enzyme concentration and positive effect with temperature whereas time has slightly negative quadratic effect. The loss modulus (G″) varied according to the equation:

$$G'' = 1146.67 - 9.75T + 58.63H - 44.13E + 2.42T^2 - 71.75T*H + 31.25T*E + 16.67H^2 - 49.5H*E + 19.67E^2$$

The enzyme concentration has negative quadratic effect on the phase angle whereas the temperature and time has positive quadratic effect. The phase angle of the filmogenic solution varied according to the equation:

$$0 = 42.42 + 0.78T + 1.07H - 0.79E - 0.95T^2 + 2.09T*H + 0.68*T*E - 1.13H^2 - 2.05T*E - 0.84E^2$$

Complex viscosity of the solution linearly decreased with time whereas enzyme concentration and temperature has no significant effect, it varied according to the equation:

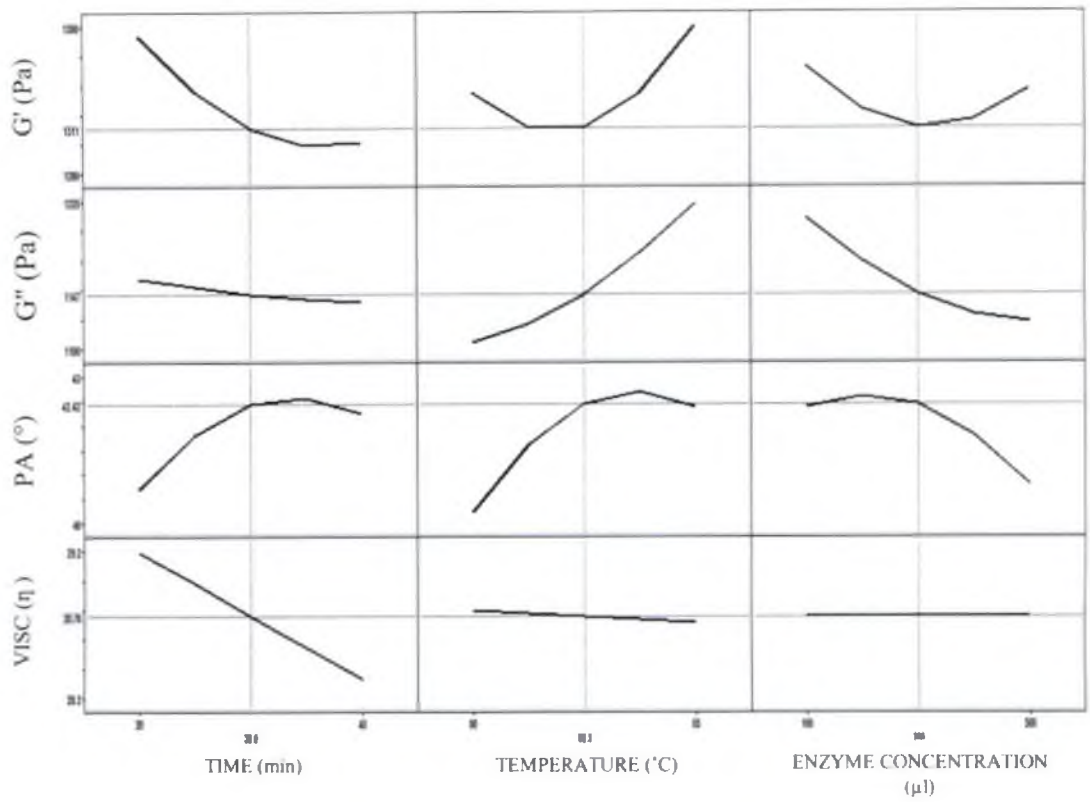
$\eta = 28.76 - 2.95\ T*H$

Maximum storage modulus of 1605 Pa was obtained for the solution made with 200µl enzyme concentration gelatinized for 20min at 90°C whereas the solution prepared at 90°C for 40min had a minimum value of storage modulus 1085 Pa.

The rheological properties were compared for the filmogenic solutions containing different enzyme concentration and it was observed that for 100µl enzyme concentration, the storage modulus of the film was found to be highest (1415 Pa) for 30 min at 90°C and lowest (1335 Pa). for 30 min at 80°C whereas for 200µl enzyme concentration, the storage modulus of the film was found to be maximum (1605 Pa) for 20 min at 90°C and minimum (1085 Pa) for 40 min at 90°C and for the 300µl enzyme concentration, storage modulus of the solution was found to be highest (1535 Pa) for 30 min at 90°C and lowest (1280 Pa) for 30 min at 80°C gelatinization temperature.

Maximum loss modulus of 1385 Pa was obtained for the starch solution added with 200µl enzyme and heated for 20min at 90°C whereas minimum of 983 Pa for the solution containing 200µl enzyme gelatinized for 20min at 80°C.

When comparing the samples with different enzyme concentrations, it was found that, for 100µl enzyme concentration, the loss modulus of the film was highest (1265 Pa) for 30 min at 90°C and lowest (1180 Pa) for 40 min at 85°C



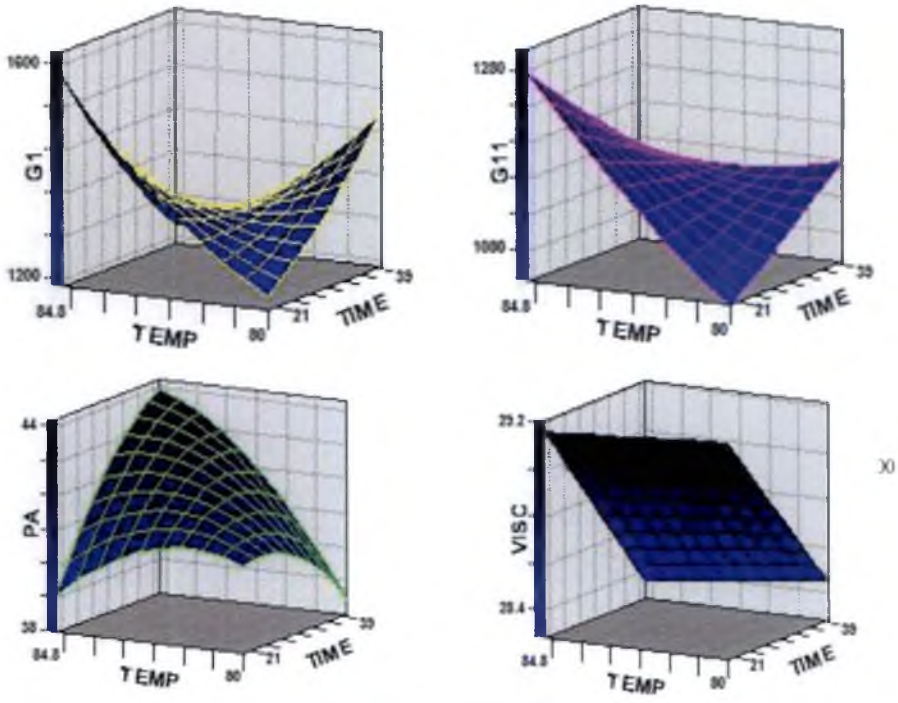


Fig.5. Rheological properties of the filmogenic solutions containing α-amylase treated cassava starch

whereas with 200µl enzyme concentration, the highest loss modulus(1385 Pa) of the film was observed for 20 min at 90 °C and lowest (983 Pa) for 20 min at 80°C and with 300 µl enzyme concentration, the value was found to be highest 1200 Pa) for 30 min at 80°C and lowest (1077 Pa) for 30 min at 90°C.

Maximum phase angle of 48° was obtained for the solution made with 200µl enzyme, heated for 40min at 90°C whereas minimum of 35.15° for the solution having 300µl enzyme prepared at 90°C for 30min. When comparing the values of phase angle of the solutions with different enzyme concentration, it was found that, for 100µl enzyme concentration, the phase angle of the solution was found to be highest (41.9°) for 30 min at 90°C and lowest for 40 min at 85°C (40.25°). In the case of 200µl enzyme concentration, the phase angle of the film was found to be highest for 40 min at 90 °C (48°) and lowest for 40 min at 80°C (35.7°) and the phase angle of the film was found to be highest for 30 min at 80°C (43.1°) and lowest for 30 min at 90°C (35.15°) for films with 300 µl enzyme concentration.

Maximum complex viscosity of 33.85 Pas was obtained for the film made with 200µl enzyme, heated for 20min at 90°C whereas minimum of 25.85 Pas for the solution having 200µl enzyme, heated at 40min at 90°C. When comparing the samples with different enzyme concentration for 100µl enzyme concentration, the complex viscosity of the film was found to be highest (30.35 Pas) for 30 min at 90°C and lowest (28.45 Pas) for 30 min at 80°C. In the case of 200µl enzyme concentration, the complex viscosity of the film was found to be highest (33.85 Pas) for 20 min at 90°C and lowest (25.85Pas) for 40 min at 90°C. It was found to be highest (30 Pas) for 30 min at 90 °C and lowest for 20 min at 85 °C (27.4 Pas) for the solution containing 300 µl enzyme concentration.

4.5.2. Pullulanase modified starch

The variation of rheological parameters of the filmogenic solutions containing the pullulanase enzyme *viz.*, storage modulus (G′), loss modulus (G″), phase angle (θ) and complex viscosity (η) with respect to enzyme concentration (E), time (T) and

temperature (H) as plotted by response surface prediction profiles are represented in Fig. 6..

The enzyme concentration has positive quadratic effect on the storage modulus of the films. Initially the temperature shows positive quadratic effect and eventually obtains negative quadratic effect. As time increased, storage modulus shows linearly decreasing trend.

The storage modulus of the films varied according to the equation:

$$G' = 1730 - 75.62*T + 13.12*H + 78.75*E - 10.62*T^2 + 63.75*T*H - 2.5*T*E - 155.62*H^2 - 47.5*H*E - 39.37*E^2$$

The loss modulus of the solutions were found to be negative quadratic effect with respect to enzyme concentration. The increase in temperature had both positive and negative quadratic effect. Initially time shows negative quadratic effect and eventually obtains a positive quadratic effect.

The loss modulus varied according to the equation:

$$G'' = 1146.66 - 30*H - 61.25*E + 179.16*T^2 + 55*T*H + 30*T*E - 75.83*H^2 + 2.5*H*E + 64.16*E^2.$$

The enzyme concentration and temperature had negative quadratic effect on the phase angle of the solutions. Initially time shows negative quadratic effect and eventually shows a positive quadratic effect.

The phase angle varied according to the equation:

$$0 = 34.03 + 1.26*T - 0.84*H - 2.85*E + 3.82*T^2 - 0.2*T*H + 0.75*T*E + 0.91*H^2 + 0.76*H*E + 2.01*E^2.$$

The enzyme concentration shows a slightly increasing trend in the complex viscosity. Initially the temperature shows positive quadratic effect and eventually obtains negative quadratic effect. The complex viscosity of the film shows negative quadratic effect with respect to time.

The complex viscosity varied according to the equation:

$$\eta = 33.11-0.94*T-0.15*H+0.30*E+1.59*T^2+1.27*T*H+0.23*T*E - 2.73*H^2 - 0.65*H*E + 0.14*E^2.$$

Maximum storage modulus of 2060Pa was obtained for the film made with 3U enzyme, 16h, 50°C whereas minimum of 1280Pa for 3U enzyme, 24h, 45°C.When comparing the samples with different enzyme concentration, for 2U enzyme concentration, the storage modulus of the film was found to be highest for 8h at 50°C (1590Pa) and lowest for 16h at 55°C (1470Pa). In the case of 3U enzyme concentration, the storage modulus of the film was found to be highest for 16h at 50°C (2060Pa) and lowest for 24h at 45°C (1280 Pa) and the storage modulus of the film was found to be highest for 8h at 50°C (1870 Pa) and lowest for 16h at 55°C (1415Pa) for films with 4U enzyme concentration.

Maximum loss modulus of 1485 Pa was obtained for the film made with 2U enzyme, 24h, 50°C whereas minimum of 1060 Pa for 4U enzyme, 16h, 45°C.When comparing the samples with different enzyme concentration for 2U enzyme concentration, the loss modulus of the film was found to be highest for 24h at 50°C (1485 Pa) and lowest for 16h at 45°C (1150 Pa). In the case of 3U enzyme concentration, the loss modulus of the film was found to be highest for 8 h at 45 °C (1440Pa) and lowest for 16h at 50 °C (1100 Pa) and the loss modulus of the film was found to be highest for 24h at 50 °C (1385 Pa) and lowest for 16h at 45 °C (1060 Pa) for films with 4U enzyme concentration.

Maximum phase angle of 44.75° was obtained for the film made with 2U enzyme, 24h, 50°C whereas minimum of 30.3° for 3U enzyme, 16h, 50°C. When comparing the samples with different enzyme concentration for 2U enzyme concentration, the phase angle of the film was found to be highest for 24h at 50°C (44.75°) and lowest for 16h at 45°C (36.8°). In the case of 3U enzyme concentration, the phase angle of the film was found to be highest for 24h at 45 °C (44.2°) and lowest for 16h at 50 °C (30.3°) and the phase angle of the film was found to be

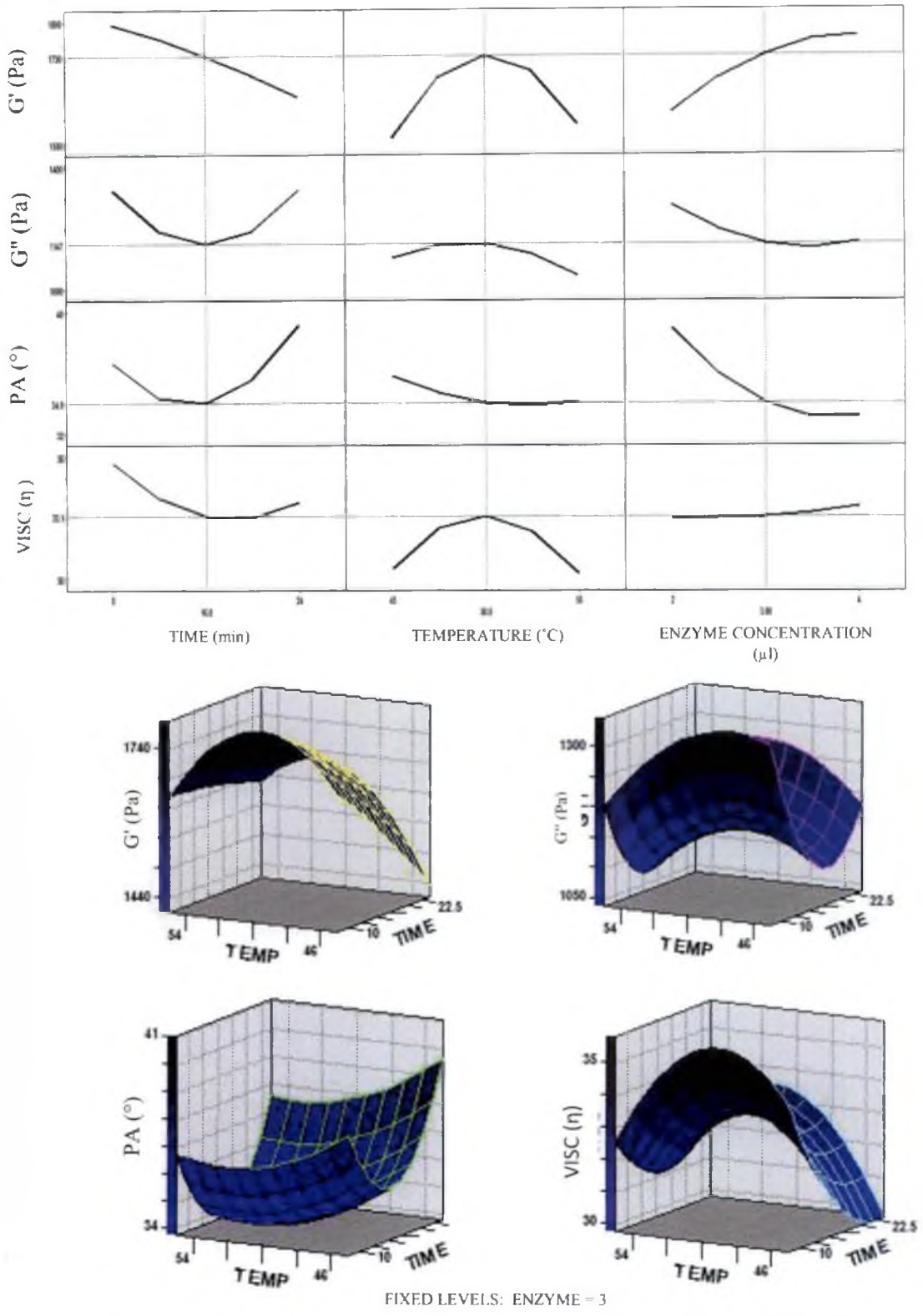


Fig.6. Rheological properties of filmogenic solutions containing pullulalanase treated cassava starch

highest for 16h at 55 °C (38.65°) and lowest for 16h at 45 °C (32.05 °) for films with 4U enzyme concentration.

Maximum complex viscosity of 37.9 Pas was obtained for the film made with 3U enzyme, 16h, 50°C whereas minimum of 28.45 Pas for 3U enzyme, 24h, 45°C. When comparing the samples with different enzyme concentration for 2U enzyme concentration, the complex viscosity of the film was found to be highest for 8h at 50°Ctemperature (34.5 Pas) and lowest for 16h at 55°C (30.5 Pas). In the case of 3U enzyme concentration, the complex viscosity of the film was found to be highest for 16h at 50 °C (37.9Pas) and lowest for 24h at 45 °C (28.45 Pas) and the complex viscosity of the film was found to be highest for 24 h at 50 °C (35.7Pas) and lowest for 16h at 55 °C (28.75 Pas) for films with 4U enzyme concentration.

4.6. PROPERTIES OF THE BIODEGRADABLE FILMS

The biodegradable films were developed as per the experimental conditions and the films were subjected to various physico mechanical, functional and water absorption properties. Representative picture of the films are represented in Plate 5.

4.6.1.Films with α-Amylase modified starch

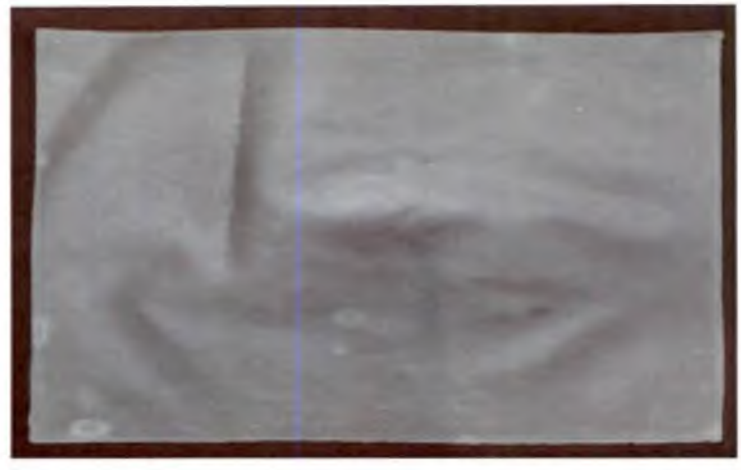
4.6.1.1. Colour of the Films

The variation of the colour parameters viz., total colour difference from the reference white sample and whiteness index with respect to different proportions of enzyme, time and temperature of gelatinization as plotted by response surface and prediction profiles are represented in Fig. 7

The enzyme concentration and time has mostly negative quadratic effect on the total colour difference of the films whereas temperature has both negative and positive quadratic effect. As expected the whiteness index values for all the combinations are just reverse of the total colour changes i.e., whiteness index of the film has positive quadratic effect with respect to enzyme concentration and time and

Native starch based film

α-amylase based film



Pullulanase based film

Plate 5. Film samples developed by using native starch, α-amylase and pullulalanse

temperature has positive and negative quadratic effect. The total colour change (TCD) and whiteness index (WI) of the films varied according to the equation:

$$\text{Total colour difference} = 62.56 - 0.74T + 0.099H - 1.24E - 0.23T^2 + 0.42T*H - 0.28T*E + 0.73H^2 - 0.11T*E + 1.58E^2$$

$$\text{Whiteness index} = 36.78 + 0.75T - 0.098T + 1.24E + 0.23T^2 - 0.43T*H + 0.28T*E - 0.73H^2 + 0.11H*E - 1.58E^2$$

Total colour change was found to be highest 66.66 for the film made with the solution having 100 µl enzymes prepared at 90°C for 30 min and lowest of 60.9 was for the film with the solution having 200 µl enzyme prepared at 85 °C for 30 min. The highest value for the whiteness index was found to be 38.45 for the film prepared with 200 µl enzyme containing solution gelatinized for 30 min at 85°C and minimum of 32.68 for 100 µl enzyme gelatinized for 30 min at 90°C.

