Final Report

Effect of Processing on Biochemical Compositions and Production of Resistant Starch (RS), Nutraceuticals and Value Added Products from Culinary Banana (*Musa* ABB) *Kachkal* of North East India

(No. ERIP/ER/0803747/M/01/1196 Dated 28th January, 2010)

Submitted to

DRDO
Defence Research & Development Organization
Ministry of Defence, Govt of India

Submitted by

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1.	Title of the Project:	Effect of processing on biochemical compositions and production of resistant starch (RS), nutraceuticals and value added products from culinary banana (*Musa* ABB) *Kachkal* of North East India
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4.	Duration of Project:	3 Years (wef 30th April, 2010 to 29th April, 2013)
5.	Financial Sanction :	Total Sanctioned Cost of the Project Rs. 14.53 Lakhs Total Grant Actually Received Rs. 14.03 Lakhs

CONTENT

Acknowledgements

We would like to extend our sincere thanks to the DRDO (Defence Research & Development Organization) Ministry of Defence, Govt of India, New Delhi for funding the project.

We would also like to thank Tezpur University for giving the permission to carry out the research work and for availing all facilities needed to complete the research works.

02/5/2013

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Introduction

Banana (*Musa* species) one of the most favourite fruits, widely grown in many countries, being the fourth most important food crop in the world as well as in India (Ganapathi, et al., 1999) is a staple food and export commodity. It contributes to the food security of millions of people in the developing world. Globally, banana (*Musa* species) is grown in 4.8 million ha area producing 99.99 million tonnes of banana and plantain with 20.8 MT/ha productivity. India is the largest producer of plantain and bananas with annual production of 29.78 MT from an area of 0.83 million ha with 35.9 MT/ha productivity accounting 29 % of the world's production (Kumar, et al., 2011). In India *Musa* spp. is well adopted in the regions varying from tropics to humid sub-tropics and semi-arid subtropics.

Both plantains and banana are the staple foods for rural and urban consumers in India and an important source of income. Traditionally bananas are usually eaten raw as desert while plantain and cooking bananas on the other hand are traditionally grown for cooking as a part of staple diet or for processing of more durable products such as flour that can be stored for later use (Wall, 2006). Banana fruit is highly nutritious and easily digestible than many other fruits. Digestion time of banana fruit is less (105 min) than apple (210 min). (Shiau, & Yeh, 2001) Banana are popular for aroma, texture and easy to peel and eat, besides rich in potassium and calcium and low in sodium content (Anhwange, 2008; Walstra, 2003; Adisa & Okey, 1987)

Table 1 Nutrient composition of plantain (USDA, 2012)

Nutrient	unit	Value per 100g	Nutrient	unit	Value per 100g
water	g	65.28	Zinc (Zn)	mg	0.14
Energy	kcal	122	Vitamin C (Total ascorbic acid)	mg	18.4
Protein	g	1.30	Thiamine	mg	0.052
Total lipid (fat)	g	0.37	Riboflavin	mg	0.054
Carbohydrate	g	31.89	Niacin	mg	0.686
Total dietary fibre	g	2.3	Vitamin B-6	mg	0.299
Total sugars	g	15.00	Vitamin A, IU	IU	1127
Calcium (Ca)	mg	3	Vitamin E (alpha-tocopherol)	mg	0.41
Iron (Fe)	mg	0.60	Vitamin K (phylloquinone)	µg	0.7
Magnesium (Mg)	mg	37	Fatty acids (Total saturated)	g	0.143
Phosphorous (P)	mg	34	Fatty acids (Total monounsaturated)	g	0.032
Potassium (K)	mg	499	Fatty acids (Total polyunsaturated)	g	0.069
Sodium (Na)	mg	4	Cholesterol	mg	0

Plantain has more calories weight than fruit banana. 100 g plantain consists of 122 calories, while banana has 89 calories (USDA, 2012). Indeed, they are very reliable sources

of starch and energy ensuring food security for millions of households worldwide. The composition of banana and plantain changes dramatically during ripening (Happi, et al., 2008; Barnell, 1940). Several authors have reported various chemical changes that occur in plantain and cooking bananas during ripening (Ferris, et al., 1996; Loeseck, 1950). The moisture content in pulp increases during ripening process due to respiratory breakdown of starch into sugar and migration from peel to pulp (Marriott et al., 1881). Post harvest changes associated with the plantain fruits are mainly derived from the ripening process.

The peel of banana represents 40 % of the total weight of fresh banana (Tein et al., 1998) and has been underutilized and discarded as waste. Like its pulp flour counterpart, banana peel flour can potentially be used in new products with standardized composition for various industrial and domestic uses (Emaga et al., 2007). Peels are the major by-products of all fruits and vegetables obtained during processing; some studies show that these are good sources of polyphenols, carotenoids and other bioactive compounds which possess various beneficial effects on human health (Ruiz-Lopez et al., 2008; Zhang et al., 2005). Of particular interest is the finding that banana peel extract contained higher antioxidant compounds than that of the pulp (Sreekanth et al., 1998), thus promising a more intense utilization of the peels in food and nutraceuticals. Potential applications of banana peel flour however depend on its chemical composition as well as physicochemical and functional properties (Nunez-Santiago et al., 2004; Emaga et al., 2007).