When comparing the films made from the solution having different enzyme concentration, for 100µl enzyme, the total colour difference of the film was found to be highest (66.66) for 30 min at 90°C and lowest (63.77) for 40 min at 85°C. The whiteness index of the film was found to be highest (35.58) for 40 min at 85°C and lowest (32.68) for 30 min at 90°C. In the case of films containing for 200µl enzyme, the total colour change value of the film was found to be highest for 30 min at 85°C (64.85) and lowest (60.9) for 30 min at 85°C. The whiteness index of the film was found to be highest (38.45) for 30 min at 85°C and lowest (34.49) for 30 min at 85°C. For the film prepared with 300µl enzyme, the total colour difference value of the film was found to be highest (64.63) for 20 min at 85°C and lowest (61.15) for 40 min at 85°C where as the whiteness index of the film was found to be highest (38.19) for 40 min at 85°C and lowest (34.71) for 20 min at 85°C.

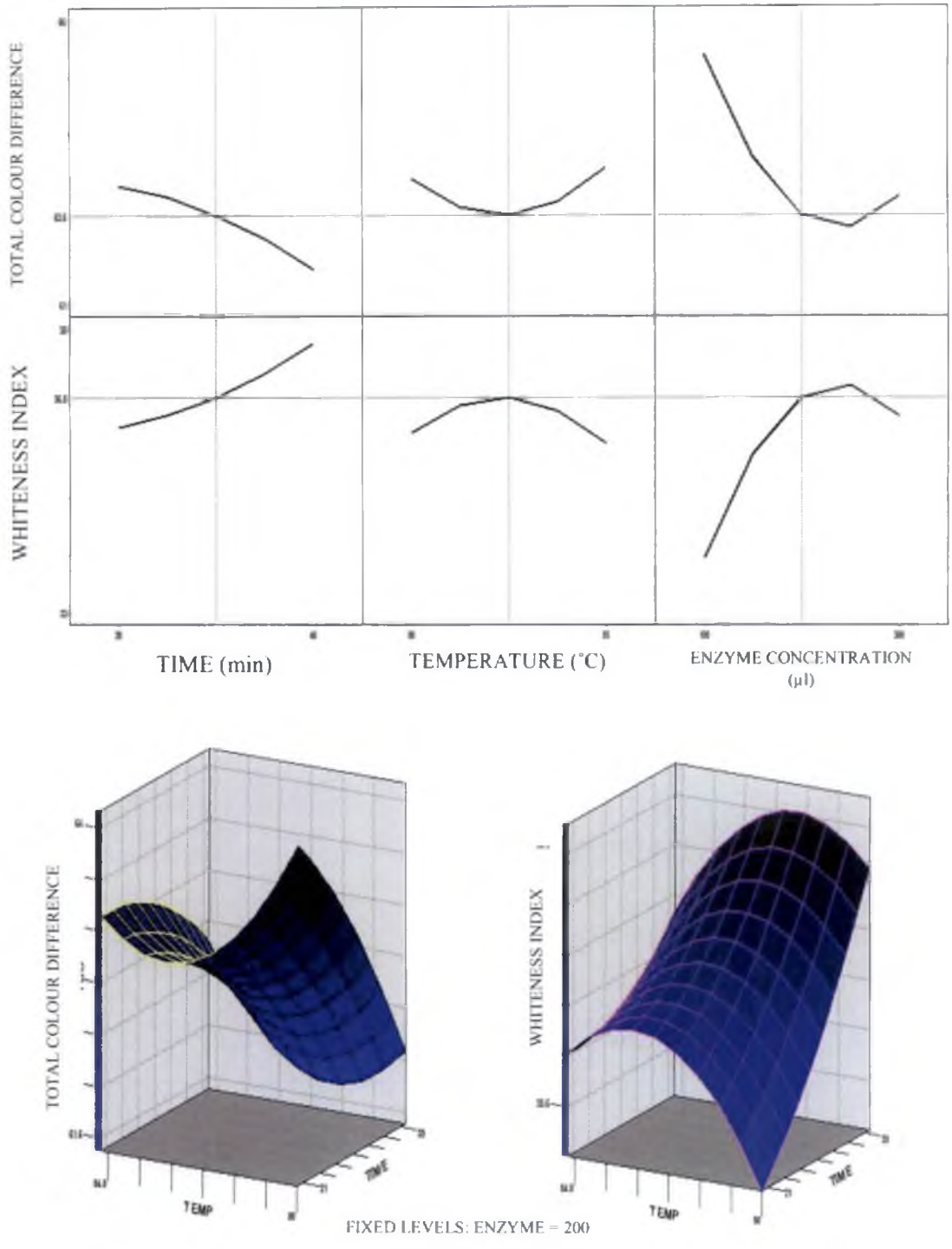


Fig. 7. Colour properties of biodegradable films made from the α-amylase treated cassava starch

4.6.1.2. Thickness

The variation of thickness with respect to different proportions of enzyme, time and temperature as plotted by response surface and prediction profiles are depicted in Fig .8..

It was found that thickness of the film was strongly influenced by both the enzyme concentration and time and was quadratically increasing with increase in enzyme concentration and time. Temperature shows a quadratic increase in the beginning and then apparently shows a quadratic decrease. The thickness of the film was influenced by enzyme concentration, time, temperature according to the equation,

Thickness = 0.18 + 0.0087*E.

Thickness was found to be highest (0.201mm) for film made from the solution with 300 µl enzyme heated for 30 min at 80°C and lowest (0.169mm) for film with having 100 µl enzyme for 30 min at 90°C. While comparing the films made with different enzyme concentration, it was found that for 100µl enzyme concentration, the thickness of the film was found to be highest(0.195mm) for 40 min at 85°C and lowest (0.169mm) for 30 min at 90°C. The thickness of films having 200 µl enzyme concentration was found to be maximum (0.198mm) for 30 min at 85°C and minimum (0.170mm). for 20 min at 80°C whereas for the films with 300 µl enzyme, thickness was found to be highest (0.201mm) for 30 min at 80°C and lowest (0.189mm)for 20 min at 85°C.

4.6.1.3. Moisture Content

The variations in moisture content of the films with respect to different proportions of enzyme, time and temperature as plotted by response surface and prediction profiles in Fig.8. From these values, it is clear that the enzyme concentration and temperature have apparently high effect on the moisture content of the film when compared to time. Enzyme concentration and temperature has negative quadratic effect on the moisture content of the films.

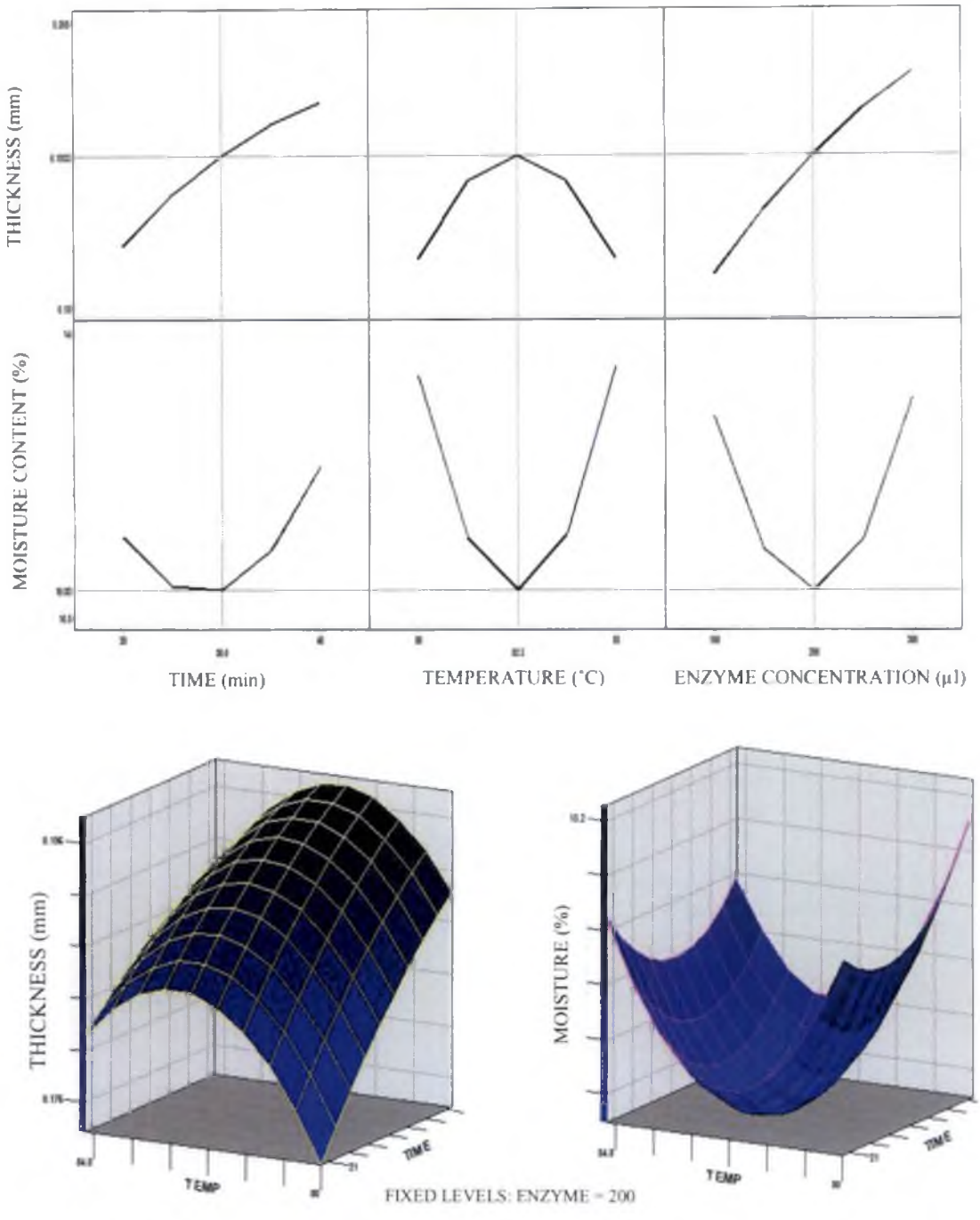


Fig. 8. Physical properties of biodegradable films made from the α-amylase treated cassava starch

The variation in moisture content of the film with reference to enzyme, time and temperature is explained by the equation,

$$\text{Moisture content} = 10.83 + 0.44T + 0.05H + 0.12E + 1.08T^2 - 0.48T*H - 0.57T*E + 2.67H^2 + 1.16H*E + 2.25E^2$$

Enzyme concentration and temperature have apparently high effect on the moisture content of the film. The moisture content of the film was found to be highest (17.6%) for the films containing 300µl enzyme prepared for 30 min at 90°C and lowest (10.47%) for 200µl enzyme, 30 min, 85°C. While comparing the films with, 100µl enzyme concentration, the moisture content of the film was found to be highest for 30 min at 80°C (16.23m%) and lowest for 20 min at 85°C (13.18%). The moisture content of films having 200 µl enzyme concentration was found to be highest for 40 min at 80°C (15.37%) and lowest for 30 min at 85°C (10.47%). And the moisture content of the films with 300 µl enzyme was found to be highest for 30 min at 90°C (17.6%) and lowest for 20 min at 85°C (13.84%).

As the enzyme concentration and temperature increases, the moisture content of the film was found out to be decreasing initially and then it began to increase. At initial state, it was found that, as time increases, moisture content of the decreases and at a further increase in time, moisture content is found to have a positive quadratic effect. The influence of time on film moisture content was less significant when compared to enzyme concentration and temperature.

4.6.1.4. Mechanical properties

The variations in tensile force, toughness and elongation at break of the films with respect to different enzyme concentration, time and temperature is represented as response surface and prediction profile in Fig.9.

Tensile force of the film was found to be quadratically decreased initially with respect to enzyme concentration and eventually shows a quadratic increase and time and temperature shows a positive quadratic effect.

The tensile force (TF) of the films varied according to the equation:

$$\text{Tensile force} = 3.91 + 0.56T + 1.4H - 1.07E + 0.69T^2 - 0.56T*H + 0.84T*E - 1.27H^2 + 0.31H*E + 5.85\,E^2$$

The toughness of the films was found to be quadratically decreased initially and eventually shows a quadratic increase with respect to enzyme concentration. The temperature shows a positive quadratic effect whereas increased linearly with the increase in time.

The toughness varied according to the equation,

$$\text{Toughness} = 6.75 + 5.73T + 5.30H - 4.37E - 0.36T^2 - 1.01T*H - 3.58T*E + 9.53\,H^2 - 3.99H*E + 19.24E^2$$

The percentage of elongation at break of the films was found to have a positive quadratic effect with respect to enzyme concentration, whereas quadratically decreased initially and eventually shows a increase with respect to temperature and with time, it showed a slightly positive quadratic effect. The percentage of elongation at break varied according to the equation:

$$\text{Elongation} = 7.52 + 0.11*T - 0.57*H + 0.96*E + 0.80*T^2 + 0.71*T*H - 1.34*T*E + 6.24*H^2 - 2.54*H*E - 0.75*E^2.$$

Maximum tensile force of 14.41N was obtained for the film made with 100µl enzyme for 30 min at 90°C whereas minimum was obtained as 1.94N for 200 µl enzyme, 20 min, 90°C. When comparing the properties of the film made from the solutions with different enzyme concentrations, it was observed that, for 100 µl enzyme concentration, the tensile force of the film was highest for 30 min at 90°C (14.41N) and lowest for 30 min at 80°C (7.84 N). In the case of 200 µl enzyme concentration, tensile force was found to be highest for 30 min at 85°C (6.11N) and lowest for 20 min at 90°C (1.94 N) and with 300 µl enzyme, it was found to be highest for 40 min at 85°C (11.74 N) and lowest for 30 min at 80°C (1.96 N).

Maximum toughness of 66.81Ns was obtained for the film made with 100µl enzyme, 30 min at 90°C whereas minimum of 1.17N of 200 µl enzyme, 30

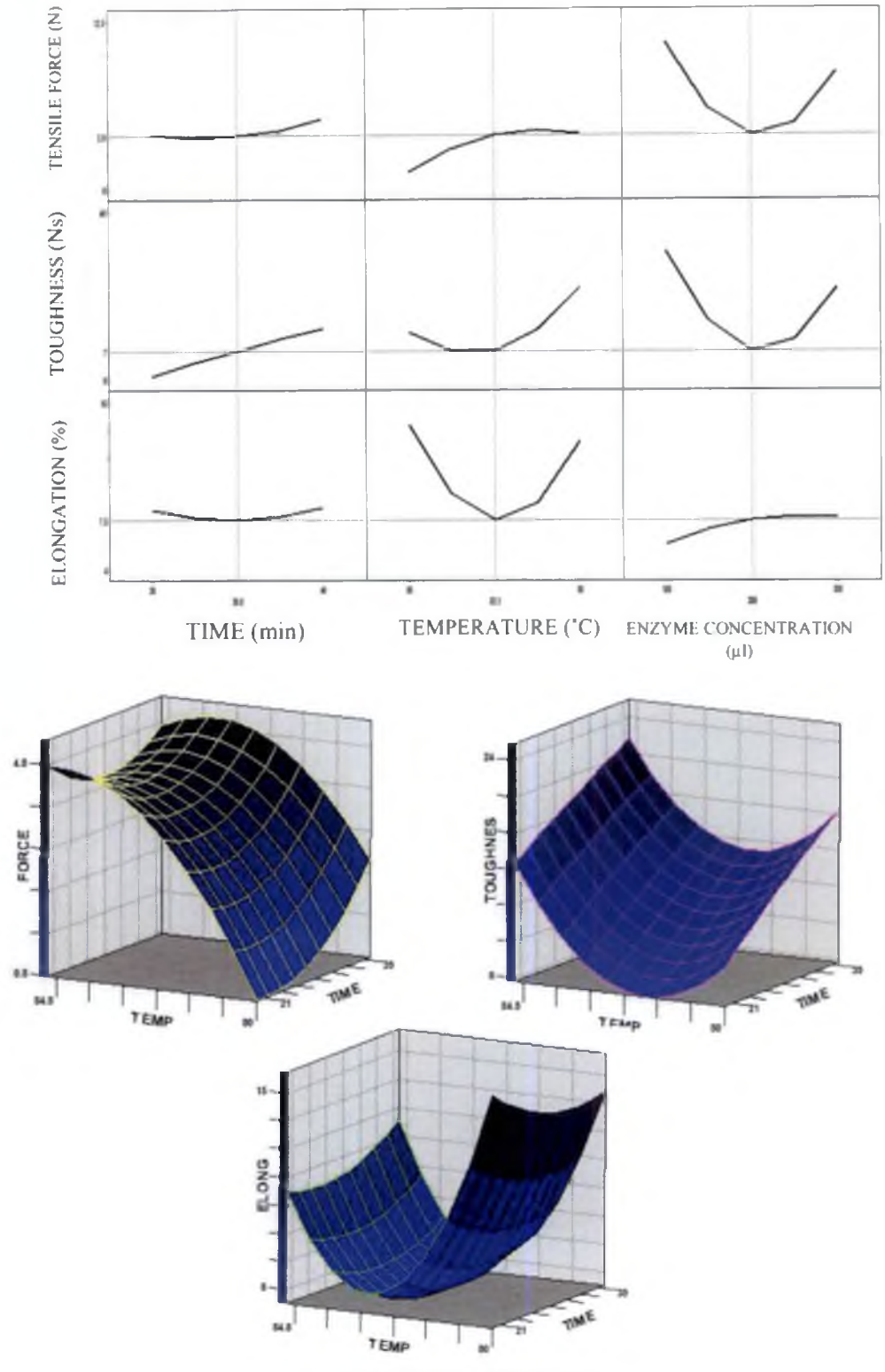


Fig. 9. Mechanical properties of biodegradable films made from the α-amylase treated cassava starch

min, 85°C. When comparing the films with different enzyme concentration, for 100 µl enzyme concentration, the toughness of the film was found to be highest for 30 min at 90°C (66.81N) and lowest for 20 min at 85°C (9.41 N). In the case of 200 µl enzyme concentration, toughness was found to be highest for 40 min at 80°C (23.74N) and lowest for 30 min at 85°C (1.17 N) and with 300 µl enzyme concentration, it was found to be highest for 40 min at 85°C (34.67 N) and lowest for 30 min at 80°C (12.2 N).

Maximum elongation at break of 19.07% was obtained for the film made with 300µl enzyme, 30 min at 80°C whereas minimum of 3.01% for 200 µl enzyme, 30 min and 85°C. When comparing the films with different enzyme concentration, for 100 µl enzyme concentration, the elongation at break of the film was found to be highest for 30 min at 90°C (12.05%) and lowest for 20 min at 85°C (4.46%). In the case of 200 µl enzyme concentration, elongation at break of the film was found to be highest for 20 min at 90°C (15.87%) and lowest for 30 min at 85°C (3.01%) and the elongation at break of the films with 300 µl enzyme was found to be maximum for 30 min at 80°C (19.07%) and minimum for 40 min at 85°C (8.01%).

4.6.1.5. Swelling capacity and Solubility

The variations in swelling capacity Volume and Solubility of the films with respect to different proportions of enzyme, time and temperature as plotted by response surface and prediction profile are represented in Fig.10.

Swelling capacity of the films has positive quadratic effect with the increase in enzyme concentration. For swelling capacity, the temperature has negative quadratic effect. It was found that the swelling volume of the film was influence by the time in an linearly increasing fashion. The swelling capacity of the films varied according to the equation:.

$$\text{Swelling capacity} = 165.57 + 20.55T + 7.01H + 23.41E - 1.48T^2 - 6.58*TH + 37.22T*E - 23.95\,H^2 + 5.08\,T*E + 46.99E^2$$

Solubility of the films was strongly influenced by the enzyme concentration and it was linearly increased with increase in enzyme concentration. For solubility, the temperature has negative quadratic effect and time showed slightly positive quadratic effect. The solubility of the films varied according to the equation:

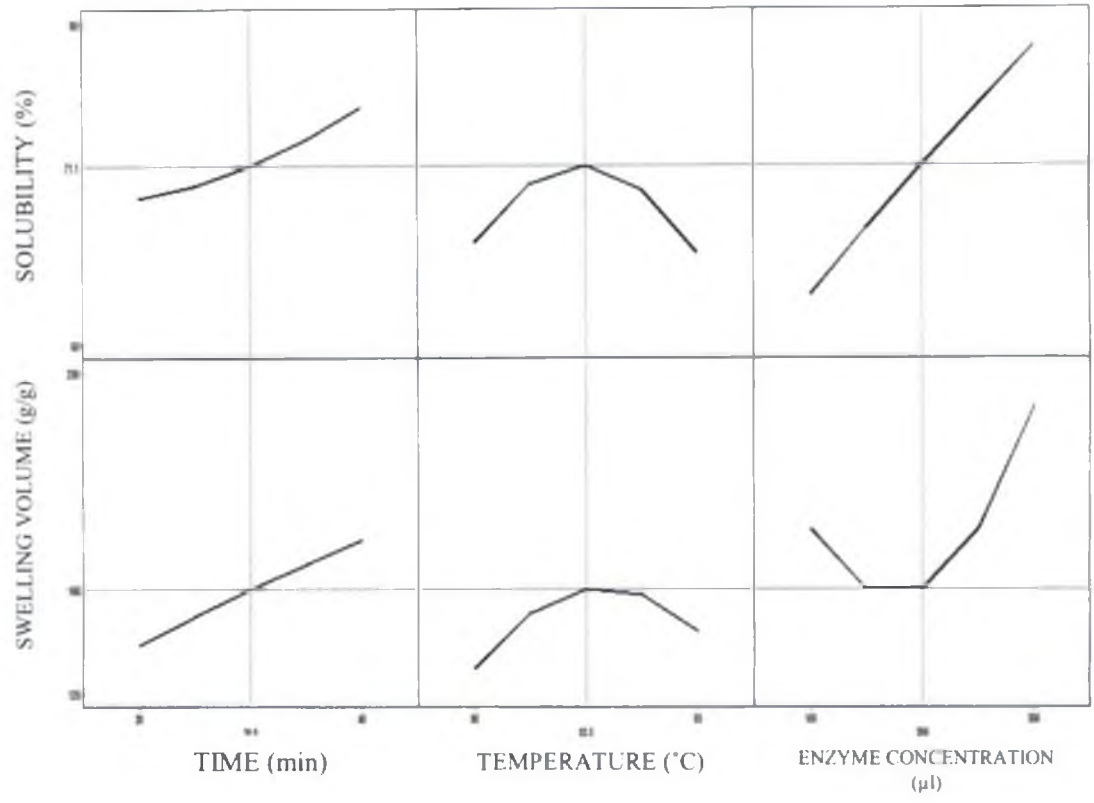
Solubility = 68.63 + 7.70*E.