The flower of banana plant is known as banana blossom or banana heart. The growing point emerges from the center of the tightly rolled bunch of leaves develop as banana blossom. It is actually the inflorescence of the banana plant, the shoot meristem transform into an inflorescence which is also called as spike or inflorescence stalk. According to Sheng et al. (2010), banana flowers have tremendous nutritional value and healthy effect. These have been supported by Oliveira et al. (2006) who found that fatty acids and sterols were the major families of the lipophilic components in the floral stalk of "Dwarf Cavendish" banana. The chloroform, water and ethanol extract of *Musa* sapientum flowers were found to exhibit hypoglycaemic activities in alloxan diabetic rat (Dhanabal et al. 2005; Grover et al. 2002; Pari and Umamaheswari, 2000). Studies on the contents of vitamin C, tannin, myoinositol phosphates and alpha tocopherol in *Musa* sapientium flower have been reported by Somsub et al. (2008).

The culinary bananas are a rich source of nutrients and the biochemical composition of fruit varies greatly with the advancement of fruit growth and maturity (Emaga *et al.*, 2008; Mohapatra *et al.*, 2010; Smith *et al.*, 1989). As per the findings of various researchers some

important nutrients such as crude fat, crude protein, most of the minerals and phenolic compounds, and also the radical scavenging activities of culinary banana are comparatively higher at early stages of ripening which declines as ripening progresses (Cheirsilp and Umsakul, 2008; Emaga *et al.*, 2008; Mohapatra *et al.*, 2010). Whereas, the content of some other compounds of interest such as sugars, pectic substances and some vitamins increases with stage of fruit development and maturity (Emaga *et al.*, 2008; Smith *et al.*, 1989). In spite of the panorama of benefits, *kachkal* is still to come under the preview of scientific exploitation. A study on the processing of *Kachkal* is the prerequisite for increasing its short shelf life along with its further industrial utilization. *Kachkal* is consumed necessarily cooked, whether green or ripe. Boiling has been the known traditional method of cooking in Assam.

However, in spite of *kachkal* being the only culinary banana found in the entire Northeastern region of India, till today no scientific effort has been put to identify its unknown features, improve its consumption or to obtain processed products. The potential applications of this fruit depend on its chemical compositions and the chemical compositions of the fruit changes with the advancement of fruit growth. Therefore, in order to obtain the maximum yield of particular compound of interest, the fruit has to be harvested at that particular stage when its concentration is in its maximum level. Traditionally in this region, *kachkal* is consumed at a stage of about 60 to 65 days after emergence. Beyond this period the texture becomes unsuitable for culinary consumption. The 65 DAE has been considered as the terminal stage because *kachkal* matures and starts ripening after 65 DAE.

Starch is the storage polysaccharides of green plants and a major dietary component in all human populations. Starch is deposited in the fruit in the form of granules, partially crystalline, whose morphology, chemical composition, and super molecular structure are characteristic of each particular plant species. Starch owes much of its functionality to two major high-molecular-weight carbohydrate components, amylose and amylopectin, as well as to the physical organization of these macromolecules into the granular structure. This biopolymer constitutes an excellent raw material to modify food texture and consistency. The amount of starch is not only important for the texture of a given food product, but starch type is equally critical. Many tropical countries have plant species which can be used as a good source of starch; unfortunately some of them have not been exploited. One such plant species is *kachkal* (*Musa* ABB), the only culinary banana or plantain found in the entire Assam and North-East India. Starch being the principal component of green bananas, which undergoes

important changes during ripening they can be considered as a resource for production of modern forms of consumption like processed snacks and precooked products.

Starch, which is the major dietary source of carbohydrates is the most abundant storage polysaccharide in plants and occurs as granules in the chloroplast of green leaves and the amyloplast of seeds, pulses, and tubers (Ellis et al., 1998). Starch, on the basis of its digestibility, has been classified into three groups, such as readily digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) (Englyst & Cummings, 1987). Readily digestible starch is the starch fraction that causes an increase in blood glucose level after ingestion immediately, whereas SDS is the starch fraction that is digested completely in the small intestine at a lower rate as compared to RDS. Resistant starch is the portion of starch and/or starch hydrolysis products that escape digestion in the small intestine, and enters the colon for fermentation (Sajilata et al., 2006).

Extensive studies have shown RS to have physiological functions similar to those of dietary fiber (Asp, 1994; Eerlingen & Delcour, 1995). The diversity of the modern food industry and the enormous variety of food products being produced require starches that can tolerate a wide range of processing techniques and preparation conditions (Visser et al., 1997). These demands are met by modifying native starches with chemical, physical, and enzymatic methods (Betancur & Chel, 1997), which may lead to the formation of indigestible residues. The availability of such starches therefore deserves consideration. Four forms of RS are distinguished: RS type I is defined as physically inaccessible starch for instance in grains; type II is granular starch in raw potato and bananas; type III is retrograded starch, arising after hydrothermal treatment of starch; and type IV is considered to be a chemically modified starch (Englyst et al., 1992). Among these four types, RS type III seems to be particularly interesting because it preserved its nutritional characteristics when it is added as ingredient to cooked food. RS type III is produced by gelatinization, which is a disruption of granular structure by heating starch with excess water, and then retrogradation occurs. The generation of RS after hydrothermal treatment is mainly due to increased interactions between starch polymers. The degree of RS formation in foods depends not only on the type of included starch and the adopted processing conditions but is also influenced by the duration and conditions of storage (Goni et al., 1996).

The global trends in rising levels of obesity, diabetes and cardiovascular disease has refueled consumer and research interest in the dietary intake of fat, protein and carbohydrate to maintain good health. The World Health Organization (WHO) and Food and Agricultural Organization (FAO) of the United Nations stated that globally, overweight populations are a

bigger problem than under nourishment and recommended people in industrialized countries base diet on low GI foods to prevent most common disease of affluence (FAO/WHO, 1998). Foods containing resistant starch (RS) generally give a low glycaemic response because RS is not digested in the small intestine. Instead RS passes into the large intestine where it is fermented (Brown et al., 2004; Englyst et al., 2007; Sajilata et al., 2006; Sharma & Yadav, 2008).

Glycaemic index (GI) according to Allen (1997) is simply a ranking of foods, based on their immediate effect on blood glucose. It is a physiological measure of how fast and to what extent a carbohydrate food affects blood glucose levels. The glycaemic index is defined as the incremental area under the blood glucose response curve of a 50g carbohydrate portion of a test food expressed as a percent of the response to the same amount of carbohydrate from a standard food taken by the same subject (Jenkins et al., 2002). It compares carbohydrates in foods as eaten on a gram per gram basis (Wolever et al., 1991).

India being the first major producer of banana, 20 – 30 % of the production is wasted every harvest season during transport or lack of compliance with the normal commercialization standards for fresh fruits. Wide ranges of processing operations are employed before plantain is consumed and they include boiling, roasting or baking, frying and drying (Abiose & Adedeji, 1992; Dadzie & Wainwright, 1995). Bananas are highly perishable, with a significant proportion of the harvested crop being lost from the farm gate to the market place, owing to poor handling, storage and transportation of the fresh fruits. Additionally, non-harvesting losses may occur in peak production periods when farmers do not harvest the entirety of their production because of saturated markets. Cooking bananas are used in a wide range of food dishes of varying regional importance. There appears to be a potential market for a wider range of snack products produced from these commodities in the target countries, including the production of several popular alcoholic and non-alcoholic beverages. The conversion of cooking bananas into flour, wine, beer, and weaning food products (extruded and high protein, low cost) is a means of adding value to the fruits as well as extending the shelf life of derived foods (Adeniji & Empere, 2001).

Flour is an important raw material in the baking and confectionery industry. The demand for flour in bakery products is increasing globally and banana flour is currently being exploited in baking and complementary weaning foods (Ogazi, 1996; Adeniji & Empere, 2001). New economical strategy to increase utilization of banana includes the production of banana flour when the fruit is unripe, and to incorporate the flour into various innovative products such as slowly digestible cookies (Aparicio-Saguilan et al., 2007), high-fibre bread

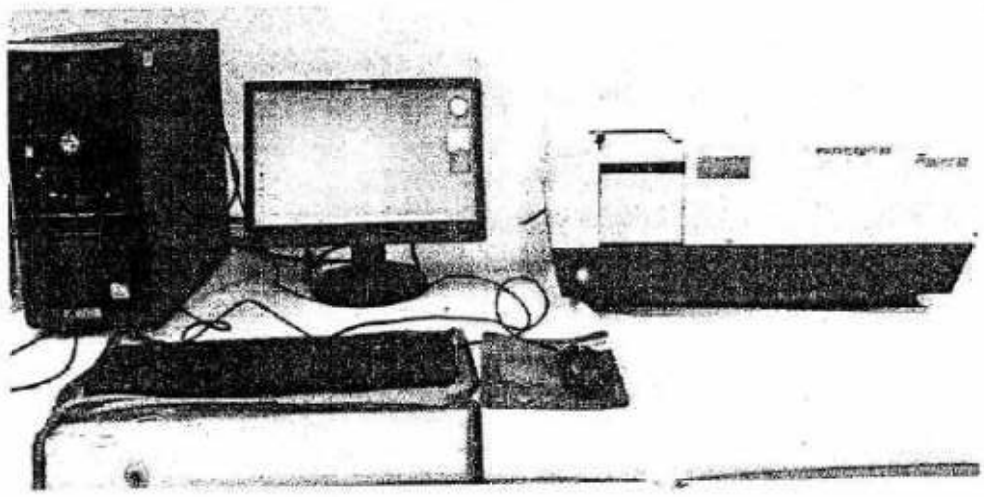
(Juarez-Garcia et al., 2006) and edible films (Sablani et al., 2006). The clear advantage presented by green banana flour includes a high total starch (73.4%), resistant starch (17.5%) and dietary fibre content (14.5%) (Juarez-Garcia et al., 2006). Due to the high content of these functional ingredients, regular consumption of green banana flour can be expected to confer beneficial health benefits for humans (Rodriguez-Ambriz et al., 2008). On the other hand, ripe banana flour that is less known banana product, may offer high sugar content to dishes requiring sweetness. Other than health and sensory reasons, the stages of ripeness are also important for technical aspect of processing. Banana pulp flour prepared using fruits at different stages of ripening has been shown to behave differently during manufacture of food products such as cakes (Yomeni et al., 2004) and extruded products (Gamlath, 2008). Flour has a longer shelf life than the raw fruits because of the reduced moisture content. Converting fresh banana fruits to flour also adds value to them. Banana flour is prepared from green unripe fruit and is therefore characterized by high starch content (Thompson, 1995). Chips or crisps are hard, brittle fried products abruptly releasing energy that gives rise to characteristic sound effects when they are bitten. They are the most popular post harvest processed products of cooking bananas in East and West Africa (Onyejegbu & Ayodele, 1995). They are one of the most important foods usually fried in the form of crisps (thin circular) or sometimes in form of French fries (stick). They are prepared by deep-frying round slices of unripe or slightly ripened plantain pulp in vegetable oil, and can be preserved for a long time given adequate packaging and storage facilities. Several studies have been conducted on the use of *Musa* species fruits for chips making (Ogazi, 1996; Adeniji, 2005; Adeniji & Tenkouano, 2007).