Maximum swelling capacity of 315.20g/g was obtained for the film made with 300µl enzyme, 40 min at 85°C whereas minimum of 128.25g/g for the film having of 200 µl enzyme, 20 min, 80°C. While comparing the films with different enzyme concentration, for 100 µl enzyme , the swelling capacity of the film was found to be highest for 30 min at 90°C (191.63g/g) and lowest for 30 min at 80°C (163.95g/g). In the case of 200 µl enzyme concentration, swelling capacity of the film was found to be highest for 30 min at 85°C (173.45g/g) and lowest for 20 min at 80°C (128.25g/g) and the swelling capacity of the films with 300 µl enzyme was found to be highest for 40 min at 85°C (315.20g/g) and lowest for 30 min at 80°C (175.41g/g).

Maximum solubility of 80.08% was obtained for the film made with 300µl enzyme, 40 min at 85°C whereas minimum was obtained for 54.85% of 100 µl enzyme, 30 min, 80°C. In the case of films with 100 µl enzyme , the solubility of the film was found to be highest for 40 min at 85°C (75.42%) and lowest for 30 min at 80°C (54.85%). In the case of 200 µl enzyme concentration, solubility of the film was found to be highest for 30 min at 85°C (74.87%) and lowest for 20 min at 90°C (65.03%) and the solubility of the films with 300 µl enzyme concentration was found to be highest for 40 min at 85°C (80.08%) and lowest for 20 min at 85°C (73.30%).

4.6.1.6. Water Permeability and Water Absorption Properties

The variations in water permeability and water sorption of the films with respect to different proportions of enzyme, time and temperature as plotted by



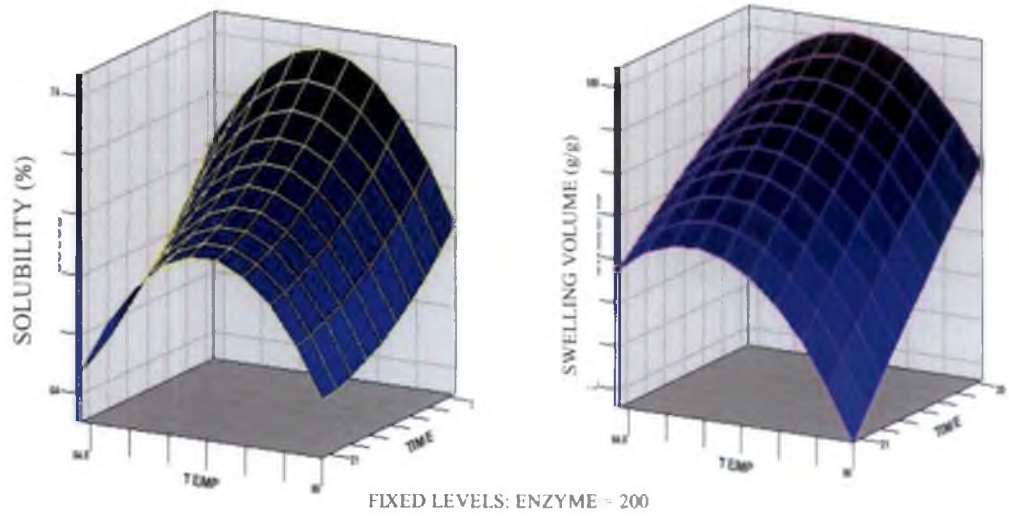


Fig. 10. Functional properties of biodegradable films made from α-amylase treated cassava starch

response surface and prediction profiles in Fig.11.

The water permeability had positive quadratic effect with respect to enzyme concentration and time whereas temperature had no significant effect on water permeability. Water permeability of the films varied according to the equation:

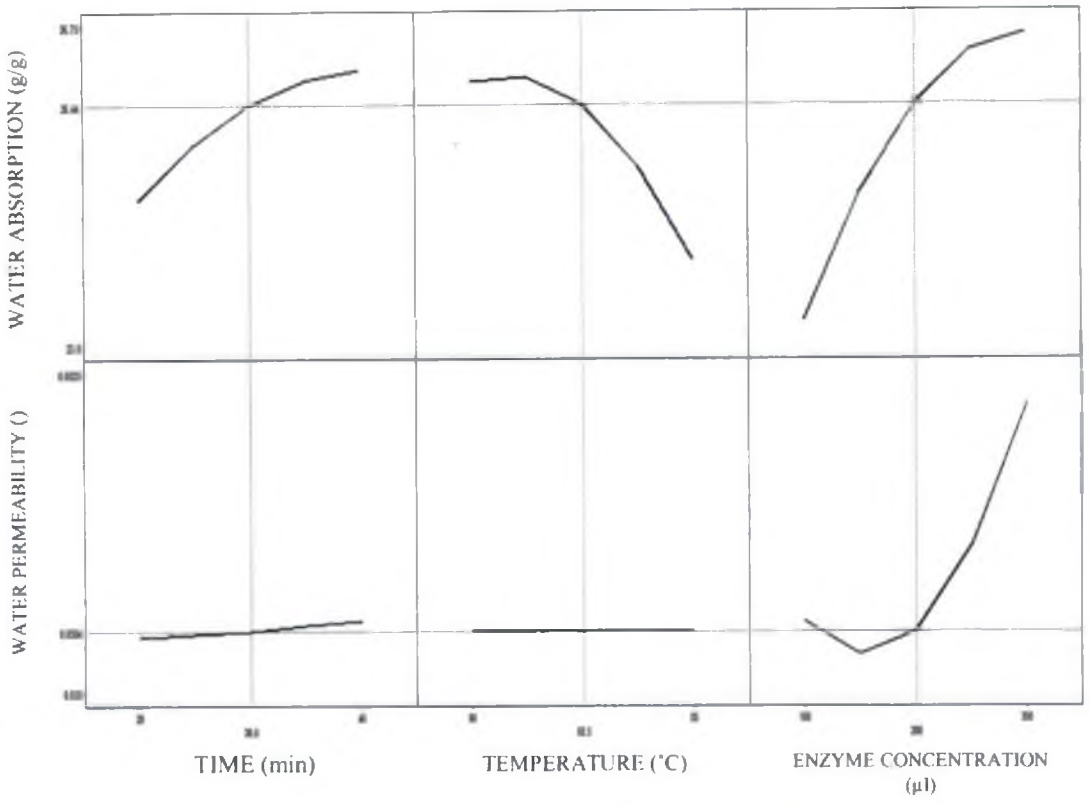
Permeability = $0.036 + 0.006E + 0.005T^2 + 0.0068E^2$

Both enzyme concentration and time shows positive quadratic effect and temperature shows negative quadratic effect on water absorption, it varied according to the equation:

Water absorption = 26.08 - 0.89*T*E.

Maximum water permeability of 0.052 was obtained for the film made with 300µl enzyme, 30 min at 90°C whereas minimum was obtained as 0.031 of 200 µl enzyme, 30 min, 85°C. When comparing the samples with different enzyme concentrations, for 100 µl enzyme, the water permeability of the film was found to be highest for 20 min at 85°C (0.043) and lowest for 30 min at 90°C (0.035). In the case of 200 µl enzyme concentration, water permeability of the film was found to be highest for 40 min at 90°C (0.044) and lowest for 30 min at 85°C (0.031) and with 300 µl enzyme, it was found to be highest for 30 min at 90°C (0.052) and lowest for 30 min at 80°C (0.048).

Maximum water absorption of 27.16g/g was obtained for the film made with 300µl enzyme, 30 min at 80°C whereas minimum was obtained as 24.75g/g for 100 µl enzyme, 20 min, 85°C. While comparing the films with different enzyme concentrations, for 100 µl enzyme, the water absorption of the film was found to be highest for 40 min at 85°C (26.77g/g) and lowest for 20 min at 85°C (24.75g/g). In the case of 200 µl enzyme concentration, water absorption of the film was found to be highest for 30 min at 85°C (26.88 g/g) and lowest for 20 min at 90°C (25.15g/g) and the water absorption of the films with 300 µl enzyme was found to be highest for 30 min at 80°C (27.16g/g) and lowest for 40 min at 85°C (25.52g/g).



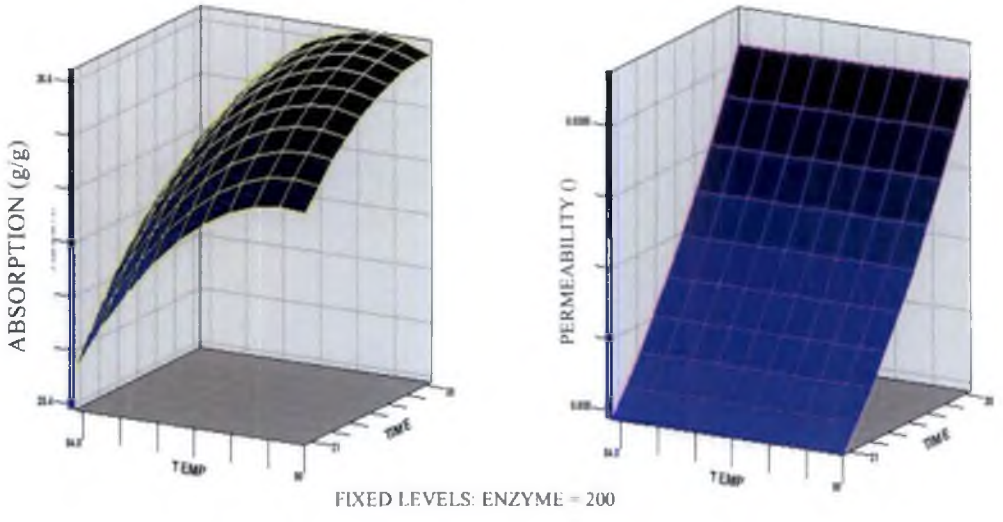


Fig.11. Water absorption and water permeability of biodegradable films made from the α-amylase treated cassava starch

4.6.2. FILMS WITH PULLULANASE MODIFIED STARCH

4.6.2.1. Colour

The variation of the colour parameters of the films containing pullulanase modified starch viz., total colour difference from the reference white sample and whiteness index with respect to different proportions of enzyme, time and temperature of gelatinization as plotted by response surface and prediction profiles are represented in Fig.12.

The enzyme concentration and time has mostly positive quadratic effect on the total colour difference of the films whereas temperature has negative quadratic effect. As expected the whiteness index values for all the combinations are just reverse of the total colour changes i.e., whiteness index of the film has negative quadratic effect with respect to enzyme concentration and time and temperature has positive quadratic effect. The total colour change (TCD) and whiteness index (WI) of the films varied according to the equation:

$$TCD = 64.12 + 0.43*T - 0.28625*H + 0.41*E + 0.27*T^2 - 0.53*T*H + 0.66*T*E + 0.18*H^2 - 0.06*H*E - 0.13*E^2.$$

$$WI = 35.21 - 0.43*T + 0.29*H - 0.42*E - 0.27*T^2 + 0.53*T*H - 0.66*T*E - 0.18*H^2 + 0.06*H*E + 0.13*E^2.$$

Total colour change was found to be highest 66.06 for the film made with the solution having 3 U enzyme prepared at 45°C for 24h and lowest of 63.31 was for the film with the solution having 4 U enzyme prepared at 50°C for 8h. The highest value for the whiteness index was found to be 36.04 for the film prepared with 4U enzyme containing solution for 8h at 50°C and minimum of 33.28 for 3U enzyme for 24h at 45°C.

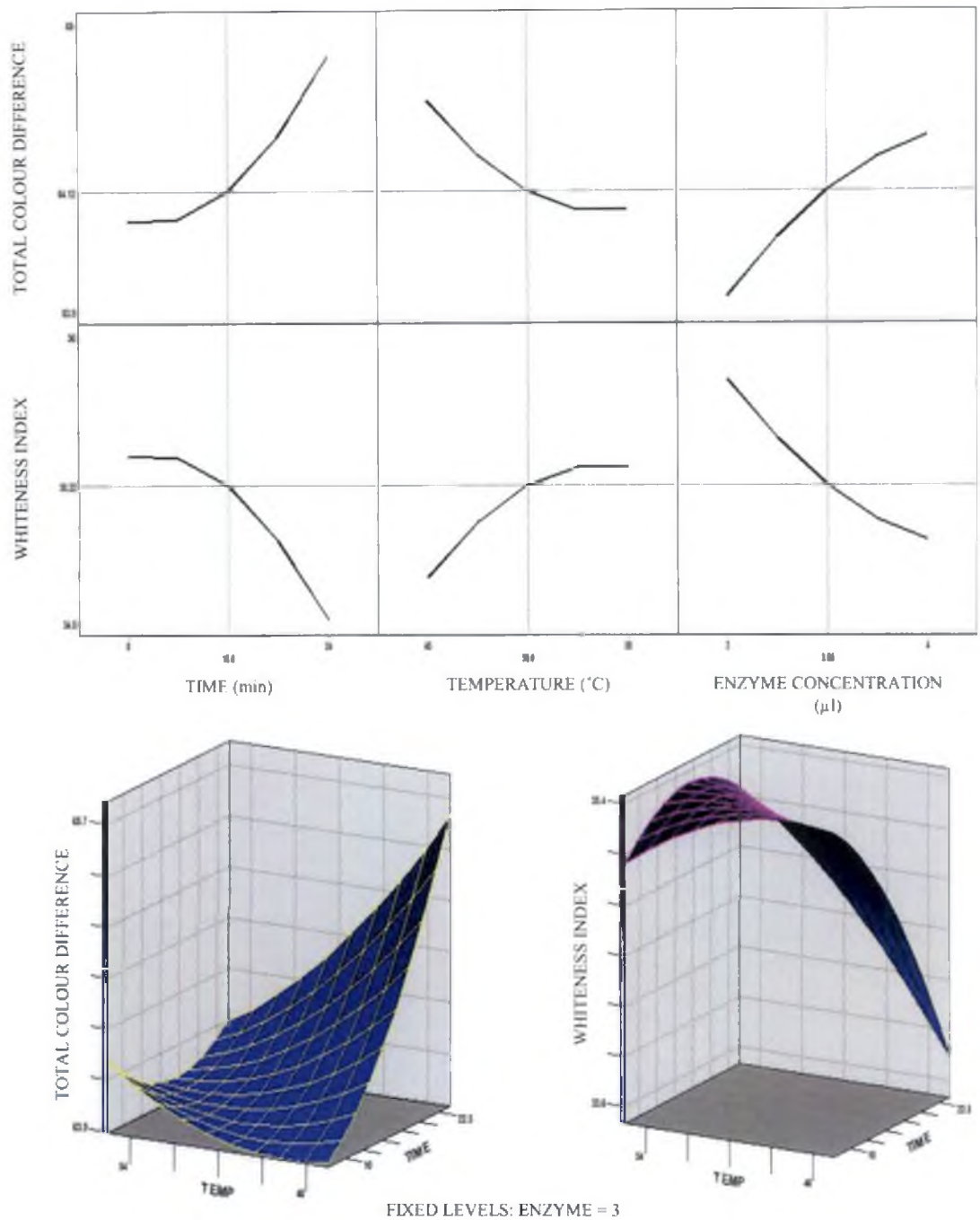


Fig. 12. Colour properties of biodegradable films made from pullulanase treated cassava starch

When comparing the films made from the solution having different enzyme concentration, for 2U enzyme, the total colour difference of the film was found to be highest (64.03) for 16h at 55°C and lowest (63.48) for 16h at 45°C. The whiteness index of the film was found to be highest (35.86) for 16h at 45°C and lowest (35.32) for 16h at 55°C.

In the case of films containing for 3U enzyme, the total colour change value of the film was found to be highest for 24h at 45°C (66.06) and lowest (63.42) for 24h at 55°C. The whiteness index of the film was found to be highest (35.92) for 24h at 55°C and lowest (33.28) for 24h at 45°C. For the film prepared with 4U enzyme, the total colour difference value of the film was found to be highest (66.05) for 24h at 50°C and lowest (63.31) for 8h at 50°C whereas the whiteness index of the film was found to be highest (36.04) for 8h at 50°C and lowest (33.29) for 24h at 50°C.

4.6.2.2. Thickness

The variation of thickness with respect to different proportions of enzyme, time and temperature as plotted by response surface and prediction profiles are represented in Fig.13.

It was found that thickness of the film was strongly influenced by both the temperature and time. And was quadratically increasing with increase in time. Temperature shows a quadratic decrease in the beginning and then apparently shows a quadratic increase. Enzyme concentration shows a quadratic increase in the beginning and then apparently shows a quadratic decrease. The thickness of the film was influenced by enzyme concentration, time, temperature according to the equation,

$$\text{Thickness} = 0.18+0.0037*T-0.0025*H - 0.0037*E + 0.0041*T^2 - 0.0075*T*H + 0.01*T*E + 0.0066*H^2 + 0.0025*H*E - 0.0058*E^2.$$

Thickness was found to be highest (0.218mm) for film made from the solution with 3U enzyme treated for 24h at 45°C and lowest (0.163mm) for film with having

4U enzyme for 8h at 50°C. While comparing the films made with different enzyme concentration, it was found that for 2U enzyme concentration, the thickness of the film was found to be highest(0.211mm) for 8h at 50°C and lowest (0.178mm) for 16h at 45°C. The thickness of films having 3U enzyme concentration was found to be maximum (0.218mm) for 24h at 45°C and minimum (0.176mm) for 16h at 50°C whereas for the films with 4U enzyme, thickness was found to be highest (0.201mm) for 16h at 55°C and lowest (0.163mm) for 8h at 50°C.

4.6.2.3. Moisture Content

The variations in moisture content of the films with respect to different proportions of enzyme, time and temperature as plotted by response surface and prediction profiles in Fig.13. From these values, it is clear that the enzyme concentration has apparently high effect on the moisture content of the film when compared to time and temperature. Initially the enzyme concentration has positive quadratic effect on the moisture content and eventually shows a negative quadratic effect. Temperature and time shows a slightly positive quadratic effect on moisture content of the films. The variation in moisture content (MC) of the film with reference to enzyme, time and temperature is explained by the equation,

$$MC = 17.90 - 0.59*T + 0.063*H - 0.39*E + 0.50*T^2 - 0.11*T*H - 0.39*T*E + 0.22*H^2 + 0.13*H*E - 1.41*E^2.$$

Enzyme concentration has apparently high effect on the moisture content of the films. The moisture content of the film was found to be highest (19.33%) for the films containing 3U enzyme prepared for 8h at 55°C and lowest (15.56%) for 4U enzyme, 24h, 50°C.