Objectives of the project:

- To study the effect of processing on biochemical composition at various growth stages of *kachkal*
- To develop Resistance starch (RS) from *kachkal* by use of heat processing and enzymatic methods.
- To develop Nutraceuticals from *kachkal* blossom
- To use banana powder as a substitute in different foods and developing value added products from *Kachkal*

Details of the equipment purchased

1.	Name of grantee institute:	Tezpur University, Department of Food Engineering and Technology, Napaam-784028, Tezpur, Assam, India.
2.	No. & Date of sanction:	ERIP/ER0803747/M/01/1196 Date: 28th January, 2012
3.	Amount of sanctioned grant:	Rs. 14.53 lakhs (Rupees fourteen lakhs fifty three thousand) only
4.	Particulars:	P.C based double beam scanning UV-visible spectrophotometer Make Thermofisher Scientific Pvt. Ltd. Model: Spetrascan UV-2600



P.C based Double beam UV-visible spectrophotometer, sponsored by DRDO, New Delhi

Review of Literature

Musa

Banana belongs to the family *Musaceae*, genus *Musa* and is a general term embracing a number of species or hybrids in this genus. The name *Musa* is from the Sanskrit, *Moca*, via its Arabic counterpaert, mauz. Bananas descended from two wild ancestors: *Musa acuminate* and *Musa balbisiana* (Lehmann et al., 2002; Stover & Simmonds, 1987), which are native from Southeast Asia. By now, 700 varieties of *Musa* are known and 100 varieties from them are cultivated.

Stover & Simmonds, 1987 reported that there are three common species of *Musa,* which include *Musa Cavendishii, Musa paradisiaca* and *Musa sapientum. Musa cavendishii* is pure triploid acuminate (AAA group) and is type of dessert banana. Cavendish is one of the most important fruit grown commercially in large scale for world export trade. *Musa paradisiaca* is a type of plantain, which is normally, cooked before eaten while *Musa sapientum* known as true banana is usually eaten raw at maturity. Both *Musa paradisiaca* and *Musa sapientum* belong to AAB group and are characterized by the higher starch concentration. There are diploid, triploid or tetraploid genome groups. The main genome groups are AA, AB, AAA, AAB and ABB. (Stover & Simmonds, 1987).

According to Morton, (1987) *Musa* is one of the cheapest food crops to produce and the cost of production is less than most other staples. Besides been used primarily as dessert, banana fruit may be processed into pulp-liquid fruit, canned slice, deep-fried chips, toffees, fruit bars, brandy and etc. In addition, the by-products of banana such as the leaves, fibres and pseudo-stem extract have been reported to have some commercial value such as animal feeds, medicines and crafts. India, with rich bio-diversity of banana and plantain, is the largest producer and consumed of banana in the world with an estimated production of 16 million tones of bananas annually.

Nutritional Values of *Musa*

A healthy diet consists of eating a variety of foods from 5 food groups but in the correct proportions. These include; foods containing starch, fruit and vegetables, milk and diary food, foods containing protein, and that containing fats and sugars. Bananas fall in the fruit and vegetable group as well as the food group which mostly contain starch. Sweet dessert bananas are generally eaten raw (fruit), while cooking bananas and plantains are boiled,

steamed, fried or roasted (food). Any food containing carbohydrates should be the main part of our daily meals. In unripe bananas the carbohydrates are mostly starches. In the process of ripening the starches are converted to sugars; a fully ripe banana has only 1-2% starch (Forsyth, 1980).

Bananas have been classified as one of the antioxidative foods by Kanazawa and Sakakibara (2000). These tropical fruits have strong ability to protect themselves from the oxidative stress caused by the intense sunshine and high temperature by increasing their antioxidant levels. They are known as a weak primary antioxidant source but a powerful secondary antioxidant source (Haripyaree et al 2010; Lim et al. 2007). The antioxidant compounds identified in bananas include ascorbic acid, tocopherol, beta carotene, phenolic groups, dopamine and gallocatechin (Qusti et al. 2010; Someya et al. 2002).