While comparing the films with, 2U enzyme concentration, the moisture content of the film was found to be highest for 8h at 50°C (17.64%) and lowest for 24h at 50°C (16.8%). The moisture content of films having 3U enzyme concentration was found to be highest for 8h at 55°C (19.33%) and lowest for 16h at 50°C (17.59%). And the

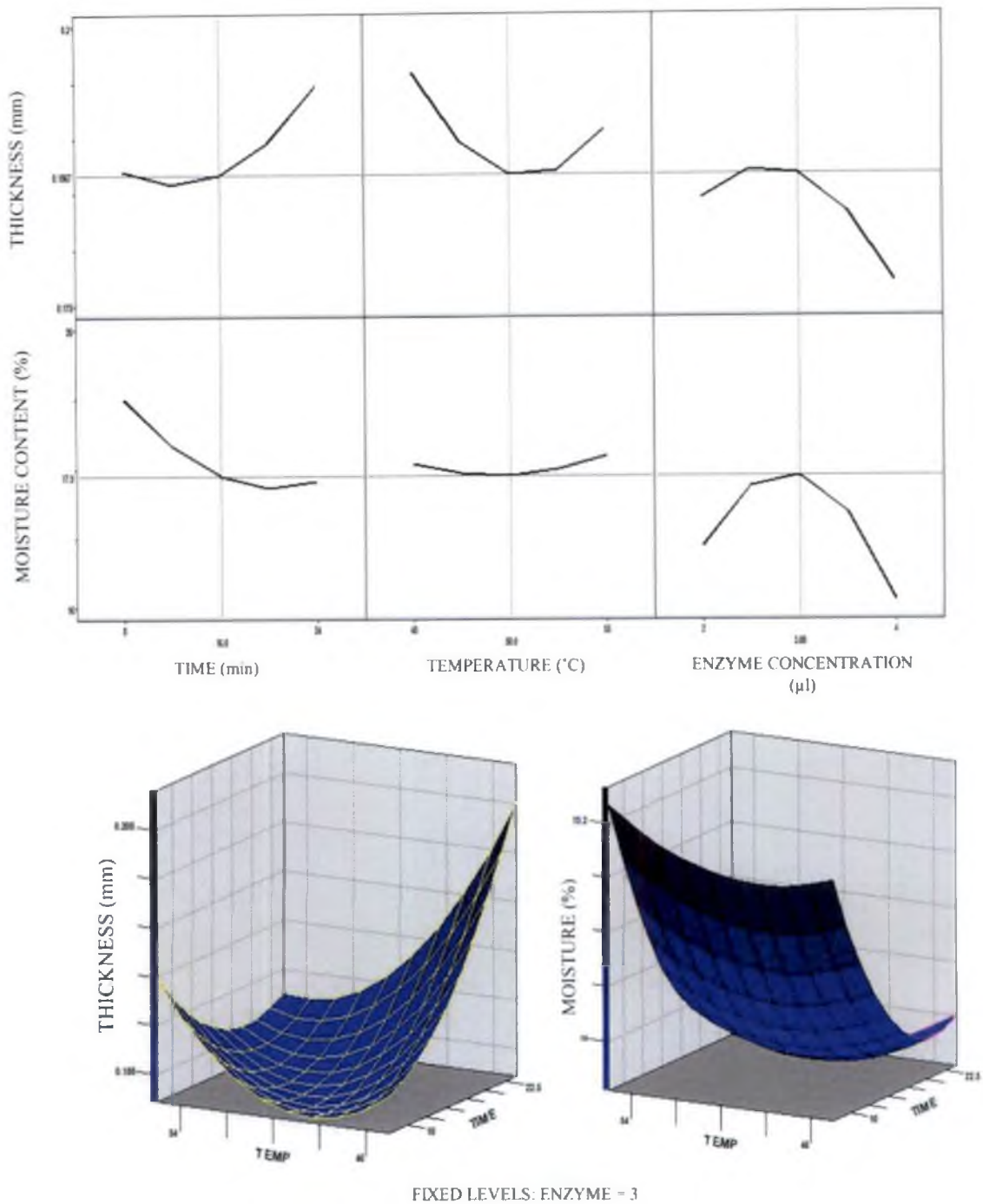


Fig.13. Physical properties of biodegradable films made from pullulanase treated cassava starch

moisture content of the films with 4U enzyme was found to be highest for 8h at 50°C (17.99%) and lowest for 24h at 50°C (15.56%).

As the time and temperature increases, the moisture content of the film was found out to be decreasing initially and then it began to increase. At initial state, it was found that, as enzyme concentration increases, moisture content of the film increases and at a further increase in enzyme concentration, moisture content is found to have a negative quadratic effect. The influence of enzyme concentration on film moisture content was significant when compared to time and temperature.

4.6.2.4. Mechanical properties

The variations in tensile force, toughness and elongation at break of the films with respect to different enzyme concentration, time and temperature is represented as response surface and prediction profile in Fig.14.

Tensile force of the film was found to be quadratically decreased initially with respect to enzyme concentration and eventually shows a quadratic increase and time and temperature shows a negative quadratic effect.

The tensile force (TF) of the films varied according to the equation:

$$TF = 7.06 - 1.81*T - 0.21*H + 0.98*E + 1.89*T^2 - 0.25*T*H + 2.09*T*E - 1.58*H^2 - 0.66*H*E + 6.00*E^2.$$

The toughness of the films was found to be quadratically decreased initially and eventually shows a quadratic increase with respect to enzyme concentration. The temperature shows a positive quadratic effect, whereas time initially decreases quadratically and eventually increases slightly .

The toughness varied according to the equation,

$$Toughness = 23.75 - 14.05*T + 9.01*H + 20.62*E + 23.20*T^2 - 6.63*T*H + 22.38*T*E + 13.74*H^2 + 24.13*H*E + 64.67*E^2.$$

The percentage of elongation at break of the films was found to have a positive quadratic effect with respect to enzyme concentration, whereas time and

temperature shows a negative quadratic effect initially and eventually shows positive quadratic effect. The percentage of elongation at break varied according to the equation:

$$\text{Elongation} = 10.94 - 0.42*T - 0.17*H + 2.44*E + 4.74*T^2 - 1.30*T*H + 1.80*T*E + 6.97*H^2 + 4.93*H*E + 3.78*E^2.$$

Maximum tensile force of 22.97N was obtained for the film made with 2U enzyme for 8h at 50°C whereas minimum was obtained as 3.91N for 2U enzyme, 16h, 55°C. When comparing the properties of the film made from the solutions with different enzyme concentrations, it was observed that, for 2U enzyme concentration, the tensile force of the film was highest for 8h at 50°C (22.97N) and lowest for 16h at 55°C (3.91 N). In the case of 3U enzyme concentration, tensile force was found to be highest for 8h at 55°C (11.18N) and lowest for 24h at 45°C (4.07 N) and with 4U enzyme, it was found to be highest for 16h at 45°C (20.38N) and lowest for 24h at 50°C (11.13 N).

Maximum toughness of 197.15Ns was obtained for the film made with 4U enzyme, 16h at 55°C whereas minimum of 18.16N of 3U enzyme, 16h, 50°C. When comparing the films with different enzyme concentration, for 2U enzyme concentration, the toughness of the film was found to be highest for 8h at 50°C (161.56N) and lowest for 16h at 55°C (20.3N). In the case of 3U enzyme concentration, toughness was found to be highest for 8h at 55°C (102.41N) and lowest for 16h at 50°C (18.16N) and with 4U enzyme concentration, it was found to be highest for 16h at 55°C (197.15N) and lowest for 8h at 50°C (70.72N).

Maximum elongation at break of 32.77% was obtained for the film made with 4U enzyme, 16h at 55°C whereas minimum of 10.07% for 3U enzyme, 16h and 50°C. When comparing the films with different enzyme concentration, for 2U enzyme concentration, the elongation at break of the film was found to be highest for 16h at 45°C (20.48%) and lowest for 16h at 55°C (15.86%). In the case of 3U enzyme concentration, elongation at break of the film was found to be highest for 8h at 45°C

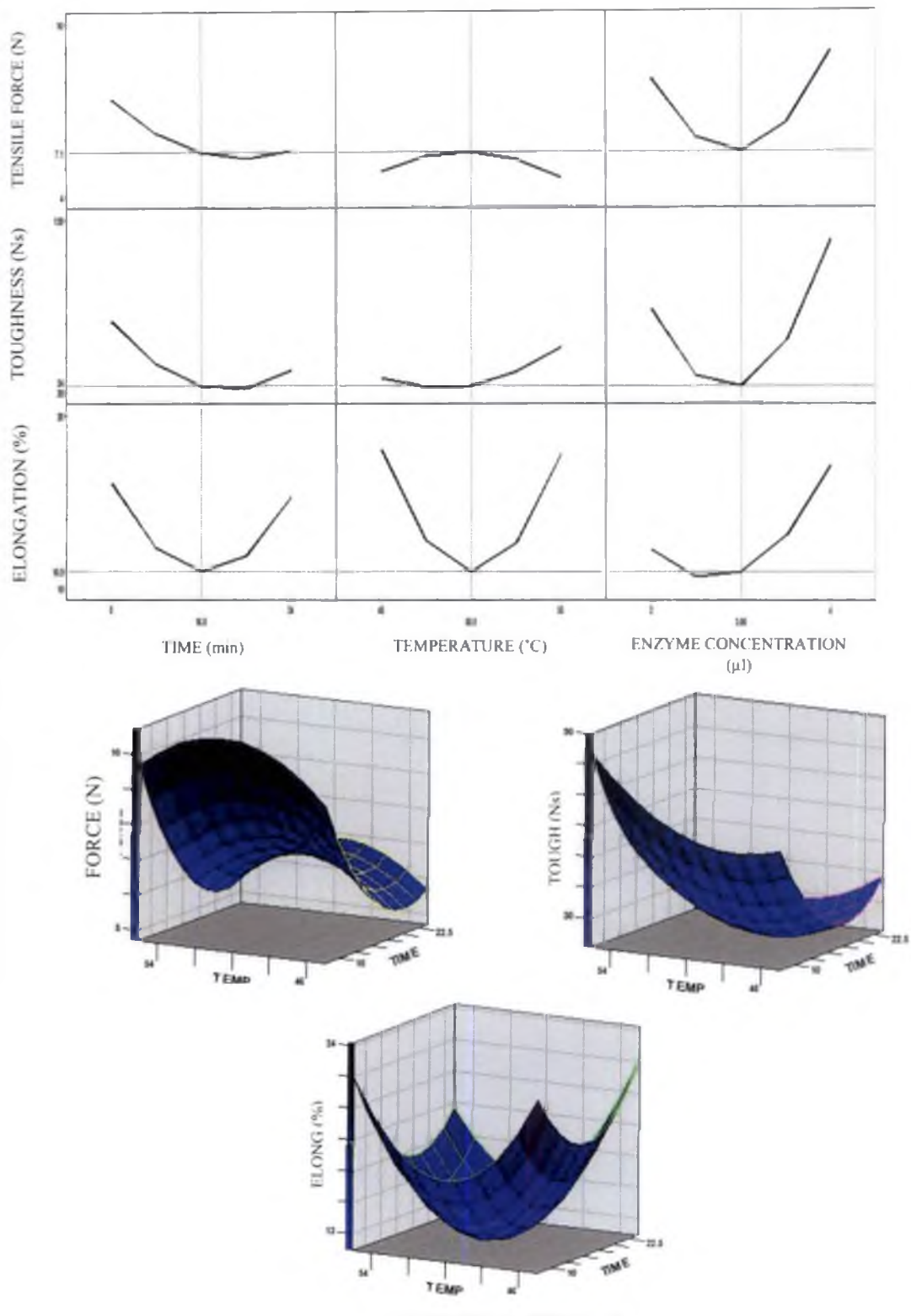


Fig.14. Mechanical properties of biodegradable films made from pullulanase treated cassava starch

(27.61%) and lowest for 16h at 50°C (10.07%) and the elongation at break of the films with 4U enzyme was found to be maximum for 16h at 55°C (32.77%) and minimum for 8h at 50°C (16.58%).

4.6.2.5. Swelling capacity and Solubility

The variations in swelling capacity Volume and Solubility of the films with respect to different proportions of enzyme, time and temperature as plotted by response surface and prediction profile are represented in Fig.15.

Initially the swelling capacity of the films has negative quadratic effect with time and temperature and has positive quadratic effect with respect to enzyme concentration, and eventually shows positive quadratic effect with time and temperature and shows negative quadratic effect with enzyme concentration. The swelling capacity of the films varied according to the equation:

$$\text{Swelling capacity} = 182.12 - 3.64*T + 3.61*H - 6.65*E + 9.62*T^2 + 28.99*T*H - 4.15*T*E + 10.33*H^2 - 9.53*H*E - 12.89*E^2.$$

Solubility of the films was strongly influenced by the enzyme concentration and it was quadratically increased with increase in enzyme concentration. For solubility, the temperature has slightly positive quadratic effect and time initially shows positive quadratic effect and eventually shows negative quadratic effect. The solubility of the films varied according to the equation:

$$\text{Solubility} = 45.04 + 1.29*T - 0.08*H + 5.96*E - 5.96*T^2 - 0.33*T*H + 1.16*T*E + 0.47*H^2 - 0.49*H*E - 2.06*E^2.$$

Maximum swelling capacity of 238.52g/g was obtained for the film made with 3U enzyme, 24h at 55°C whereas minimum of 148.41g/g for the film having of 4U enzyme, 16h, 55°C. While comparing the films with different enzyme concentration, for 2U enzyme , the swelling capacity of the film was found to be highest for 16h at 55°C (199.40g/g) and lowest for 8h at 50°C (173.87g/g). In the case of 3U enzyme concentration, swelling capacity of the film was found to be highest for 24h at 55°C (238.52g/g) and lowest for 24h at 45°C (154.76g/g) and the swelling

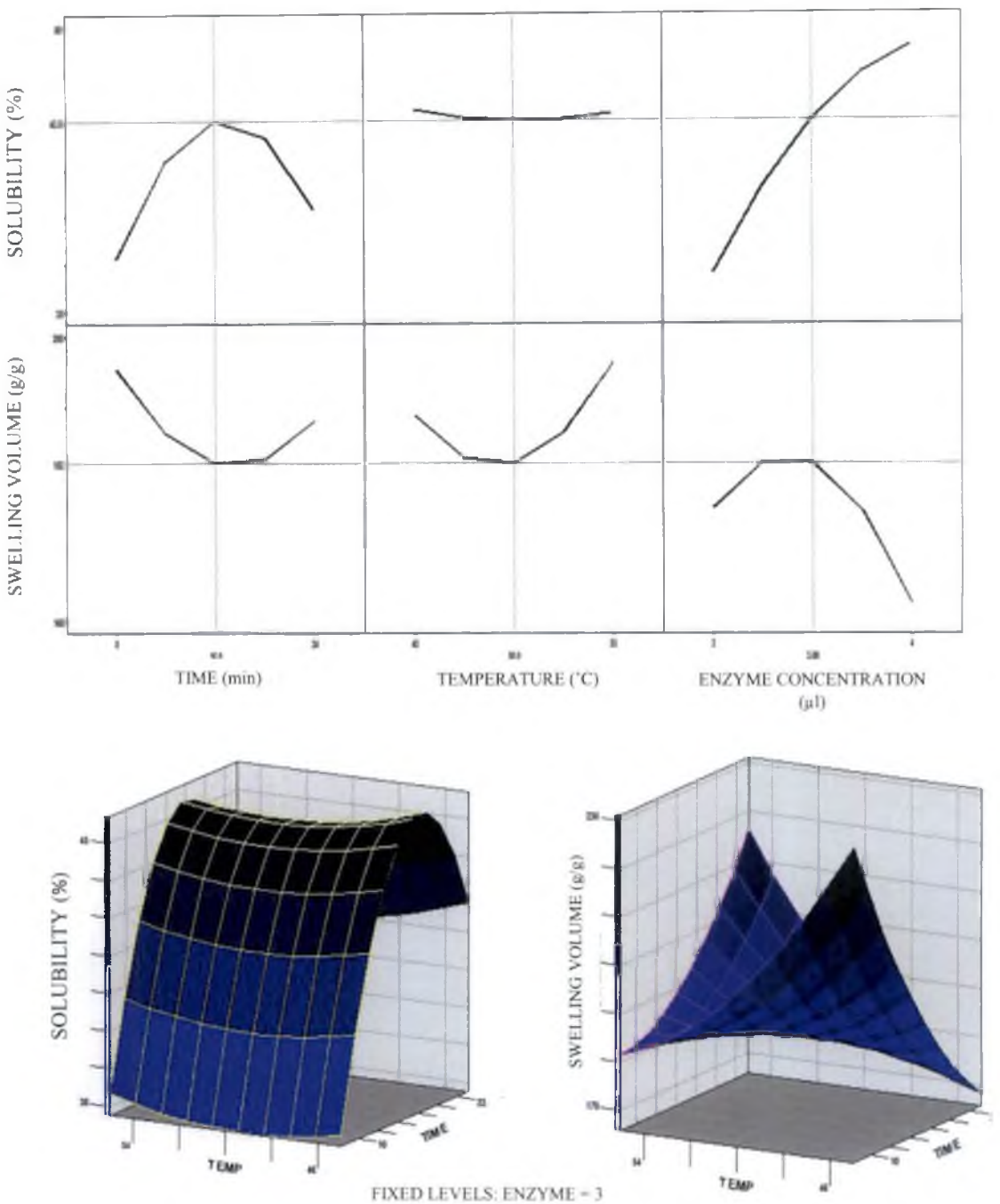


Fig.15. Functional properties of biodegradable films made from pullulanase treated cassava starch

capacity of the films with 4U enzyme was found to be highest for 8h at 50°C (187.51g/g) and lowest for 16h at 55°C (148.41g/g).

Maximum solubility of 51.79% was obtained for the film made with 4U enzyme, 16h at 45°C whereas minimum was obtained for 32.30% of 2U enzyme, 8h, 50°C. In the case of films with 2U enzyme, the solubility of the film was found to be highest for 16h at 55°C (36.12%) and lowest for 8h at 50°C (32.30%). In the case of 3U enzyme concentration, solubility of the film was found to be highest for 16h at 50°C (49.14%) and lowest for 8h at 55°C (38.91%) and the solubility of the films with 4U enzyme concentration was found to be highest for 16h at 45°C (51.79%) and lowest for 8h at 50°C (37.79%).

4.6.2.6. Water Permeability and Water Absorption Properties

The variations in water permeability and water sorption of the films with respect to different proportions of enzyme, time and temperature as plotted by response surface and prediction profiles in Fig.16.

The water permeability initially had positive quadratic effect with respect to enzyme concentration and negative quadratic effect with temperature, and eventually shows a negative quadratic effect with respect to enzyme concentration and positive quadratic effect with temperature. Time shows positive quadratic effect on water permeability. Water permeability of the films varied according to the equation:

$$\text{Permeability} = 0.035+0.0016*T+0.0005*H+0.00062*E+0.00091*T^2-0.0017*T*H+0.002*T*E+0.0021*H^2+0.0012*H*E-0.0020*E^2.$$

Enzyme concentration shows positive quadratic effect and temperature shows negative quadratic effect on water absorption. Water absorption increases with increase in time and eventually decreases, it varied according to the equation,

$$\text{Water absorption} = 27.34 + 0.65*T - 0.43*H + 0.65*E - 0.72*T^2 - 0.027*T*H - 0.52*T*E - 0.43*H^2 + 0.53*H*E - 0.22*E^2.$$

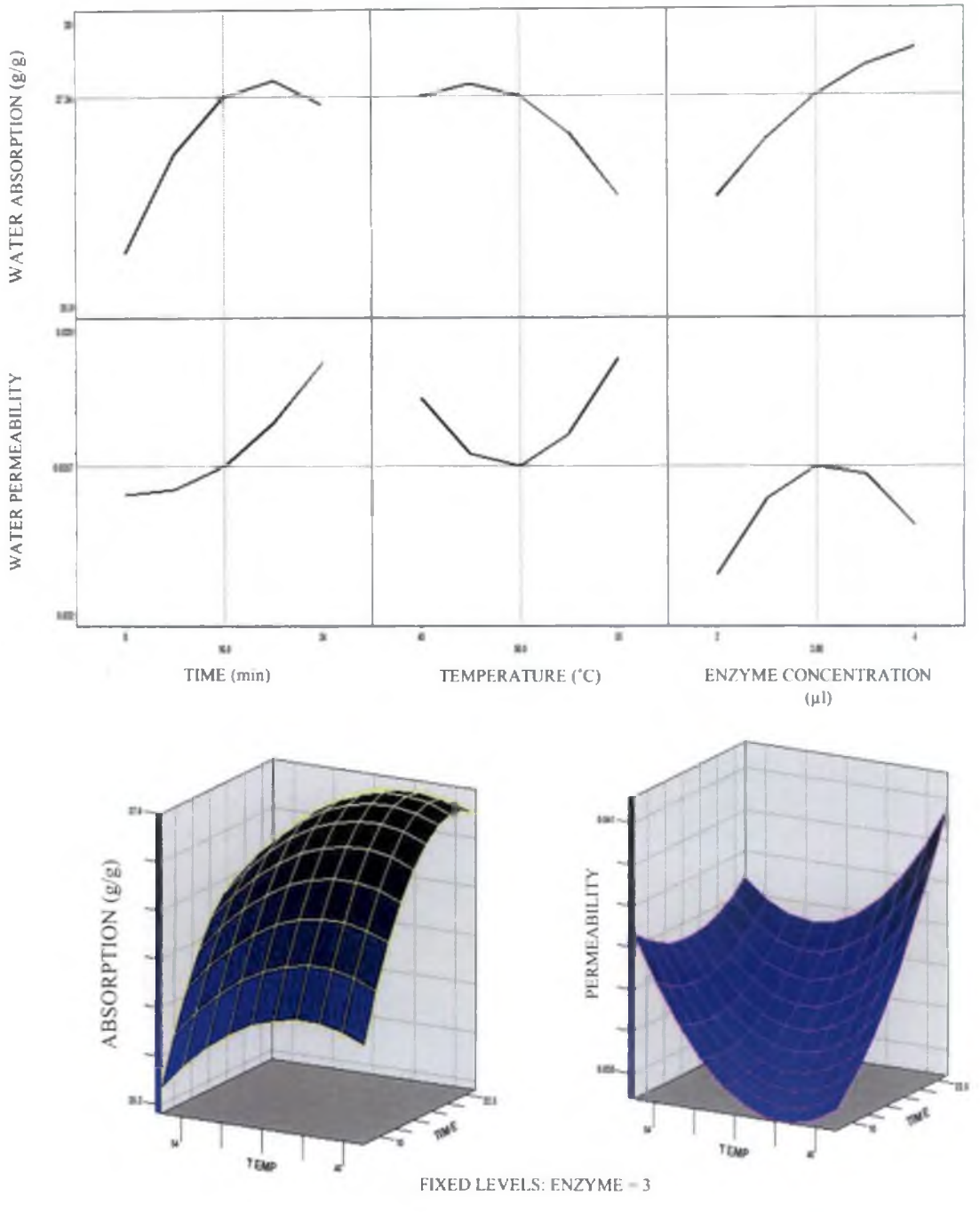


Fig. 16. Water absorption and permeability of biodegradable films made from pullulanase treated cassava starch

Maximum water permeability of 0.043 was obtained for the film made with 3U enzyme, 24h at 45°C whereas minimum was obtained as 0.030 of 4U enzyme, 8h, 50°C. When comparing the samples with different enzyme concentrations, for 2U enzyme, the water permeability of the film was found to be highest for 8h at 50°C (0.038) and lowest for 16h at 55°C (0.031). In the case of 3U enzyme concentration, water permeability of the film was found to be highest for 24h at 45°C (0.043) and lowest for 16h at 50°C (0.032) and with 4U enzyme, it was found to be highest for 16h at 55°C (0.040) and lowest for 8h at 50°C (0.030).

Maximum water absorption of 28.28g/g was obtained for the film made with 3U enzyme, 16h at 50°C whereas minimum was obtained as 24.32g/g for 2U enzyme, 16h, 55°C. While comparing the films with different enzyme concentrations, for 2U enzyme, the water absorption of the film was found to be highest for 24h at 50°C (27.48g/g) and lowest for 16h at 55°C (24.32g/g). In the case of 3U enzyme concentration, water absorption of the film was found to be highest for 16h at 50°C (28.28g/g) and lowest for 8h at 45°C (25.77g/g) and the water absorption of the films with 4U enzyme was found to be highest for 16h at 45°C (27.95g/g) and lowest for 8h at 50°C (26.34g/g).

4.7. FILM DEGRADABILITY

The degradability of the films were observed by visual observation of the films buried in the soil at regular intervals of one week. In the first week of the study, the alpha amylase treated film showed a degradability of 40-50% as evidenced from the fragmented segment of the films in the soil. In the second week of the study, the film was observed to have degraded to a range of 90-95% and a very few residues were observed in the soil and in the third week no film residues were seen (Plate 5)

While studying the degradability of pullulanase treated film, degradability was comparatively slow in the first week, it was only 20-25% and there were many film residues retained in the soil. In the second week, we could observe 40-50%

Biodegradable films

Placing of films in the soil

After 1 week

After 2 week

After 3 week

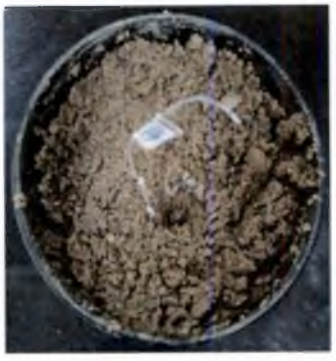
Plate 6: Soil burial test for α-amylase treated films

Biodegradable films

Placing films in the soil

After 1 week

After 2 week



After 3 week

After 4 week

Plate 7: Soil burial test for pullulanase treated films

degradability and the third week showed 80-90% degradability and a very few film residues were seen and the fourth week was found to have no film at all (Plate 6)

4.8. MICROBIAL POPULATION ASSOCIATED WITH THE DEGRADABILITY OF THE FILM

The microbial population in the soil in which the film samples were buried for degradation with respect to the soil moisture content is shown in the Table. 8 and 9. The microbial populations in the respective plates shown in Plate 8. are counted to analyze the microbial flora.

The control showed a value of 4.26×10^6 CFU/g for bacteria, 2.11×10^4 CFU/g for fungi, and 0.47×10^5 CFU/g for actinomycetes. The soil used to degrade α-amylase treated film was found to have a highest bacterial, fungal and actinomycetes population with a value of 88.26 $\times10^6$ CFU/g, 66.47 $\times10^4$ CFU/g and 17.58×10^5 CFU/g and a lowest value of 1.18×10^6 CFU/g, 0.09×10^4 CFU/g and 0.02×10^5 CFU/g respectively according to the film combinations as mentioned in the Table 8.

The soil used to degrade pullulanase treated film was found to have the highest bacterial, fungal and actinomycetes population with a value of 60.86 $\times10^6$ CFU/g, 4.5 $\times10^4$ CFU/g and 52.11×10^5 CFU/g and a lowest value of 1.18×10^6 CFU/g, 0.03×10^4 CFU/g and 0.29×10^5 CFU/g respectively according to the film combinations as mentioned in the Table 9

Table. 8. Microbial population in the soil associated with the degradation offilms made from amylase treated starch

Sample	Sl. No.	Moisture (%)	Bacteria CFU/g	Fungi CFU/g	Actinomycetes CFU/g
Control	1	14.68	16.409×10^{6}	0.4467×10^{4}	2.9773×10^{5}
	2	14.02	4.2646×10^{6}	2.1104×10^{4}	0.4738×10^{5}
	1	15.22	11.009×10^{6}	0.3025×10^{4}	3.306×10^{5}
	2	15.26	6.6871×10^{6}	0.7971×10^{4}	2.0827×10^{5}
	3	15.38	6.6966×10^{6}	0.5465×10^{4}	1.8297×10^{5}
	4	15.41	1.1822×10^{6}	9.3049×10^{4}	0.1075×10^{5}
	5	16.12	3.1791×10^{6}	10.169×10^{4}	0.2291×10^{5}
	6	14.14	2.7176×10^{6}	66.478×10^{4}	0.02×10^{5}
	7	14.64	16.011×10^{6}	0.6452×10^{4}	2.0614×10^{5}
	8	15.31	1.5744×10^{6}	10.372×10^{4}	0.1282×10^{5}
	9	14.69	9.3776×10^{6}	5.6155×10^{4}	0.1781×10^{5}
	10	15.1	8.245×10^{6}	12.451×10^{4}	0.0803×10^{5}
	11	16.09	2.7808×10^{6}	8.1489×10^{4}	0.1632×10^{5}
	12	14.26	2.7214×10^{6}	2.6935×10^{4}	0.3713×10^{5}
	13	15.51	40.241×10^{6}	0.0994×10^{4}	10.06×10^{5}
	14	15.78	88.261×10^{6}	0.0944×10^{4}	17.589×10^{5}
	15	16.11	1.192×10^{6}	40.821×10^{4}	0.0245×10^{5}

Table 9. Microbial population in the soil associated with the degradation of films made from pullulanase treated starch

Sample	Sl.no.	Moisture (%)	Bacteria CFU/g	Fungi CFU/g	Actinomycetes CFU/g
Control	1	14.68	16.409×10^{6}	0.4467×10^{4}	2.9773×10^{5}
	2	14.02	4.2646×10^{6}	2.1104×10^{4}	0.4738×10^{5}
	1	14.29	26.057×10^{6}	0.0384×10^{4}	52.114×10^{5}
	2	14.57	26.142×10^{6}	0.3569×10^{4}	4.6512×10^{5}
	3	14.12	3.4932×10^{6}	2.0039×10^{4}	1.3274×10^{5}
	4	14.9	20.368×10^{6}	0.0815×10^{4}	20.368×10^{5}
	5	14.78	2.738×10^{6}	1.7042×10^{4}	0.5868×10^{5}
	6	15.68	3.1626×10^{6}	1.6853×10^{4}	0.5933×10^{5}
	7	14.79	4.6943×10^{6}	1.0651×10^{4}	1.5585×10^{5}
	8	15.1	7.8524×10^{6}	4.5846×10^{4}	0.2901×10^{5}
	9	15.25	5.8997×10^{6}	1.2424×10^{4}	0.8049×10^{5}
	10	16.11	20.265×10^{6}	0.8058×10^{4}	1.2409×10^{5}
	11	14.21	16.707×10^{6}	0.1592×10^{4}	6.281×10^{5}
	12	15.39	1.1819×10^{6}	1.6922×10^{4}	0.5909×10^{5}
	13	16.21	60.866×10^{6}	0.0821×10^{4}	16.19×10^{5}
	14	14.1	5.4327×10^{6}	1.5333×10^{4}	0.6522×10^{5}
	15	16.52	5.9895×10^{6}	0.8348×10^{4}	1.1979×10^{5}

RB medium KK medium NA medium

Plate 8. Microbial populations in the soil associated with the films from amylase treated cassava starch

RB medium KK medium NA medium

Plate 9. Microbial populations in the soil associated with the films from pullulanase treated cassava starch

4.9. PACKAGING STUDIES

Alpha amylase films show less packaging quality, because of its high water sorption property. And the film was kept in room temperature for 3 weeks to study packaging quality. In the first week of study, because of the interference of moisture the weight of the film was increased by 4.2-5% and in the second week the value reduced from 3.1-2.3% and no significant change was observed during the third week. Some of the packaging was unsealed or rupture was found to be formed after even 2 days of packaging as shown in Table. 10

Table 10. Packaging study of the films made from amylase treated starch

Sample no.	Time (min)	Temperature (°C)	Enzyme concentration,(µl)	No: of days for rupture
1	20	80	200	retained
2	20	90	200	3
3	40	80	200	retained
4	40	90	200	3
5	30	80	100	retained
6	30	80	300	2
7	30	90	100	3
8	30	90	300	2
9	20	85	100	retained
10	40	85	100	3
11	20	85	300	2
12	40	85	300	2
13	30	85	200	retained
14	30	85	200	retained
15	30	85	200	retained

Pullulanase films show a high packaging quality and good sealability, because of its less water sorption property. And the film was kept in room temperature for 3 weeks to study packaging quality. In the first week of study, because of the interference of moisture the weight of the films increased by 1.5-2.1% and in the second week the value reduced from 1.3-1.8% and no significant change was observed during the third week (Table 11).

Table 11. Packaging study of the films made from pullulalanase treated starch

Sample no.	Time (h)	Temperature (°C)	Enzyme concentration (u)	No: of days for rupture
1	8	45	3	retained
2	8	55	3	retained
3	24	45	3	retained
4	24	55	3	retained
5	16	45	2	retained
6	16	55	4	retained
7	16	45	2	retained
8	16	55	4	retained
9	8	50	2	retained
10	24	50	2	retained
11	8	50	4	retained
12	24	50	4	retained
13	16	50	3	retained
14	16	50	3	retained
15	16	50	3	retained

DISCUSSION

5. DISCUSSION

The main objective of this study was to enzymatically modify cassava starch using liquefying and debranching enzymes viz., α-amylase and pullulanase respectively, their rheological characterisation and development and physic-mechanical, functional and biodegradability characterization of the films from it. The starch was extracted by wet milling process. Starch is a carbohydrate source essentially consisting of linear amylase and the branched amylopectin (Manners, 1989). In the study, the liquefying enzyme viz., α-amylase and the debranching enzyme viz., pullulanase is used for the enzymatic modification of starch considering their difference in action on the starch ie., α-amylase can hydrolyze starch, glycogen and related polysaccharides by randomly cleaving internal α-1,4-glucosidic linkages to produce different sizes of oligosaccharides and pullulanase debranch starch by cleaving its alpha 1,6-glycosidic bond and convert starch into its dextrins. The studies on the enzymatically modified starch for the development of biodegradable films are scanty and hence the study was undertaken.

5.1. Recovery of starch

The starch was extracted by wet milling process and a recovery of 27.5% starch was obtained. This recovery was higher than that reported by Sajeev and Balagopalan, (2005) wherein they got 20.5 percent recovery for cassava tubers using the same machines, mainly due to the difference in the varieties used.

5.2. Dextrose equivalent of filmogenic solutions

The dextrose equivalent (DE) of the filmogenic solutions containing cassava starch, modified with α-amylase and pullulanase was done and the DE value was 1.6-8.4 with the amylase treated starch and 2.2-16.0 with the pullulanase treated starch.

Alpha amylase break the glycosidic linkages to produce dextrins. Maltodextrins are partially hydrolysed starch with a DE of 20 (Balagopalan,1996). The cassava mash undergone gelatinization and then to liquefaction experiments by treating with α-amylase obtained DE value of 20 (Lambri *et al.*, 2014). Barnett *et al.*, 1999 worked on potato starch and yielded a dextrose equivalent of 20 which is comparatively higher than that of our values, may be mainly due to the variety and genetic make up of the tubers used in the present study.

5.3. Rheological properties of enzymatically modified starch

The variation of rheological parameters viz., storage modulus, loss modulus, phase angle and complex viscosity with respect to enzyme concentration, time and temperature was studied. Storage modulus and complex viscosity showed higher values for the solution containing pullulanase treated starch films. Loss modulus was more or less same for the solutions having α-amylase and pullulanase treated starch whereas phase angle is low for the pullulanase treated starch solutions. The higher values of storage modulus, complex viscosity and low phase angle of the solution containing pullulanase treated starch compared to α-amylase treated solutions showed that the gel formed during gelatinization of the solution is having more solid nature in their visco elastic character. Modification of starch invariably alter the rheological properties of the starch solutions (Jyothi and Moorthy, 2012,; Sajeev *et al.*, 2009).

5.4. Physical properties of biodegradable films

Alpha amylase was found to have a low total colour difference value when compared to pullulanase treated films and whiteness index had an opposite effect as envisaged. This showed that films containing α-amylase treated cassava starch showed better whiteness character when compared to the films with pullulanase treated starch. Preparation of novel biodegradable edible film obtained from psyllium seed was studied by Ahmadi *et al.*, (2012) and got a whiteness index of 6.

Ghanbarzadeh *et al.*, 2010 studied the whiteness index in biodegradable film produced from cellulose films and found to have a value of 70. In the present study, the whiteness index was in between these two cases, mainly due to the colour of the starch, glycerol and the gelatinization conditions etc.

Thickness o f the films containing pullulanase treated starch was higher than that of the films with α-amylase treated starch showing a good film characteristics with pullulanase treatment. Thickness of the film mainly depends on the volume of the filmogenic solutions, source of starch and type of modification. The higher value of the storage modulus and complex viscosity of the pullalanase treated starch solution invariably resulted in higher thickness of the films too. Ezeoha and Ezenwanne, 2013 studied on cassava starch based biodegradable films and got a thickness of the film as 0.20mm. The pure amylose structure is very stable, with strong molecular orientation, forming films denser and stronger than amylopectin films (Lourdin *et al.*, 1995).

The best film should have minimum moisture content to have better strength storability. The present study showed that films having α-amylase treated cassava starch had minimum moisture content ranging from 10.47 to 17.60% compared to pullulanase treated starch having 15.56 to 19.33%. Ezeoha and Ezenwanne, 2013 studied on cassava starch films and found that the moisture content was 12% which is comparable to our study. The higher moisture content of the pullulanase treated film is mainly because of the high solid nature of the visco-elastic solution as evidenced from the rheological properties. In the study, the drying time for both the films were kept same irrespective of the modified starch included and hence increased drying time may help to get low moisture films from pullulanase treated starch.

5.5. Mechanical properties of the biodegradable films

The tensile force of the film made from the pullulanase treated starch was higher (3.91 to 22.97N) compared to the amylase treated starch based films (1.94 to

14.41) and the elongation at break ranged from 10.07 to 32.77% for the pullulanase based film compared to 3.01 to 19.07%. The higher value of the tensile force for the pullulanase based film is due to the high solid nature of the solution as evidenced from the high storage modulus and viscosity values. Also for the higher elongation, the high moisture content in the pullulanase based film plays a role. Assefa *et al.*, (2013) found out that the tensile strength of taro based starch films varied from 24-28N. Dias *et al.*, 2010 studied biodegradable films based on rice starch and rice flour and they obtained a tensile strength ranging from 2-22N with an elongation at break ranged from 2-66.

5.6. Swelling capacity and Solubility

The swelling capacity of the α-alpha amylase based film varied from 128.25 to 223.41% and for the pullulanase based film from 148.42 to 238.52% whereas respective solubility ranged from 54.85 to 80.09% and from 32.31 to 51.79%. Though the swelling capacity was higher for the pullulanase based film, solubility was found to be low showing higher degradation time to be taken. Whereas in packaging studies, because of low solubility, pullulanase based films showed better promise. Assefa *et al.*, 2013 found out that the swelling volume of taro films ranged from 28-120 which is comparable to our study and a solubility value was only of 2.5%.

5.7. Water Permeability and Water absorption properties

Pullulanase based films showed less water permeability (0.030-0.043) when compared to α-amylase based films (0.032-0.052) and the corresponding water absorption values were 24.46 to 28.29% and 25.15 and 27.16% showing not much difference between these two films. These results showed that good results were obtained in the case of pullulanase based film when compared to α-amylase modified film offering better packaging ability of the pullulanase based films. The various in thickness, moisture content and mechanical properties are all attributed to this. Water

sorption isotherms showed that above 43% RH the equilibrium water content of starch films with glycerol was higher than those of non-plasticized films (Mali *et al.*, 2002). Water vapor permeability values ranged from 9.6 to 108 g water/h.m.Pa, for the rice starch films plasticized with 0.2 g sorbitol/g dry starch (SS20) to 63.6 108g water/h.m.Pa, for the rice flour films plasticized with 0.3 g glycerol/g dry flour (Dias *et al.*, 2010).

5.8. Film degradability

Films, once disposed into soil should be degraded fast and easily is considered as best when all other properties meet. In this study the α-amylase based films showed the best result when comparing to pullulanase treated films. The alpha amylase treated films show a high rate of degradability within three weeks, whereas the pullulanase treated films took 4 weeks. However, both these films are highly biodegradable when disposed to the soil. The higher degradability of the α-amylase based film is mainly due to its high solubility. Bastioli, (1995) reported the enhanced biodegradation of PCL in the presence of starch by providing a larger surface area for microbial attack.

5.9. Microbial population in the film degraded soil

The control showed a low value for bacteria, fungi and actinomycetes when compared to α-amylase treated films and pullulanase treated films. The soil which was used to degrade α-amylase treated film was found to have a highest bacterial, fungal and actinomycetes population when compared to soil which was used to degrade pullulanase treated films.

5.10. Packaging studies

α-amylase films showed less packaging quality, because of its high water sorption and solubility whereas pullulanase films show a high packaging quality and good sealability, because of its less water sorption property. So the pullulanase films offers better scope for a good packaging material because of its good sealability and less water permeability when compared to α-amylase treated films.

SUMMARY

6. SUMMARY

The production and consumption of synthetic polymeric packaging materials are increasing at a phenomenal rate during the last few decades. Most of these plastics are crude oil based, and an increase in their production results in an increase of oil use and causes serious environmental pollution, due to undegradability. Biological recycling of polymers is an alternative to more traditional recycling procedures which has stimulated researchers to synthesize new polymers that can be returned to the biological cycle after use. There is an increasing interest in biodegradable plastics as food packaging material. Starch is a biopolymer that is an attractive alternative for packaging material. Starch and starch derivatives are considered to be promising candidates for the development of biopolymer based environment friendly packaging materials mainly due to their renewability, abundance, low cost, film forming properties, bland taste and colour, low solubility, biodegradability etc. In recent years, starches have been applied alone or as part of a composite for the manufacture of films. Research has been performed concerning the use of these films as a way of improving shelf life of food. Biodegradable films are not meant to totally replace synthetic packaging films. However, they do have potential to replace the conventional packaging in some applications.

Cassava is an abundant and cheap agricultural source of starch. It is the most important among the tuber crops, having high amount of starch (25-40%). Cassava starch has very desirable physico-chemical and functional properties and has extensive use in the food, feed and industrial sector. Cassava starch has drawn lot of interest by the researchers for the development of edible or biodegradable films. Native starch based films have limitations because of their poor physico-mechanical properties and poor water-resistance, making starch products very sensitive to the relative humidity at which they are stored and used.

Starches can be modified by physical, chemical and enzymatic methods to customise the starch properties and the resulting products have improved functional properties to suit various end uses. Enzymatic modification, brings about changes in viscosity and gel strength of the gelatinised starch suspension. The selective enzymatic hydrolysis of starch produces range of products with varying chain length and dextrose equivalent enabling the production of a variety of end use specific products. Hence, enzymatically modified starches offer better scope for the production of biodegradable films with desirable properties. The present study aims at analyzing the physico-mechanical, hygroscopic and functional properties of films made from cassava starch modified with liquefying and debranching enzymes.

The starch used for this study was extracted from cassava variety CMR256 by wet processing method. Cassava starch modified using α-amylase which is a liquefying enzyme and pullulanase which is a debranching enzyme. A combination of 15 modification by changing time, temperature and enzyme concentrations were obtained through response surface methodology for each enzyme.