According to Ramcharan and George (1999), the role of banana and plantain is becoming more important with the increasing emphasis today on diets that are low in sodium but high in potassium and vitamins. Both banana and plantains are good source of potassium and vitamin A, vitamin C, vitamin B_6 and low in sodium. They are highly digestible and contain fiber, they are also considered as blood pressure stabilizer. They also concluded that plantains are also of the fruit sources of chromium, a nutrient vital for combating diabetes because it stimulates the metabolism of glucose.

Marriott et al., 1981 reported that moisture content in banana pulp increases during ripening process due to respiratory breakdown of starches into sugar and migration of moisture from peel to pulp. Even when banana is fully ripe, still some starch is left in pulp tissue.

Ketiku (1973), studied the proximate chemical composition, the carbohydrate constituents and the amino acid make-up of green and ripe plantain and the results showed that the quantity of total sugars considerably increased during ripening from 3.0 to 31.6% in the peel and from 1.3 to 17.3% in the pulp while starch concentration decreased from 50 to 35% and from 83 to 66% in the skin and the pulp respectively.

Yang and Hoffman 1984 stated that during ripening process, starch is converted into sugar, through enzymatic breakdown process. In Musa AAB group, starch contains declines from 20 – 30 % to 1 – 2 %, but starch amount could be as high as 11 % depending on plantain variety.

According to Cheirslip & Umsakul (2008), sugar content of fully matured banana is quite high that makes banana an ideal substrate for wine making.

According to the findings of Lehmann & Robin (2007), carbohydrate type in banana is resistant starch and non-starch polysaccharides, which have low glycemic index or low digestibility. This property makes banana excellent ingredient for different functional and convenience foods like cookies and chips (Aparicio-Saguilan et al., 2007).

Izonfuo & Omuaru (1988) studied the effect of ripening on the chemical composition of plantain peels and pulps (*Musa paradisiaca*) and reported that the plantain pulp is low in protein with estimated values of 4g per kg in green unripe finger, and 9g per kg in the fully ripe finger. A higher level of about 72g per kg is found in the peels, which makes the peel a suitable feeding stuff for ruminants, especially in ripe form.

Baoxiu et al., (2000), studied the textural and biochemical changes in two distinct *Musa* types, plantain and dessert banana, during cooking and concluded that the plantain pulp softened at a slower rate than banana and remained firmer throughout cooking.

According to Emaga et al., 2008, ripe banana pulp contains (0.7 – 1.2 %) pectin. During ripening, insoluble proto-pectin is converted into soluble pectin that causes loosening of cell wall and texture degradation of fruit.

Prasanna et al., (2007) stated that gel forming ability of pectin has varied use as additives in jams, jellies and marmalades as thickeners, texturizers, emulsifiers, fat or sugar replacers.

Kanazawa & Sakakibara (2000) reported that banana is rich in phenolic compounds and flavonoids, which have antioxidant properties. Astringent taste of unripe banana is due to phenolic compounds. Bananas are also rich in dopamine antioxidant.

Value Added Products

Plantains, and banana (*Musa* ABB, AAA, AAB) are major starchy staples of considerable importance. They are consumed as energy yielding food and as dessert, providing more than 200 calories (food energy) a day (Stover and Simmonds, 1987).

New economical strategy to increase utilization of banana includes the production of banana flour when the fruit is unripe, and to incorporate the flour into various innovative products such as slowly digestible cookies (Aparicio-Saguilan et al., 2007), high-fiber bread (Juarez-Garcia et al., 2006) and edible films (Rungsinee & Natcharee, 2007). The clear advantage presented by green banana flour includes a high total starch (73.4%); resistant starch (17.5 %) and dietary fiber content (~ 14.5%) (Juarez-Garcia et al., 2006). Due to the high content of these functional ingredients, regular consumption of green banana flour can be expected to confer beneficial health benefits for human (Rodriguez-Ambriz et al., 2008).

Shiau & Yeh (2001) stated that it would be possible to utilize the green pulp as a functional ingredient in starch-rich products such as the yellow noodles. Yellow noodles are typically made by adding alkaline salt to the ingredients. The alkaline salt added imparts the unique features of Chinese noodles with pH 9.0- 11.0 where the yellowness of the noodles is produced when the flavones react with the alkaline water.

According to Shiau & Yeh (2001), the grade of yellow noodles can be evaluated from their color, shape, texture and eating qualities. High quality yellow noodles should be free from discoloration, having symmetry dimensions, should not be sticky after being cooked as well as to show sufficient firmness and springiness.

Emperatriz et al., 2008 stated that by converting plantain into its flour it could contribute to reduce losses and allow the food industry to store the product throughout the year. In order to use plantain flour as ingredient for food industry it is necessary to characterize their chemical and nutritional composition, as well as their physical, physiochemical, rheological and functional properties.

Ukhun & Ukpebor (1991) prepared instant plantain flour from ripe and unripe plantain fingers by cooking and subsequent oven dehydration at 76^0 C and 88 – 92^0C respectively. They found that products having commercial potential on their own or as an ingredient for other foods such as baby weaning foods, puddings, soups and gravies.