The obtained combinations were prepared to make different filmogenic solutions and thereby corresponding films. The colour of the filmogenic solutions were measured using colourimeter and from the primary colour parameters 'L', 'a' and 'b', the total colour difference and whiteness index values were calculated. The dextrose equivalent of the filmogenic solution was calculated by NS method which is used for determining reducing sugar content and thereby calculating the dextrose equivalent from it. The highest value of dextrose equivalent of 16 was obtained for pullulanase treated filmogenic solution. α-amylase based filmogenic solutions have low DE value. Quantitative analysis of sugars present in filmogenic solution by HPLC shows The dynamic rheological properties of the filmogenic samples were measured using rheometer and the rheograms gives storage modulus, loss modulus, phase angle and complex viscosity of filmogenic solutions. Pullulanase treated films show good rheological properties like having 2060Pa storage modulus, 37.9 Pas

complex viscosity showing that it have more solid nature in its visco elastic properties.

Films were prepared by solution casting methods. The filmogenic solution prepared as in the experimental plan was allowed to dry in levelled Teflon plates in a film dryer overnight to get the starch films. Alpha amylase shows better colour properties when compared to pullulanase treated films whereas pullulanase treated film showed highest thickness when compared to α-amylase treated films. A-alpha amylase based films show minimum moisture content than the pullulanase based films. In our study best results for tensile strength was obtained by pullulanase treated films obtaining highest tensile force whereas elongation at break was minimum for α-amylase treated films. Best results for swelling volume was obtained by α-amylase when compared to pullulanase treated films and best solubility was highest for α-amylase treated films. Pullulanase films shows less water permeability when compared to alpha amylase treated films and water absorption quality of pullulnase show minimum value which is a desirable effect. And degradability is highest for alpha amylase treated films when compared to pullulanase treated films. The alpha amylase treated films degrade easily. The microbial population of alpha amylase film degraded soil and pullulanase film degraded soil was also analysed. Packaging quality is comparatively higher for pullulanase treated films.

REFERENCES

7. REFERENCE

Abbas, K. A., Sahar, K. K. and Hussin, M. A. S. 2010. Modified starches and their usages in selected food products: A review study. *J. of Agricultural Sci.* 2: 2.

Abdorreza, M. N., Cheng, L. H. and Karim, A. A. 2011. Effects of plasticizers on thermal properties and heat sealability of sago starch films. *Food Hydrocolloids* 25: 56-60.

Abdullah, M. and French, D. 1966. Reverse action of pullulanase. *Nature,* 200: 210.

Acharya, D. P., Hossain, M. K., Jin-Feing, Sakai, T. and Kunieda, H. 2004. Phase and rheological behaviour of viscoelastic worm like micellar solutions formed in mixed nonionic surfactant system. *Phys. Chem. Chem. Phys.* 6:1627-1631.

Adejumo, A. L., Aderibigbe, F. A. and Owolabi, R. U. 2013. Comparative studies of starch susceptibilities to α-amylase degradation of different cereal and root crops of Nigeria. *Afr. J. of Biotechnol.* 12(29): 4663-4669.

Adzahan, N. M. 2002. Modification on wheat, sago and tapioca starches by irradiation and its effect on the physical properties of fish cracker (keropok). *Food Technol.* Selangor, University of Putra Malaysia. Master of Science: pp. 1-222.

Ahmadi, R., Kalbasi-Ashtari, A., Oromiehie, A., Yarmand, M. S. and Jahandideh, F. 2012. Development and characterization of a novel biodegradable edible film obtained from psyllium seed (*Plantago ovata* Forsk). *J. of Food Eng.* 109: 745-751.

Ak, M. M. and Gunesekharan, S. 2001. Linear viscoelastic methods. In:. Nondestructive food evaluation: *Techniques to analyse properties and quality.* Gunasekharan, S. (ed). Marcel Dekker Inc, New York.

Akhila, R., Sudha, T. and Emelia, A. 2008. Enzymatic modification of cassava starch by fungal lipase. *Indus. Crp. Products* 27: 50-59.

Akoh, C.C., Chang, S., Lee, G. and Shaw, J. 2007. Enzymatic approach to biodiesel production. *J. Agric. Food Chem.* 55: 8995-9005.

Alves, V. D., Hilliou, L., Larotonda, F. D. S., Coelhoso, I. M., Goncalves, M. P. and Sereno, A. M. 2005. Characterization of model edible films using hydrocolloids and pectins. *9th international chemical engineering conference, Chempor applications: A review. Agron. Sustain. Dev.* 32: 501-529.

Alves, V. D., Mali, S., Beleia, A. P. and Grossmann, M. V. E. 2007. Effect of glycerol and amylose enrichment on cassava starch film properties. *J. of Food Eng.* 78(3): 941-946.

Anjana, S. P., Khan, A., T. K., Jayasree, J. T., Priyamvada, T., Jyothi, A. N., Sajeev, M. S. and Indu, B. 2013. Development of Hydroxypropylated cassava starch- nanokaoline clay composite Biodegradable films. 2013 International conference on food technology: "*Food processing technologies - challenges and solution for sustainable food security*". IICPT, Thanjavur.

Arvanitoyannis, I. and Biliaderis, C. G. 1998. Physical properties of polyol-plasticized edible films made from sodium caseinate and soluble starch blends. *Food Chem.* 62(3): 333-342.

Arvanitoyannis, I., Kalichevsky, M. T., Blanshard, J. H. V. and Psomiadou, E. 1994. Study of diffusion and permeation of gases in undrawn and uniaxially drawn films made from potato and rice starch conditioned at different relative humidities. *Carbohydr. Polym.* 24: 1-15.

Arvanitoyannis, I., Nakayama, A. and Aiba, S. 1998. Edible films made from hydroxypropyl starch and gelatin and plasticized by polyols and water. *Carbohyd. Polym.* 36: 105-119.

Arvanitoyannis, I., Psomiadou, E. and Nakayama, A. 1996. Edible films made from sodium caseinate, starches, sugars or glycerol. *Carbohydr. Polym.* 31: 179-192.

Arvanitoyannis, I., Psomiadou, E., Nakayama, A., Aiba, S. and Yamamoto, N. 1997. Edible films made from gelatin, soluble starch and polyols. *Food Chem.* 60: 593-604.

Arvanitoyannis, S., Sablami, A., Datta, M. S., Rhaman, A. and Mujumdar, 2006. (eds.), Multivariate analysis, Handbook of food bioprocess modelling techniques, CRC Press, Boca Ranton, pp. 323-335.

Asaoka, M., Blanshard, J. M. V. and Rickard, J. E. 1991. Seasonal effects on the physico-chemical properties of starch from flour cultivar of cassava. *Starch* 43:455-459.

Asaoka, M., Blanshard, J. M. V. and Rickard, J. E. 1992. Effect of cultivar and growth season on the gelatinization properties of cassava (*Manihot esculenta*) starch. *J. Sci. Food Agric.* 59:53-58.

Assefa, Z. and Admassu, S. 2013. Development and characterization of antimicrobial packaging films. *J. Food Process Technol.* 4-6.

Atwell, W. A., Hood, L., Lineback, D., Varriano-Marston, E. and Zohel, H. 1988. The terminology and methodology associated with basic starch phenomenon. *Cereal Foods World* 33: 306-311.

Averous, L. and Boquillon, N. 2004. Biocomposites based on plasticized starch: thermal and mechanical behaviours. *Carbohyd. Polym.* 56(2): 111-122.

Averous, L. and Fringant, C. 2001. Association between plasticized starch and polyesters: processing and performances of injected biodegradable systems. *Polym. Eng. Sci.* 41: 727-734.

Averous, L., Fringant, C. and Moro, L. 2001. Plasticized starch–cellulose interactions in polysaccharides composites. *Polym.* 42: 6565-6572.

Azeredo, H. M. C., Faria, J. A. F. and Azeredo, A. M. C. 2000. Embalagens ativas para alimentos. *Ciencia e Tecnologia de Alimentos,* 20(3): 337-341.

Balagopalan, C. 1996. Improving the nutritional value of cassava by solid state fermentation: CTCRI Experiences. *J. Sci. and Ind. Res.* 5: 479-482.

Balagopalan, C., Padmaja, G., Nanda, S. K. and Moorthy, S. N., 1987. Cassava based industries. In: cassava in food, feed industry. Boca Raton, Florida: CRC Press Inc. 169-172.

Balat, M. and Balat, H. 2009. Recent trends in global production and utilization of bio-ethanol fuel. *Appl. Energy* 86(11): 2273-2282.

Baldwin, E. A., Nisperos-carriedo, M. O. and Baker, R. A. 1995. Edible coatings for lightly processed fruits and vegetables. *Hort. Sci.* 30: 35-38.

Bangyekan, C., Aht-Ong, D. and Srikulkit, K. 2006. Preparation and properties evaluation of chitosan-coated cassava starch films. *Carbohydr. Polym.* 63: 61-71.

Barnett, C., Smith, A., Scanlon, B. and Israilides, C. J. 1999. Pullulan production by *Aureobasidium pullulans* growing on hydrolysed potato starch waste. *Carbohyd. Polym.* 38: 203-209.

Bastioli, C. 1995. In: Scott, G. and Gilead, D. (ed.), *Degradable polymers, principles and applications*. 1st edition, Chapman and Hall, London, 112p.

Bataille, I., Huguet, J., Muller, G., Mocanu, G. and Carpov, A. 1997. Associative behaviour of hydrophobically modified carboxymethylpullulan derivatives. *Int.l J. Biol. Macromol.* 20(3): 179-191.

Belibi, P. C., Daou, T. J., Ndjaka, J. M., Michelin, L. and Brendié, J. 2013. Tensile and water barrier properties of cassava starch composite films reinforced by synthetic zeolite and beidellite. *J. Food Eng.* 115: 339-346.

BeMiller, J. N. 1997. Starch modification: Challenges and prospects. *Starch* 49: 127-131.

Bengtsson, M., Koch, K. and Gatenholm, P. 2003. Surface octanylation of high-amylose potato starch films. *Carbohyd. Polym.* 54: 1-11.

Bergo, P. and Sobral, P. J. A. 2007. Effects of plasticizer on physical properties of pigskin gelatin films. *Food Hydrocolloid* 21(8): 1285-1289.

Bijttebier, A., Goesaert, H. and Delcour, J. 2008. Amylase action pattern on starch polymers. Biologia, 63(6): 989-999.

Black, H. N. and melton, L. I. 1966. Temporary well plugging agent with predetermined life U.S. Patent, 3: 212-227.

Carvalho, A., Zambon, M., Da Silva, A. and Gandini, A. 2005. Thermoplastic starch modification during melt processing: Hydrolysis catalyzed by carboxylic acids. *Carbohyd. Polym.* 62(4): 387-390.

Cereda, M. P., Bertolini, A. C., Silva, A. P., Oliveira, M. A. and Evangelista, R. M. 1995. Películas de almidón para la preservación de frutas. In: *Congresso Polimeros Biodegradables: Avances y Perspectivas (Anais... Buenos Aire)* 42p.

Chang, Y.P., Cheah, P. B. and Seow, C. C. 2000. Plasticizing-antiplasticizing effects of water on physical properties of tapioca starch films in the glassy state. *J. of Food Sci.* 65(3): 445-451.

Cheetham, N. W. H. and Tao, L. 1998. Variation in crystalline type with amylose content in maize starch granules: An X-ray powder diffraction study. *Carbohyd. Polym.* 36: 277-284.

Chen, M. C., Yeh, G. H. and Chiang, B. H. 1996. Antimicrobial and physicochemical properties of methylcellulose and chitosan films containing a preservative. *J. Food Process. Preserv.* 20(5): 279-390.

Chen, T., Embree, H. D., Brown, E. M., Taylor, M. M. and Payne, G. F. 2003 Enzyme-catalyzed gel formation of gelatin and chitosan: Potential for *in situ* applications. *Biomaterials* 24: 2831-2841.

Cheng, H. N. and Gu, Q. 2012. Enzyme-catalyzed modifications of polysaccharides and poly (ethylene glycol). *Polymers* 4: 1311-1330.

Colla, E., Sobral, P. J. A. and Menegalli, F. C. 2006. *Amaranthus cruentus* flour edible films: Influence of stearic acid addition, plasticizer concentration, and emulsion stirring speed on water vapour permeability and mechanical properties. *J. of Agric. Food Chem.* 54: 6645-6653.

Colonna, P., Buleon, A. and Lemane, F. 1988. Action of *Bacillus subtilis* α-amylase on native wheat starch. *Biotechnol. Bioeng.* 31: 895-904.

Colonna, P., Tayeb, J. and Mercier, C. 1984. Extrusion cooking of starch and starchy products. In: Mercier, C., Linko, P. and Harper, J. M. (eds.), *Extrusion Cooking*. American Association of Cereal Chemists, St. Paul, 247p.

Cone, J. W. and Wolters, G. E. 1990. Some properties and degradability of isolated starch granules. *Starch/Starke,* 42: 298-301.

Cortes, A. B., Valiente, M. and Rodenas, E. 1999. Properties of the lyotropic phases in CTAB/Glycerol/water and CTAB/Glyceraldehyde/water systems. Langmuir 15: 6658-6663.

Cuppola, L., Gianferri, R., Nicotera, I., Olivero, C. and Ranieri, G. A. 2004. Structural changes in CTAB/ H_2O mixtures using a rheological approach. *Phys. Chem. Chem. Phys.* 6: 2364-2372.

Cuq, B., Gontard, N. and Guilbert, S. 1998. Proteins as agricultural polymers for packaging production. *Cereal Chem.* 75(1): 1-9.

Dave, H., Rao, P. V. C. and Desai, J. D. 1997. Biodegradation of starch polyethylene films in soil and by microbial cultures. *J. Microb. Biot.* 13(6): 655-658.

Davies, L. 1995. Starch-composition, modifications, applications and nutritional value in foodstuffs. *Food Technol. Europe*, 6(7): 44-52.

Defloor, I., Dehing, I., and Deliour, J. A. 1998. Physicochemical properties of cassava starch. *Starch/ Stärke* 50: 58-64.

Dias, A. B., Muller, C. M. O., Larotonda, F. D. S. and Laurindo, J. B. 2010. Biodegradable films based on rice starch and rice flour. *J. of Cereal Sci.* 51: 213-219.

Díaz-Sobac, H., García, C., Beristain, I. and Vernon-Carter, E. J. 2002. Morphology and water vapour permeability of emulsion films based on mesquite gum. *J. of Food Processing and Preservation*, 26(2): 129-141.

Donhowe, G. and Fennema, O. 1994. Edible films and coatings: characteristics, formation, definitions, and testing methods. In: Krochta, J. M, Baldwin, E. A. and Nisperos-Carriedo, M. O. (eds), Lancaster: Technomic Publishing Co. pp. 1-24.

Donhowe, I. G. and Fennema, O. 1993b. The effects of plasticizers on crystallinity, permeability, and mechanical properties of methylcellulose films. *J. Food Process. Preserv.* 17: 247-257.

Drauz, K. and Waldmann, H. 1995. Enzyme Catalysis in Organic Synthesis *Curr. Opin. Biotechnol.* 10: 324-330.

Dreher, M. L., Barry, J. W. and Dreher, C. J. 1984. Starch digestibility of foods a nutritional perspective. *Crit. Rev. Food Sci. Nutr.* 20: 47-71.

Edison, S., Unnikrishnan, M., Vimala, B., Pillai, S. V., Sheela, M. N., Sreekumari, M. T. and Abraham, K. 2006. Biodiversity of tropical tuber crops in India. NBA Scientific Bulletin Number-7. National Biodiversity Authority, Chennai, TamilNadu, India, 60.

Ellis, P. R., Roberts, F. G., Low, A. G. and Morgan, L. M. 1995. The effect of high-molecular-weight guar gum on net apparent glucose absorption and net apparent insulin and gastric inhibitory polypeptide production in the growing pig: relationship to rheological changes in jejunal digesta. *Br. J. Nutr.* 74: 539-556.

Ellis, R. P., Cochrane, M. P. and Dale, M. F. B. 1998. Starch production and industrial use. *J. Sci. Food Agric.* 77: 289-311.

Ezeoha, S. L. and Ezenwanne, J. N. 2013. Production of biodegradable plastic packaging film from cassava starch. *IOSR J. of Eng.* 3(10): 14-20.

Famá, L., Flores, S. K., Gerschenson, L. and Goyanes, S. 2006. Physical characterization of cassava starch biofilms with special reference to dynamic mechanical properties at low temperatures, *Carbohyd. Polym.* 66: 8-15.

Fama, L., Goyanes, S. L. and Gerschenson, B. 2007. Influence of storage time at room temperature on the physicochemical properties of cassava starch films. *Carbohyd. Polym.* 70: 265-273.

Fama, L., Rojas, A. M., Goyanes, S. and Gerschenson, L. 2005. Mechanical properties of tapioca-starch edible films containing sorbates. *LWT- Food Sci. Technol.* 38: 631-639.

Fang, J. M., Fowler, P. A., Escrig, C., Gonzalez, R., Costa, J. A. and Chamudis, L. 2005. Development of biodegradable laminate films derived from naturally occurring carbohydrate polymers. *Carbohyd. Polym.* 60(1): 39-42.

Fanta, G. F., Felker, F. C., Shogren, R. L. and Salch, J. H. 2002. Formation of hydrophilic starch coatings on polyethylene films. *J. appl. polym. sci.* 84: 1781-1788.

FAOSTAT, 2015. http://www.fao.org/statistics/databases/en/ 2015.

Fasina, O. O., Walter, Jr. W. M., Fleming, H. P. and Simunovic, N. 2003. Viscoelastic properties of restructured sweet potato puree, *Int. J. Food Sci. Technol.* 38: 421-425.

Fishman, M. L., Friedman, R. B. and Huang, S. J. Polymers from agricultural coproducts. *Am. Chem. Soc. Symp. Ser.* 575p.

Fourmann, S.B., Carrot, C. and Mignard, N. 2003. Gelatinization and gelation of corn starch followed by dynamic mechanical spectroscopy analysis. *Rheol. Acta.* 42:110-117.

Frohberg, C. and Baeuerlein, M. 2006. Method for generating maize plants with an increased leaf starch content, and their use for making maize silage. US Patent application no. US 2006/0150278 A1.

Gallant, D. J., Bouchet, B., Bulion, A. and Perez, S. 1992. Physical characteristics of starch granules and susceptibility to enzymatic degradation. *European J. of Clinical Nutrition,* 46: 3-16.

Gautam, N. and Kaur, I. 2013. Soil burial biodegradation studies of starch grafted polyethylene and identification of *Rhizobium meliloti* therefrom. *J. Environ. Chem. and Ecotoxicol.* 5(6):147-158.

Geigenberger, P., Stamme, C., Tjaden, J., Schulz, A., Quick, P. W., Betsche, T., Kersting, H. J. and Neuhaus, H. E. 2001. Tuber physiology and properties of starch from tubers of transgenic potato plants with altered plastidic adenylate transporter activity, *Plant Physiol.* 125: 1667-1678.

Gerard, C., Colonna, P., Buleon, A. and Planchot, V. 2001. Amylolysis of maize mutant starches. *J. of Sci. and Food Agric.* 81: 1281-1287.

Ghanbarzadeh, B., Almasi, H. and Entezami, A. A. 2010. Physical properties of edible modified starch/carboxymethyl cellulose films. *Innovative Food Sci. and Emerging Technologies,* 11: 697-702.

Ghosh, A., Azam, A. M. and Walls, R. 2010. Modification of microstructural morphology and physical performance of chitosan films. *Int. J. of Biol. Macromol.* 46(2): 179-186.

Ghosh, R. N., Jona, T., Adhikari, B. and Roy, B. 2002. Proceedings of national conference: Tissue replacement materials and devices and biodegradable polymers and composites for a millennium ahead, IIT Kharagpur, 195p.

Glinel, K., Huguet, J. and Muller, G. 1999. Comparison of the associating behaviour between neutral and anionic alkyl perfluorinated pullulan derivatives. *Polym.* 40(25): 7071-7081.

Goheen, S. M. and Wool, R. P. 1991. Degradation of polyethylene-starch blends in soil, *J. Appl. Polym. Sci.* 42: 2691-2701.

Gontard, N., Guilbert, S. and Cuq, J. L. 1993. Water and glycerol as plasticizers affect mechanical and water vapour barrier properties of an edible wheat gluten film. *J. Food Sci.* 58(1): 206-211.

Gouda, M. K., Swellam, A. E. and Omar, S. H. 2012. Biodegradation of synthetic polyesters (BTA and PCL) with natural flora in soil burial and pure cultures under ambient temperature. *Res. J. Environ. Earth Sci.* pp. 325-333.

Goutard, N., Gulibert, S. and Cuq, J. L. 1993. Water and glycerol as plasticizers affect the mechanical and water vapour barrier properties of an edible wheat gluten film, *J. Food Sci.* 58(1): 206-211.

Griffin, G. J. L. 1974. Advances in chemistry series, fillers and reinforcements for plastics. *Am. Chem. Society.* Pp. 134-159.

Griffin, G. J. L. 1994. Chemistry and Technology of Biodegradable Polymers, Blackie Academic & Professional, London, pp. 135-149.