Gwanfogbe et al. (1988) had shown the usage of plantain flour at an industrial level, with full or low starch content, in order to maintain the texture of certain frequently frozen and defrosted foodstuff.

Dietary fiber, resistant starch, proteins and mineral contents increase in industrially elaborated cookies when substituting wheat flour by 7% of unripe plantain flour, as shown by Maldonado and Pacheco-Delahaye (2002), they also showed that starch is the main component (84%) of unripe plantain flour, and reported the content of proteins (6.8%), fats (0.3%), ash (0.5%), and dietary fiber (7.6%).

Juarez-Garcia et al. (2006) also reported that banana flour was mainly total starch (73.36%) and dietary fiber (14.52%). These authors prepared banana flour bread with a higher content of protein, total starch, resistant starch and indigestible fraction than the control made without banana flour, and indicated that the banana flour is a potential ingredient for bakery products containing slowly digestible carbohydrates.

Morton (1987) stated that a novel way of utilizing these green bananas is to process the fruit into flour. In the flour form, the shelf life can be extended and provide easy storage. Banana or plantain flour is made domestically by sun-drying the slices of unripe banana and pulverizing. Commercially, it is produced by spray-drying, or drum-drying the mashed fruits. The plantain cultivars 'Saba', 'Tundoc' and 'Latundan' are very suitable for making flour.

Saifullah et al., 2009 prepared Banana pulp noodles by partial substitution of wheat flour with green Cavendish banana pulp flour and studied pH, color, tensile strength and elasticity, and *in-vitro* hydrolysis index (HI) and estimated glycemic index (GI). In their study they found that banana flour noodles had higher tensile strength and elasticity modulus than control noodles. They also reported that *in-vitro* starch hydrolysis study, it was found that GI of banana flour noodles was lower than control noodles.

Banana and plantain can be produced in many different ways, such as frozen puree, juice, figs, jams and canned banana slices (Thompson, 1995). Seasonal gluts and perishability of ripe bananas and plantains caused great economic losses and therefore there is tremendous interest in the development of modes of processing and preserving these fruits.

Works Completed Objective Wise

Objective 1: To study the effect of processing on biochemical composition at various growth stages of *kachkal*

(i) Nutritional composition of *kachkal* at different developmental stage

Materials and methods

Sample collection and preparation

The same *kachkal* genotype was collected from Horticultural Orchard, Assam Agricultural University, Jorhat and multiplied (in some specific farmers field) in all the six agro climatic zones of Assam. Based on the rainfall pattern, terrain and soil characteristics, Assam has been delineated into six agro-climatic zones viz.(1) North Bank Plain Zone (Darrang, Sonitpur, Lakhimpur, Dhemaji districts) (2) Upper Brahmaputra Valley Zone (Golaghat, Jorhat, Sivasagar, Dibrugarh, Tinsukia districts) (3) Central Brahmaputra Valley Zone (Nagaon, Marigaon districts) (4) Lower Brahpamutra Valley Zone (Goalpara, Dhubri, Kokrajhar, Bongaigaon, Kamrup, Nalbari, Barpeta districts) (5) Barak Valley Zone (Cachar, Karimganj, Hailakandi districts) (6) Hill Zone (North Cachar Hills, Karbi Anglong districts). This helped us to know the effect of genotype, environmental conditions, cultivation, harvest and transport which has been shown to be of great importance in determining the bioactive components of foods. The use of randomized sampling techniques from several defined locations over a period of time (harvest years/seasons) was taken into account.

The culinary banana (*kachkal)* samples were harvested at every 15 days interval and studies were carried out to find out the best stage for further processing. The *kachkal* finger samples were harvested at four different growth stages viz; 20 days after emergence (DAE) considered as stage I, 35 DAE as stage II, 50 DAE as stage III and 65 DAE as stage IV and 80 DAE which was considered as terminal stage of ripening

The samples were washed thoroughly under running water followed by distilled water and then spread out on absorbent tissue papers to remove the surface moisture. The pulp and peel of the samples were separated using a stainless steel knife. The pulp of the samples were sliced into thickness of about 5mm approximately and dried in a tray drier at 40 °C for 12 hours. The dried samples were then grounded, sieved and stored in air tight containers till the time of analysis.

Chemical analysis

Proximate compositions

Moisture content was determined according to the method described in AOAC (2010a). Ash content was determined by ignition in a muffle furnace (LabTech, Korea) at 550^{0}C for 6

hours (AOAC, 2010b). The nitrogen content of the sample was determined using the Kjeldahl apparatus (KelPlus, Pelican Equipment, India) and the amount of nitrogen obtained was then multiplied by factor 6.25 (AOAC, 2010c). Crude fat was determined using the Soxhlet extractor (SocsPlus, Pelican Equipment, India) with n-hexane as solvent (AOAC, 2010d). Crude fiber was determined for the loss on ignition of dried residue remaining after digestion of banana flour (2 g) with 1.25% H_2SO_4 and 1.25% NaOH. The total carbohydrate content was measured by hydrolyzing the polysaccharides (acid hydrolysis) into simple sugars and estimating the resulting monosaccharide by anthrone method (Hodge and Hofreiter, 1962).