Gu, Q. M. and Cheng, H. N. 2005. Enzyme-catalyzed condensation reactions for polymer modification. *ACS Symp. Ser.* 900: 427-436.

Guilbert, S. and Gontard, N. 2005. Agro polymers for edible and biodegradable films: review of agricultural polymeric materials, physical and mechanical characteristics. In Han, J. H. (ed.). *Innovations in food packaging.* Elsevier Academic, Oxford, England, pp. 263-276.

Guilbert, S., Gontard, N. and Cuq, B. 1995. Technology and applications of edible protective films. *Packag. Technol. Sci.* pp. 339-346.

Guilbot, A. and Mercier, C. 1985. Starch, In: Aspinall, G. D. (ed.). *The Polysaccharides*, Academic Press Inc., London, pp. 209-282.

Guohua, Z., Ya, L., Cuilan, F., Min, Z., Caiqiong, Z. and Zongdao, C. 2006. Water resistance, mechanical properties and biodegradability of methylated-cornstarch/poly (vinyl alcohol) blend film, polymer degradation and stability. *Polym. Degrad. Stabil.* 91(4): 703-711.

Gupta, R., Gigras, P., Mohapatra, H., Goswami, V. K. and Chauhan. B. 2003. Microbial α-amylase: A biotechnological perspective. *Process Biochem.* 38: 1599-1616.

Guraya, H. S., James, C. and Champagne, T. 2001. Effect of cooling and freezing on the digestibility of debranched non-waxy and waxy starch and physical properties of the resulting material. *Starch/Stark* 53: 64-74.

Henrissat, B. 1991. A classification of glycosyl hydrolases based on amino acid sequence similarities. *Biochem. J.* 280: 309-316.

Hii, S. L., Tan, J. S., Ling, T. C. and Ariff, A. B. 2012. Pullulanase: Role in starch hydrolysis and potential industrial applications. *Enzyme Resear.* Hindawi Publishing Corporation, 14p.

Hirsch, J. B. and Kokini, J. L. 2002. Understanding the mechanism of cross-linking agents (POCl3, STMP, and EPI) through swelling behaviour and pasting properties of cross-linked waxy maize starches. *Cereal Chem.* 79: 102-107.

Hoover, R. and Sosulki, F. W. 1985. Studies on the functional characteristics and digestibility of starches from *Phaseolus vulgaris* biotypes. *Stark* 37: 181-191.

Horner, V. V. and walker, R. E. 1965 (eds) for plugging all the gas bore holes. US patent 3208, 524 (chem. Abstr. 64: 5036, 1966).

Hoseney, R. C. 1986. Principles of cereal science and technology. *Cereal Chem.* 63(3): 285-286.

Hudson, B. J. F. and Ogunsua, A. O. 1976. The effect of fibre, starch damage and surfactants on the baking quality of wheat cassava composite flours. *J. Food Technol.* pp. 129-136.

Iefuji, H., Chino, M., Kato, M. and Iimura, Y. 1996. Raw-starch-digesting and thermostable alpha-amylase from yeast *Cryptococcus* sp. S-2: purification, characterization, cloning and sequencing. *Biochem J.* 318: 989-996.

Ikawa, Y., Glover, D. V., Sugimoto, Y. and Fuwa, H. 1978. Amylose percentage and distribution of unit chain-length of maize starches having a specific genetic background. *Carbohyd. Res.* 61: 211-216.

Jane, J., Wong, K. S. and McPherson, A. E. 1997. Branch-structure difference in starches of A- and B-type X-ray patterns revealed by their Naegali dextrins, *Carbohyd. Res.* 300: 219-227.

Jobling, S. 2004. Improving starch for food and industrial applications. *Curr. Opin. Plant Biol.* 7: 210-218.

Jokay, L., Nelson, G. E. and Powell, E. L. 1967. Development of edible amylaceous coating for foods, *Food technol.* 21: 1064-1066.

Jyothi, A.N. and Moorthy, S.N. 2012. Modified tuber starches in industry. Technical bulletin. ICAR-Central Tuber Crops Research Institute, Sreeakariyam, Trivandrum.

Karkalas, J., Tester, R. F. and Morrison, W. R. 1992. Properties of damaged starch granules. I. Comparison of a new micro method for the enzymatic determination of damaged starch with the standard AACC and Farrand methods. *J. Cereal Sci.* 16: 237-251.

Kasirajan, S. and Ngouajio, M. 2012. Polyethylene and biodegradable mulches for agricultural edible wheat protein films. *J. of Food Sci.* 62(1): 160-164.

Kaur, B., Ariffin, F., Bhat, R. and Karim, A. A. 2012. Progress in starch modification in the last decade. *Food Hydrocolloid.* 26: 398-404.

Kayisu, K. and Hood, L. F. 1979. Effects of dehydration and storage conditions on the pancreatic a-amylase susceptibility of various starches. *J. of Food Sci.* 44: 1728-1731.

Ke, T. and Sun, X. S. 2003. Starch, poly(lactic acid), and poly(vinyl alcohol) blends. *Polym. Environ.* 11(1): 7-14.

Kester, J. J. and Fennema, O. 1986. Edible films and coatings: A review. *Food Technol.* 40: 47-59.

Khatoon, S., Sreerama, Y. N., Raghavendra, D., Bhattacharya, S. and Bhat, K. K. 2009. Properties of enzyme modified corn, rice and tapioca starches. *Food Resear. Int.* 42: 1426-1433.

Kim, C. H., Nashiru, O. and Ko, J. H. 1996. Purification and biochemical characterization of pullulanase type I from *Thermus aldophilus* GK-24. *FEMS Microbiol. Lett.* 138, 147-152.

Koch, H., Roper, H. and Hopcke, R. 1993. New industrial uses of starch. *Roy. Sot. Chem., Spec. Publ.* 134:157-179.

Krochta, J. M. and Miller, K. S. 1997. Oxygen and aroma barrier properties of edible films: A review. *Trends Food Sci. Technol.* 8: 228-237.

Krossmann, J. and Lloyd, J. 2000. Understanding and influencing starch biochemistry. *Crit. Rev. Biochem. Mol. Biol.* 35: 41-196.

Krupp, L. and Jewell, W. 1992. Biodegradable Polymers. *Environ. Sci. Tech.* 26: 193-198.

Kuriki, T. and Imanaka, T. 1999. The concept of the α-amylase family: Structural similarity and common catalytic mechanisms. *J. Biosci. Bioeng.* 87: 557-565.

Lagarigue, S. and Alvarez, G. 2001. The rheology of starch dispersions at high temperatures and high shear rate: a review. *J. Food Eng.* 50:189-202.

Lajolo, F. M., Finardi-Filho, F. and Menezes, E. W. 1991. Amylase inhibitors in *Phaseolus vulgaris* beans. *Food Technol.* 45: 119-121.

Lambri, M., Dordoni, R., Roda, A. and De Faveri, D. M. 2014. Process development for maltodextrins and glucose syrup from cassava. *Chemical Engineering Transactions,* 38: 469-474.

Laohakunjit, N. and Noomhorm, A. 2004. Effect of plasticizers on mechanical and barrier properties of rice starch film. *Starch/Starke,* 56: 348-356

Larotonda, F. D. S., Matsui, K. N., Sobral, P. J. A. and Laurindo, J. B., 2005. Hygroscopicity and water vapour permeability of Kraft paper impregnated with starch acetate. *J. Food Eng.* 71: 394-402.

Lauro, M., Forssell, P. M. P., Suortti, M. T., Hulleman, S. H. D. and Poutanen, K. S. 1999. α-Amylolysis of large barley starch granules. *Cereal Chem.,* 76: 925-930.

Leach, H. W. 1965. Gelatinization of starch. In: Whistler, R. L. and Paschall, E. F. (eds.), *Starch chemistry and technology*, Academic Press, New York.

Leloup, V. M., Colonna, P. and Ring, S. G. 1990. Studies on probe diffusion and accessibility in amylose gels. *Macromols.* 23: 862-866.

Light, J. 1990. Modified food starches: why, what, where and how. *Cereal Food World.* 35 (11): 1081-1092.

Lindahl, L. and Eliasson, A. C. 1986. Effects of wheat proteins on the viscoelastic properties of starch gels, *J. Sci. Food Agric.* 37: 1125-1132.

Ling, S. H., Tan, J. S., Ling, T. C. and Ariff, A. B. 2012. "Pullulanase: Role in Starch Hydrolysis and Potential Industrial Applications," *Enzyme Research,* 1-14.

Liu, Z. 2005. Edible films and coatings from starches. In: Han, J. H. (ed.) *Innovations in food packaging. Elsevier* pp. 318-337.

Lloyd, J. R. *et al.* 1999. Simultaneous antisense inhibition of two starch synthase isoforms in potato tuber leads to accumulation of grossly modified amylopectin. *Biochem. J.* 338: 515-521.

Lloyd, N.E. and Kirst, L.C. 1963. Some factors affecting the tensile strength of starch films. *Cereal Chem.* 40: 154.

Lomako, J., Lomako, W. M., Whelan, W. J. and Marchase, R. B. 1993. Glycogen contains phosphodiester groups that can be introduced by UDPglucose: glycogen glucose 1-phosphotransferase, *FEBS Lett.* 329: 263-67.

Loos, K. 2011. *Biocatalysis in Polymer Chemistry*, Wiley-VCH, Weinheim, Germany.

Lourdin, D., Della Valle, G. and Collona, P. 1995. Influence of amylose content on starch films and foams. *Carbohyd. Polym.* 27: 261-270.

Lu, Y., Tighzert, L., Berzin, F. and Rondot. 2005. Innovative plastizide starch film modified with water born polyurethane from renewable sources. *Carbohyd. polym.* 61: 174-182.

Ma, X., Chang, P. R. and Yu, J. 2008. Properties of biodegradable thermoplastic pea starch/carboxymethyl cellulose and pea starch/microcrystalline cellulose composites. *Carbohyd. Polym.* 72: 369-375.

Maarel, V. M. J. E. C., Veen, V. B., Uitdehaag, J. C. M., Leemhuis, H. and Dijkhuizen, L. 2002. Properties and applications of starch-converting enzymes of the α-amylase family. *J. Biotechnol.* 94: 137-155.

Mac Gregor, A. N. and Fincher, G. B. 1993. Carbohydrates of the barle grain. In: Barley Chemistry and Technology. A. N. Mac Gregor and R. S. Bhatty (eds). Am. Assoc. *Cereal Chem.* St. Paul, M. N. pp. 73-130.

MacAllister, R. V. 1979. Nutritive sweeteners made from starch. In: Tipson, S. and Horton, D. (eds), *Advances in Carbohydrate Chemistry and Biochemistry*, Academic Press, New York, 36: 15-56.

Madzlan, K., Hasnisa, H., Sabeetha, S. and Dayana, M. N. 2012. Extraction of starch and enzymatic production of high amylose starch from sweetpotato (*Ipomea batatas*) var. Telong. *J. Trop. Agric. Food Sci.* 40(2): 203-210.

Maharana, T. and Singh, B. C. 2006. Synthesis and characterization of biodegradable polyethylene by graft copolymerization of starch using glucose-Ce (IV) redox system. J. *Appl. Polym. Sci.* 100(4): 3229-3239.

Maldonado, G. H. and Lopez, P. O. 1995. Amylolytic enzymes and products derived from starch: A review. *Crit Rev. Food Sci. Nutr.* 35: 373-403.

Mali, S., Grossmann, M. V. E., Garcia, M. A., Martino, M. N. and Zaritzky, N. E. 2005. Mechanical and thermal properties of yam starch films. *Food Hydrocolloid.* 19: 157-164.

Mali, S., Grossmann, M. V. E., Garcia, M. A., Martino, M. N. and Zaritzky, N. E. 2002. Microstructural characterization of yam starch films. *Carbohyd. Polym.* 50: 379-386.

Mali, S., Grossmann, M. V. E., Garcia, M. A., Martino, M. N. and Zaritzky, N. E. 2006. Effects of controlled storage on thermal, mechanical and barrier properties of plasticized films from different starch sources, *J. Food Eng.* 75: 453-460.

Mali, S., Karam, L. B., Ramos, P. L. and Grossmann, M. V. E. 2004. Relationships among the composition and physicochemical properties of starches with the characteristics of their films. *J. of Agric. Food Chem.* 52: 7720-7725.

Mali, S., Sakanaka, L. S., Yamashita, F. and Grossmann, M. V. E. 2005. Water sorption and mechanical properties of cassava starch films and their relation to plasticizing effect. *Carbohyd. Polym.* 60: 283-289.

Manners, D. J. 1989. Some aspects of the structure of starch and glycogen. *Denpun Kagaku* 36: 311-323.

Maran, J. P., Sivakumar, V., Sridhar, R. and Thirugnanasambandham, K. 2013. Development of model for barrier and optical properties of tapioca starch based edible films. *Carbohyd. Polym.* 92(2): 1335-1347.

Marcotte, M., Taherian, A. R., Trigui, M. and Ramaswamy, H. S. 2001. Evaluation of rheological properties of selected salt enriched food hydrocolloids, *J. Food Eng.* 48: 157-167.

Marija, S. M., Radosavljevic, M. M. and Dokic, L. P. 2010. Effects of autoclaving and pullulanase debranching on the resistant starch yield of normal maize starch. *J. Serb. Chem. Soc.* 75(4): 449-458.

Mariniello, L., Pierro, P., Esposito, C., Sorrentino, A., Masi, P. and Porta, R. 2003. Preparation and mechanical properties of edible pectin-soy flour films obtained in the absence or presence of transglutaminase. *J. of Biotechnol.* 102(2): 191-198.

Mark, A. M., Roth, W. B., Mehltretter, C. L. and Rist, C. E. 1966. Oxygen permeability of amylomaize starch films, *Food technol.* 20: 75-77.

Marotta, M., Martino, A., De Rosa, A., Farina, E., Carten, M. and De Rosa, M. 2002. Degradation of dental plaque glucans and prevention of glucan formation using commercial enzymes. *Process Biochem.*, 38(1): 101-108.

Marz, U. 2006. World market for starches/glucose, *Emphasizing Cassava*. Report Code: FOD037A.

Meagher, M., Desphande, M. V. and Sadanam, J. C. 1989. Purification and characterization of a cellulase-free endo-1,4β-d-xylanase of *Sclerotium rolfsii* UV-8 mutant. *Enzyme Microb. Technol.* 11: 678-684.

Means, W. J. and Schmidt, G. R. 1986. Algin/Calcium Gel as a Raw and Cooked Binder in Structured Beef Steaks, *J. Food Sci.* 51: 60-65.

Milašinović, M., Radosavljević, M. and Dokić, L. J. 2010. Effects of autoclaving and pullulanase debranhing on the resistant starch yield of normal starch, *J. Serb. Chem. Soc.* 75(4): 449-458.

Min, B. J. and Oh, J. H. 2009. Antimicrobial activity of catfish gelatin coating containing origanum (*Thymus capitatus*) oil against gram-negative pathogenic bacteria, *J. Food Sci.* 74(4): 143-148.

Mitchell, J. R. and Blanshard, J. M. V. 1976. Rheological properties of pectate gels, *J. of Texture Studies*, 7: 341-351.

Miyazaki, M. and Morita, N. 2005. Effect of heat moisture treated maize starch on the properties of dough and bread. *Food Resear. Int.* 38: 369-376.

Miyazaki, M. R., Hung, P. V., Maeda, T. and Morita, N. 2006. Recent advances in application of modified starches for bread making, *Trends Food Sci. Tech.* 17: 591-599.

Montagnac, J. A., Christopher, R. D. and Tanumi, S. A. 2009. Processing technique to reduce toxicity and antinutrients of cassava for use as staple food. *Comprehensive Rev. Food Sci. Food Safety* 8: 17-27.

Montalvo, G., Valiente, M. and Rodenas, E. 1996. Rheological properties of the L phase and the hexagonal lamellar and cubic liquid crystals of the CTAB/ Benzyl alcohol/ water system. Langmuir, 12: 5202-5208.

Moorthy, S. N. 2001. Tuber Starches. Technical Bulletin No18, Central Tuber Crops Research Institute, Thiruvananthapuram-695017, India.

Moorthy, S. N. 1994. *Tuber crop starches*. Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram, Kerala.

Mukerjea, R., Slocum, G., Mukerjea, R. and Robyt, J. F. 2006. Significant differences in the activities of α-amylases in the absence and presence of polyethylene glycol assayed on eight starches solubilized by two methods. *Carbohydr. Res.* 341: 2049-2054.

Muller, C. M. O., Yamashita, F. and Laurindo, J. B. 2007. Evaluation of the effect of glycerol and sorbitol concentration and water activity on the water barrier properties of cassava starch films through a solubility approach. *Carbohyd. Polym.* 10: 1016-1021.

Müller, C. M. O., Yamashita, F. and Laurindo, J. B. 2008. Evaluation of the effects of glycerol and sorbitol concentration and water activity on the water barrier properties of cassava starch films through a solubility approach. *Carbohydr. Polym.* 72: 82-87.

Nair, S. U., Singhal, R. S. and Kamat, M. Y. 2007. Induction of pullulanase production in *Bacillus cereus* FDTA-13. *Bioresource Technol.* 98(4): 856-859.

Nakamura, E. M, Cordi, L., Almedida, G. S. G., Duran, N. and Mei, L. H. I. 2005. Study and development of LDPE/starch partially biodegradable compounds. *J. Mater. Process Tech.* 162-163.

Narayan, R. 1994. Polymeric Materials from Agricultural Feedstocks. In: Fishman, M. L., Friedman, R. B. and Huang, S. J. (ed.), *Polymers from agricultural coproducts. Am. Chem. Soc. Symp. Ser.* 575p.

Narayan, R. 1998. Commercialization technology: A case study of starch based biodegradable plastics. In: Sessa, D. J. and Willett, J. L. (eds), *Paradigm for successful utilization of renewable resources,* Champaign, IL: AOCS Press. 78p.

Narayan, R. 2001. Drivers for biodegradable/compostable plastics and role of composting in waste management and sustainable agriculture. *Bioprocessing of Solid Waste and Sludge* 1(1).

Narayanan, A. S. and Shanmugasundaram, E. R. B. 1967. Studies on amylase of *Fusarium vasinfectum. Arch. Biochem. Biophys.* 118: 317.

Neelam, K., Vijay, S. and Lalit, S. 2012. Various techniques for the modification of starch and the applications of its derivatives. *Int. J. Pharma.* 3: 5.

Nelson, K. L. and Fennema, O. R. 1991. Methylcellulose films to prevent lipid migration in confectionery products, *J. Food Sci.* 56: 504-509.

Nishinari, K. 2007. Rheological and related studies on industrially important polysaccharides and protein. *J. Cent. South Univ. Technol.* D01:10/1007/S 11771-007-0315-6.

Noel, T. R., Ring, S. G. and Whittman, M. A. 1992. The structure and gelatinization of starch. *Food Sci. and Technol. Today,* 6: 159.

Norman, B. E. 1982. A novel debranching enzyme for application in the glucose syrup industry. *Starch* 34(10): 340-346.

Nweke, F. 2004. *New Challenges in the Cassava Transformation in Nigeria and Ghana*. Environment and Production Technology Division (EPTD) Discussion Paper No. 118, International Food Policy Research Institute, Washington, D. C., USA, pp 118.

Nweke, F. I., Spencer, D. S. C. and John, K. L. 2002. *The cassava transformation; Africa's best kept secret*. Michigan State University Press, East Lansing, pp. 10-21.

Oates, C.G. 1997. Towards an understanding of starch granule structure and hydrolysis. *Trends Food Sci. Technol.* 8: 375-382.

Obasi, H. C., Onuoha, F. N., Eze, I. O., Nwanonenyi, S. C., Arukalam, I. O. and Uzoma, P. C. 2013. Effect of soil burial on properties of polypropylene (pp)/ plasticized potato starch blends. *Int. J. Eng. Sci.* 2(8): 14-18.

Ofman, M., Campos, C. and Gerschenson, L. 2004. Effect of preservatives on the functional properties of tapioca starch: Analysis of interactions. *Lebensmittel Wissenschaft und Technologie* 37: 355-361.

Ogura, T. 2004. Modified starch and utilization. In: Hidetsugu, F., Komaki, T., Hizukuri, S. and Kainuma, K. (eds), *Encyclopedia on starch science Tokyo* pp. 393-427.

Okada, M. 2002. Chemical syntheses of biodegradable polymers. *Progress in Polym. Sci.* 27: 87-133.

Olsen, H. S., Goddard, P. and Nordisk, A. S. N. 2000. *Enzymes at work: A concise guide to industrial enzymes and their uses*, Novo Nordisk A/S.

Oluwole, O. B., Olatunji, O. O. and Odunfa, S. A. 2004. A process technology for conversion of dried cassava chips into gari. *Nigerian Food J.* 22: 65-77.

Onuoha, F. N., Obasi, H. C., Eze, I. O., Nwanonenyi, S.C., Arukalam, I. O. and Uzoma, P. C. 2013. Effects of Soil Burial on Properties of Polypropylene (Pp)/ Plasticized, *Int. J. Of Eng. Sci.* 2: 2319-1805.