Ascorbic acid, titrable acidity and pH

Ascorbic acid and titrable acidity of the samples were estimated using the method of Ranganna (2008a). The pH of the fruit samples were measured by blending 10 g portion of fruit pulp with 40 ml of deionised water (AOAC, 2010e). The mixture was allowed to stand for 15 min, with shaking at 5 min intervals and then centrifuged at 3000g for 15 min. The supernatant was decanted and its pH was determined using a pH meter (pH510, Eutech, Malyasia).

Starch, amylose and sugar contents

Starch and amylose contents were determined as per the method of Hodge and Hofreiter (1962) and Sadasivam and Manikam (2008a) respectively. Soluble sugars were extracted from 1gm of sample in 80% ethanol (hot) and sugars content were quantified by phenol-sulphuric acid method (Dubois *et al.*, 1956). The reducing sugar content was estimated by the Nelson- Somogyi method (Somogyi, 1952).The amount of non-reducing sugar was determined by subtracting the amount of reducing sugars from the amount of total sugars in the sample.

Cellulose and lignin contents

Cellulose content was estimated by reacting with acetic-nitric reagent and then measuring the absorbance at 630 nm whereas the lignin content was estimated by extracting in NaOH solution and aliquot samples were adjusted to pH 7.0 and 12.3. The absorbance of the aliquots was measured at 245 and 350 nm. The amount of lignin content was calculated by a difference between A_{245} (pH 7.0) and A_{350} (pH 12.3) (Stafford, 1960).

Pectin content

Pectin content was determined by extracting from plant material and saponified (Ranganna, 2008b). It was then precipitated as calcium pectate by calcium chloride. After removal of chloride ions, the precipitate was dried and weighed.

Tannin content

Tannin content was determined by Folin-Denis method (Schanderi, 1970) in which tannin like compounds reduces phosphotungstomolybdic acid in alkaline solution to produce a highly coloured blue solution, and the intensity was measured spectrophotometrically (Spectrascan UV-2600, Thermo Fisher Scientific, India).

Thiamine, vitamin A and total carotenoid contents

The thiamine content was estimated by flurometric method, (Sadasivam and Manickam, 2008b). Vitamin A content was measured by a rapid colorimetric method of Bayfield and Cole (1980). The carotenoids were extracted and partitioned in organic solvents on the basis of their solubility. The separation of individual components was done by chromatography on activated magnesia. The carotene passed rapidly through the column; bands of xanthophylls, carotene oxidation products and chlorophylls were retained. Elutes were transferred and diluted to volume with acetone-hexane mixture. The absorbance of the solution was measured spectrophotometrically (Spectrascan UV-2600, Thermo Fisher Scientific, India) at 436 nm against different concentration of high purity β-carotene. Carotenoid contents were expressed as carotene content in mg/100g sample material (Sadasivam and Manickam, 2008c).

Phytic acid content

Phytic acid content was estimated by extracting phytate with tricholoroacetic acid and precipitating as ferric salt (Wheeler and Ferrel, 1971).

Total phenolic content and DPPH radical scavenging activity

Total phenolic content of the sample extracts was determined with Folin–Ciocalteu colorimetric method (Malick and Singh, 1980), where 0.5 ml extract was mixed with 0.5 ml Folin–Ciocalteu reagent. The contents were mixed by manual shaking for 15–20 seconds. After 3 min, 2 ml of saturated sodium carbonate solution was added to each tube. The reaction mixture was placed in a boiling water bath for 1 min, cooled and its absorbance was

measured at 650 nm against deionized water using a dual beam UV–Visible spectrophotometer (Spectrascan UV-2600,Thermo Fisher Scientific, India) and the result expressed in mg of (+/-) catechin/100 g dry weight.

The DPPH radical scavenging activity was measured as per method described by Brand-Williams *et al.* (1995). This assay is based on the ability of antioxidant to scavenge the DPPH cation radical. This method determines the hydrogen donating capacity of molecule and does not produce oxidative chain reactions or react with free radical intermediates. Scavenging activity (SA) was calculated as percent inhibition relative to control, using following equation and expressed as: SA % (30 min) = control absorbance at 517 nm − extract absorbance at 517 nm / control abs at 517 nm × 100.

Estimation of minerals

The minerals content of *kachkal* was estimated by ICP/OES (Optima 2100 DV). The concentrations were determined in the aqueous solution of acid digest of the samples. Powdered sample weighing 1 g was taken and 30 ml concentrated nitric acid and 5 ml concentrated hydrochloric acid was added. The vessels were immediately closed after the addition of oxidants. The samples were digested in a hot plate at 100°C. At the end of the digestion process, the digests were cooled and diluted up to 50 ml with distilled water.

Fatty acid profiling

Fatty acid profiling was done by GLC (Varian, CP - 3800). Fatty acids may be found in scarce amounts in free form but, in general they are combined in more complex molecules through ester or amide bonds. Before GLC analysis non-reactive derivatives of fatty acids methyl esters was prepared (Luddy *et al.*, 1968). Samples were treated with 0.4N sodium methylate and shaken vigorously at water bath for 2 - 3 min at 65^0C followed by addition of 1 ml carbon disulphide and shaken for 1 - 2 min and filtered through activated charcoal. The filtrate constituted all the methyl esters of fatty acids. Methyl esters of fatty acids were separated employing Gas Chromatograph (Varian, CP - 3800) equipped with two flame ionization detector and electron capture detector. The column temperature was 190°C and flow of the nitrogen carrier gas was maintained at 35 ml min^{-1}. The peaks were identified by comparison of their retention times with those of standard fatty acid esters.