Ortega-Ojeda, F. E., Larsson, H. and Eliasson, A. C. 2004a. Gel formation in mixtures of high amylopectin potato starch and potato starch. *Carbohyd. Polym.* 56: 505-514.

Ortega-Ojeda, F. E., Larsson, H. and Eliasson, A. C. 2004b. Gel formation in mixtures of amylose and high amylopectin potato starch. *Carbohyd. Polym.* 57: 55-66.

Ortega-Ojeda FE, Larsson H, Eliasson AC 2005. Gel formation in mixtures of hydrophobically modified potato and high amylopectin potato starch. *Carbohyd. Polym.* 59: 313-327.

Paes, S. S., Yakimets, I. and Mitchell, J. R. 2008. Influence of gelatinization process on functional properties of cassava starch films. *Food Hydrocolloid* 22(5): 788-797.

Parra, D. F., Tadini, C. C., Ponce, P. and Lugao, A.B. 2004. Mechanical properties and water vapour transmission in some blends of cassava starch edible films. *Carbohyd. Polym.* 58: 475-481.

Pilling, E. and Smith, A. M. 2003. Growth ring formation in the starch granules of potato tubers. *Plant Physiol.* 132: 365-371.

Pongjanta, J., Utaipattanaceep, A., Naivikul, O. and Piyachomkwan, K. 2009. Debranching enzyme concentration effected on physicochemical properties and α-amylase hydrolysis rate of resistant starch type III from amylose rice starch. *Carbohyd. Polym.* 78: 5-9.

Powell, E. L. 1965. Production and uses of pre-gelatinized starches. In: Whistler, R. L. and Paschall, E. F. (eds.), *Starch chemistry and technology*, vol. II. Academic Press, New York Press.

Psomiadou, E., Arvanitoyannis, I. and Yamamoto, N. 1996. Edible films made from natural resources; microcrystalline cellulose (MCC), methyl cellulose (MC) and corn starch and polyols, *Carbohyd. Polym.* 31: 193-204.

Purseglove, J. W. 1988. *Tropical Crops dicotyledons*. ELBS, Longman, U.K.

Rachtanapun, P., Pankan, D. and Srisawat, D. 2012. Edible films of blended cassava starch and rice flour with sorbital and their mechanical properties. *J. of Agricultural Sci. and Technol.* 2: 252-258.

Rajan, A., Sudha, J. D. and Abraham, E. T. 2008. Enzymatic modification of cassava by fungal lipase. *Industrial crops and products,* 27: 50-59.

Rao, M. A. 1999. Rheology of fluid and semi solid foods. Principles and application, Springer Publ, New York, p.59-152.

Ratanakamnuan, U. and Aht-Ong, D. 2006. Photobiodegradation of low density polyethylene/banana starch films. *J. Appl. Polym. Sci.* 100(4): 2725-2736.

Rayas, L. M. and Hernandez, R. J. 1997. Development and characterization of biodegradable starch films. *J. Appl. Polym. Sci.* 100: 2577.

Regierer, B., Fernie, A. R., Springer, F., Perez-Melis, A., Leisse, A., Koehl, K., Willmitzer, L., Geigenberger, P. and Kossmann, J. 2002. Starch content and yield increase as a result of altering adenylate pools in transgenic plants. *Nat. Biotech.* 20: 1256-1260.

Rendleman, J. A. 1997. Enhancement of cyclodextrin production through use of debranching enzymes. *Biotechnol. Appl. Biochem.* 26: 51-61.

Rickard, J. E., Asaoka, M. and Blanshad, J. M. V. 1991. The physico-chemical properties of cassava starch. *Trop. Sci.* 31:189-207.

Rindlav-Westling, A., Stading, M. and Gatenholm, P.. 2002. Crystallinity and morphology in films of starch, amylose and amylopectin blends. *Biomacromolecules,* 3: 84-91.

Ring, S. G., Gee, M. J., Whittam, M., Orford, P. and Johnson, I. T. 1988. Resistant starch: its chemical form in food stuffs and effect on digestibility *in vitro*. *Food Chem.* 28: 97-109.

Roberts, H. J. 1965. *Nondegradative reactions of starch*, vol. I, 1st edn, pp. 439-493.

Robertson, G. H., Wong, D. W. S., Lee, C. C., Wagschal, K., Smith, M. R. and Orts, W. J. 2006. Native or raw starch digestion: a key step in energy efficient biorefining of grain. *J. Agric. Food Chem.* 54: 353-365.

Rohn, S., Rawel, H. M. and Kroll, J. 2004. Antioxidant activity of protein- bound quercetin. *J. of Agricultural and Food Chem.* 52(15): 4725-4729.

Romero-Bastida, C. A., Bello-Pérez, L. A., García, M. A., Martino, M. N., Solorza-Feria, J., and Zaritzky, N. E. 2005. Physicochemical and microstructural characterization of films prepared by thermal and cold gelatinization from nonconventional sources of starches. *Carbohyd. Polym.* 60(2): 235-244.

Rommens, C. M., Haring, M. A., Swords, K., Davies, H. V. and Belknap, W. R. 2007. The intragenic approach as a new extension to traditional plant breeding. *Trends Plant Sci.* 12:397-403.

Rudiger, A., Jorgensen, P. and Antranikan, G. 1995. Isolation and characterization of a heat stable pullulanase from the hyperthermophilic Archeon *Pyrococcus woesei* after cloning and expression of its gene in *Escherichia coli. Appl. Environ. Microbiol.* 61: 567-575.

Sajeev, M. S., Jyothi, A. N., Seena, S. and Sheriff, J. T. 2010. Modification of rheological and pasting properties of cassava starch with hydrocolloids and glycerol. International Conference on Recent trends in Material Science and Technology, ICMST 2010, 29-31, 2010, Trivandrum.

Sajeev, M. S., Jyothi, A. N. and Sheriff, J. T. 2013.Development of Biodegradable film from cassava starch – nano clay composites. International conference on food technology: "Food processing technologies- challenges and solution for sustainable food security" IICPT, Thanjavur.

Sajeev, M. S., Sreekumar, J., Unnikrishnan, M., Moorthy, S. N. and Shanavas, S. 2009. Kinetics of thermal softening of cassava tubers and rheological modeling of the starch. *J. of Food Sci. and Technol.* 47(5): 507-518.

Sajeev, M.S. and Balagopalan, C. 2005. Performance evaluation of multi-purpose mobile starch extraction plant for small scale processing of tuber crops, *J. Root Crops*, 31(2):106-110.

Sajeev, M. S., Jyothi, A. N. and Sheriff, J. T. 2013.Development of Biodegradable film from cassava starch – nano clay composites. International conference on food technology: "Food processing technologies- challenges and solution for sustainable food security" 4-5, January 2013. IICPT, Thanjavur.

Sajeev, M. S., Jyothi, A. N. and Sheriff, J. T. 2013. Physico-mechanical and hygroscopic properties of modified cassava starch based biodegradable films International Conference on Tropical Roots and Tubers for Sustainable Livelihood Under Changing Agro-Climate (ICTRT 2013), Indian Society for Root Crops (ISRC) and Central Tuber Crops Research Institute (CTCRI), Thiruvananthapuram, Kerala, India.

Sajilata, M. G. and Singhal, R. S. 2004. Specialty starches for snack foods, *Carbohyd. Polym.* 59: 131-151.

Sajilata, M. G., Singhal, R. S. and Kulkarni, P. R. 2006. Resistant starch - a review. *Comprehensive Rev. Food Sci and Food Safety* 5: 1-17.

Sanchez-Garcia, M. D., Gimenez, E. and Lagaron, J. M. 2008. Morphology and barrier properties of solvent cast composites of thermoplastic biopolymers and purified cellulose fibers. *Carbohyd. Polym.* 71: 235-244.

Santamara, R. I., Del Rio, G., Saab, G., Rodriguez, M. E., Soberon, X. and Lopez-Mungua, A. 1999. Alcoholysis reactions from starch with α amylases. *FEBS Letters* 452: 346-350.

Schmitz, P. M. and Kavallari, A. 2009. Crop plants versus energy plants-on the international food crisis, *Bioorg. Med. Chem.* 17: 4020-4021.

Shaw, J. F. and Sheu, J. R. 1992. Production of high-maltose syrup and high-protein flour from rice by an enzymatic method. *Biosci. Biotech. Biochem.* 56: 1071-1073.

Shuren, J. 2001. In: Cassava's potential in Asia in the 21st centuary: Present situation and future research and development needs (eds). Howeler, R. H. and tan, S. H. *Proc – 6th regional workshop*, held in Ho Chi Minh city, Vietnam, pp. 553-563.

Singh, S. K., Johnson, L. A., Pollak, L. M. and Hauburgh, C. R. 2001. Compositional, Physical and wet milling properties of accessions used in Germplasm enhancement of Maize project, *Cereal chem.* 78: 330-335.

Siritunga, D., Arias-Garcon, D., White, W. and Sayre, R. T. 2004. Overexpression of hydroxynitrile lyase in transgenic cassava roots accelerates cyanogenesis and food detoxification. *J. Plant Biotechnol.* 2: 37-43.

Smith, A. M. 1999. Making starch. *Curr. Opin. Plant Biol.* 2: 223-229.

Snow, P. and O'Dea, K. 1981. Factors affecting the rate of hydrolysis of starch in foods. *Am. J. Clin. Nutr.* 34: 2721-2727.

Sobral, P. J. A., Menegalli, F. C., Hubinger, M. D. and Roques, M. A. 2001. Mechanical, water vapour barrier and thermal properties of gelatin based edible films. *Food Hydrocolloid.* 15: 423-432.

Sriroth, K., Chollkup, R. Chotineeranat, S. Piyachomkwan, K. and Oates, C. 2000. Processing cassava waste for improved biomass utilization. *Biores. Technol.* 71: 63-69.

Stark, D. M., Timmerman, K. P., Barry, G. F., Preiss, J. and Kishore, G. M. 1992. Regulation of the amount of starch in plant tissues by ADP-glucose pyrophosphorylase, *Science* 258: 287-292.

Steffe, J. F. 1996. Rheological methods in food process engineering. 2nd edn. Freeman Press, East Lansing, MI, 48823, USA.

Subramanian, N. and Gunasekharan, S. 1997. Small amplitude oscillatory shear stress on mozzarella cheese. Part II. Relaxation spectrum. *J. Text Stud.* 28: 643-656.

Swanson, C. L., Shogren, R. L., Fanta, G. F. and Imam, S. H. 1993. *J. Environ. Polym. Degrad.* 1: 155.

Talja, R. A., Helen, H., Roos, Y. H. and Jouppila, K. 2007. Effect of various polyols and polyol contents on physical and mechanical properties of potato starch-based films. *Carbohyd. Polym.* 67: 288-295.

Tang, H., Wang, A., Salley, S. O. and Simon, K. Y. 2008. The Effect of Natural and Synthetic Antioxidants on the Oxidative Stability of Biodiesel. JAOCS, 85 (4): 373-382.

Tapia-Bla 'cido, D., Sobral, P. J. A. and Menegalli, F. C. 2005. Development and characterization of edible films based on amaranth flour (*Amaranthus caudatus*). *J. Food Eng.*, 67: 215-223.

Tester, R. F. and Karkalas, J. 2002. Starch. In: Steinbuchel, A. (series ed.), Vandamme, E. J., De Baets, S. and Steinbuchel, A. (volume eds.), *Biopolymers Volume 6, Polysaccharides II: Polysaccharides from Eukaryotes*. Wiley-VCH, Weinheim. pp. 381-438.

Tester, R. F., Qi, X. and Karkalas, J. 2006. Hydrolysis of native starches with amylases. *Animal Feed Sci. and Technol.* 130: 39-54.

Tewe, O. 1984. Cyanogenic glycoside: Protein interaction in cassava peel based rations. *Nutr. Rep. Int.* 30: 425-431.

Thakore, I. M., Iyer, S., Desai, A., Lele, A. and Devi, S. 1999. Morphology, thermomechanical properties, and biodegradability of low density polyethylene/starch blend. *J. Appl. Polym. Sci.* 74(12): 2791-2802.

Tharanathan, R. N. 2003. Biodegradable films and composite coatings: Past, present, and future. *Trends Food Sci. Technol.* 14: 71-78.

Thiem, J. and Bachmann, F. 1993. Macromolecules and polymers, *Chem.* 194: 1035-1057.

Thomas, D. J. and Atwell, W. A. 1997. In: Starches, Practical guides for the food industry. St Paul, Minnesota, USA, Eagan Press, pp. 25-29.

Thompson, L. U. and Gabon, J. E. 1987. Effects of lectins on salivary and pancreatic amylase activities and the rate of starch digestion. *J. of Food Sci.* 52: 1050-1053.

Uthumporn, U., Zaidul, I. S. M. and Karim, A. A. 2010. Hydrolysis of granular starch at sub-gelatinization temperature using a mixture of amylolytic enzymes. *Food Bioprod. Process.* 88: 47-54.

van der Maarel, M. J. E. C., van der Veen, B., Uitdehaag, J. C. M., Leemhuis, H., Dijkhuizen, L. 2002. Properties and applications of starch converting enzymes of the α-amylase family, *J. Biotechnol.* 94:137-155.

Van, D. and Willem, R. 1990. Dishwashing detergents containing enzyme capable of breaking β-1,6-glucoside linkage, *Brit. UK Patent Appl.* GB2, 228, 945p.

Vasanthan, T. and Bhatty, R. S. 1996. Physicochemical properties of small and large granule starches of waxy, regular, and high amylose barleys. *Cereal Chem.* 73: 199-207.

Vatanasuchart, V., Tungtrakul, P., Wongkrajang, K. and Naivikul, O. 2010. Properties of pullulanase debranched cassava starch and type-III resistant starch. *Kasetsart J. Nat. Sci.* 44: 131-141.

Veiga-Santos, P., Oliveira, L. M., Cereda, M. P. and Scamparini, A. R. P. 2007. Sucrose and inverted sugar as plasticizer. Effect on cassava starch-gelatin film mechanical properties, hydrophilicity and water activity, *Food Chem.* 103: 255-262.

Vijaya Bhaskar, Y., Venkata Mohan, S. and Sarma, P.N., 2008. "Effect of substrate loading rate of chemical wastewater on fermentative biohydrogen production in biofilm configured sequencing batch reactor", *Biores. Technol.* 99: 6941-8.

Vorwerg, W., Radosta, S. and Leibnitz, E. 2002. Study of a preparative-scale process for the production of amylose. *Carbohyd. Polym.* 47(2): 181-189.

Wang, W. J., Powell, A. D. and Oates, C. G. 1995. Pattern of enzyme hydrolysis in raw sago starch: Effects of processing history. *Carbohydr. Polym.* 26: 91-97.

Whistler, R. L. and BeMiller, J. N. 1997. Carbohydrate chemistry for food scientists. St. Paul: Eagan Press, pp. 117-151.

Williams, P. C. and Lesselleur, G. C. 1970. Determination of damaged starch in flour, a comparative study of present day procedures. *Cereal Science Today* 15: 4.

Wool, P. R. 1994. Perspectives on standard test methods for biodegradable plastics, In: (eds.). Doi, Y. and Fukuda, K. *Biodegradable plastics and polymers.* University of Illanios, USA, pp. 250.

Wu, Y., Weller, C. L., Hamouz, F., Cuppett, S. and Schnepf, M. 2001. Moisture loss and lipid oxidation for precooked ground-beef patties packaged in edible starch-alginate-based composite films, *J. of Food Sci.* 66(3): 486-493.

Wursch, P., Dal Vedovo, S. and Koellreuter, B. 1986. Cell structure and starch nature as key determinants of the digestion rate of starch in legume. *American J. Clin. Nutr.* 43: 25-29.

Wurzburg, O. B. 1986. (eds), *Modified starches, properties and uses*. Boca Raton, FL: CRC.

Xiong, H. G., Tang, S. W., Tang, H. L. and Zou, P. 2008. The structure and properties of a starch-based biodegradable film. *Carbohyd. Polym.* 71(2): 263-268.

Yin, H. L., Alias, A. K. and Mond, H. N. 2007. Effect of pullulanase debranching of Sago starch at sub gelatinization temperature on the yield of resistant starch. *Starch/Starke* 59: 21-32.

Yu, P. H., Chua, H., Huang, A. L., Lo, W. and Chen, G. Q. 1998. Conversion of food industrial wastes into bioplastics, *Appl. Biochem. Biotechnol.* 72: 603-614.

Yun, Y. H., Wee, Y. W., Byun, H. S. and Yoon, S. D. 2008. Biodegradability of chemically modified starch (RS4)/ PVA blend films: Part 2. *J. Polym. Environ.* 16(1): 12-18.

Zhang, J. and Greasham, R. 1999. Chemically defined media for commercial fermentations. *Appl. Microbiol. Biotechnol.* 51: 407-421.

Zobel, H. F. 1988a. Starch crystal transformations and their industrial importance. *Starch* 40: 1-7.

Zobel, H. F. 1988b. Molecules to granules—a comprehensive starch review. *Starch* 40: 44-50.

Zobel, H. F. and Stephen, A. M. 1995. Starch: structure, analysis and application. In: STEPHEN, A. M. (Ed.). *Food polysaccharides and their applications*. New York: Marcel Decker, pp. 19-66.

APPENDICES

APPENDIX-1

Nutrient Agar Medium (NA)

Compostion	Concentration (%)
Peptone	0.5
Beef Extract	0.3
Agar	1.5
NaCl	0.5
Distilled water	100 ml

pH adjusted to neutral (6.8) at 25 °C.

APPENDIX- 2

ROSE BENGAL AGAR MEDIA

Compostion	Concentration (g / L)
Papain digest of soyabean meal	5.0
Dextrose	1.0
Monopotassium phosphate	1.0
Magnesium sulphate	0.050
Rose bengal	0.050
Agar	15.0

Final pH (at 25°C) adjusted at 7.2±0.2. Suspend these in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

APPENDIX-3

KEN KNIGHT MEDIUM

Compostion	Concentration (g / L)
Dextrose	1.0
Sodium nitrate	0.100
Monopotassium dihydrogen phosphate	0.100
Potassium chloride	0.100
Magnesium sulphate	0.100
Agar	15.00

Suspend these in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

DEVELOPMENT OF BIODEGRADABLE FILMS FROM ENZYMATICALLY MODIFIED CASSAVA STARCH

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ABSTRACT

Abstract

Cassava forms an important food crop in the tropical countries and are rich in starch (20-40%) having desirable physico-chemical and functional properties. Starch and starch derivatives form an important constituent in biodegradable film preparation due to its renewability, abundance availability, low cost, film forming properties, high oxygen barriers, odorless, tasteless, colourless, nontoxic, low solubility, biodegradability etc. But films from the native cassava starch often possess poor physico-mechanical and hydrophobic properties. Hence modified starches by chemical, physical and enzymatic methods offer better scope for the production of biodegradable films which has got wide applications in the food packaging industry. The objectives of the present study was to find out the film forming properties of enzymatically modified cassava starch viz., liquefaction by α-amylase and debranching by pullulanase enzymes added with glycerol as plasticiser. Rheological properties were measured in terms of the dynamic mechanical spectra of the film forming solutions viz., storage modulus, loss modulus, phase angle and complex viscosity. Filmogenic solutions based on α-amylase was prepared with starch 5%, glycerol 20%, amylase concentration: 100, 200 and 300 µl from the stock solution (0.1 ml amylase in 100 ml distilled water), temperature: 80, 85 and 90°C and time: 20, 30 and 40 min for gelatinization. For developing the pullulanase modified starch based films, the starch (5%) was incubated with pullulanase at 2, 3 and 4 units concentrations at 45, 50, 55 °C for 8, 16 and 24 h and the filmogenic solutions added with 20% glycerol were gelatinised at 90 °C for 20 min. Both the experiments were designed using response surface methodology using Box- Behnken design. The physico-mechanical, functional hygroscopic, biodegradation and storage studies of the films were carried out.

The dextrose equivalent of the filmogenic solutions varied between 1.6-8.4 with the amylase and 2.2-16.0 with the pullulanase treated starch. The higher values of storage modulus, complex viscosity and low phase angle of the solution containing

pullulanase treated starch compared to α-amylase treated solutions showed that the gel formed during gelatinization of the solution is having more solid nature in their visco-elastic character. The films containing α-amylase treated cassava starch showed better whiteness properties. Thickness, moisture content, tensile force, elongation at break and swelling capacity of the films containing pullulanase treated starch was higher than that of the films with α-amylase treated starch. The higher solubility of the α-amylase based starch films helps easy degradation of the films in the soil whereas offers poor packing ability. Pullulanase film's packing ability is better owing to low permeability and solubility. Though the films with both the modified starch is easily biodegradable, pullulalanase took 4 weeks for completely degrade into the soil as evidenced from the soil burial test. The microbial analysis studies showed that the soil in which α-amylase treated film buried for degradation had highest bacterial, fungal and actinomycetes population than that of the soils with pululanase treated films. Considering various physical, mechanical and functional properties, the pullulanase modified starch offers better scope for the production of biodegradable packing materials.