Amino acid analysis by amino acid analyzer (AAA)

Amino acid analysis was done by hydrolyzing with 6 N HCl and measured by ion exchange chromatography using ninhydrin post column derivatization. Sample equivalent to 5mg protein was taken in a 20ml glass ampoule, kept in the dry ice to avoid from clumps and 10ml 6N HCl was added. Nitrogen gas was flushed to remove oxygen from the ampoule for 1min and closed with para - film. Samples were kept in oven at 110°C for 22 hours for the hydrolysis. After hydrolysis, samples were removed and allowed to come down to room temperature. Neck of the ampoule was broken and samples were transferred to a 25 ml volumetric flask. Volume was made up to 25 ml with distilled water and mixed thoroughly and filtered through nitrogen free Whatman No.1 filter paper. The aliquot (0.5 ml) was evaporated at 45 - 50°C. After complete drying of the sample, 5 ml deionized water was added and evaporated again. Drying and evaporation of the sample was repeated for three times. Crude dried sample was dissolved with 2.5 ml of sodium citrate loading buffer (pH 2.2). Samples were then filtered using syringe driven filter (0.45 µm) and kept in an auto sampler. Standards (100 pmol) and samples were run in an automated amino acid analyzer (Beckman 119 CL). The amino acid content were calculated and expressed as g/100g protein.

Colour measurement

The colour measurement of the *kachkal* samples at different growth stages were done by analyzing the colour of the samples in a Hunter Lab Color Quest (Model Ultrascan Vis-Model, USA). The results were expressed in L, a and b systems.

Statistical analysis

Experiments were carried out in four replicates. The software Origin 8.5 was used for all the statistical analysis. ANOVA and Fisher Least Significant Difference (LSD) were used to calculate the significant difference.

Results

Proximate compositions

The proximate compositions of *kachkal* at four different developmental stages are presented in Table 2. The moisture content decreased (87.03 % to 78.02 %) gradually up to stage III and then an increase in moisture content (89.07%) was observed at stage IV. There was a significant difference in moisture content in stage I, II and IV, but no any difference was observed between stage II and III.

The ash content showed a decreasing trend with the advancement of fruit growth. The highest ash content was recorded at the stage I (1.64 g/100g) which then decreased gradually to 0.83 g/100g at fully matured stage.

The present work evinced a gradual decrease of protein content with maturity. The protein content decreased from 10.56g/100g at stage I to 4.99 g/100g at stage IV. *Kachkal* fruit was found to contain a relatively low amount of fat, which varied from 1.50 - 0.58 g/100g from 20 DAE to 65 DAE. The fiber content which evinced a gradual increase with maturity fluctuated in the range of 0.61 to 2.66g/100g showing that there was significant difference among all the stages. The carbohydrate content increased from 32.21 - 80.03 g/100g from stage I to stage IV. It is observed that there was significant difference among all the stages in case of protein, fat and total carbohydrate (Table 2). The high carbohydrate content of *kachkal* makes them one of the non oil calorie rich fruit.

Table 2 Proximate compositions of *kachkal* (*Musá* ABB) (g/100g) at different developmental stages

Stages	Moisture content (% wb)	Ash	Protein	Fat	Crude fiber	Total carbohydrate
Stage I	87.03 ± 0.80^{b}	1.64 ± 0.35^{c}	10.56 ± 0.86^{e}	1.50 ± 0.11^{d}	0.61 ± 0.10^{a}	32.21 ± 0.05^{a}
Stage II	76.75 ± 0.42^{a}	1.10 ± 0.73^{c}	8.61 ± 0.96^{b}	1.27 ± 0.08^{c}	0.99 ± 0.08^{b}	39.77 ± 0.29^{b}
Stage III	78.02 ± 0.5^{a}	1.32 ± 0.49^{b}	7.56 ± 0.84^{b}	0.94 ± 0.05^{b}	1.50 ± 0.12^{c}	58.14 ± 0.20^{c}
Stage IV	89.07 ± 0.77^{c}	0.83 ± 0.20^{a}	4.99 ± 0.57^{a}	0.58 ± 0.06^{a}	2.66 ± 0.05^{d}	80.03 ± 0.06^{d}

Results in mean±SD (n=4), Means in each column followed by different superscript letters were significantly different ($p < 0.05$)

pH, browning potential and titrable acidity

The pH of the *kachkal* fruit (Table 3 decreased to a minimum 5.01 at stage II, thereafter the pH increased to 5.25 at stage IV. It was found that there was no significant difference in pH in any of the four stages. Browning potential was found in the range of 0.51 – 0.31 from stage I to stage IV. The titrable acidity increased from 0.16 to 0.32g/100g from stage I to stage IV (Table 3). It was observed that in case of titrable acidity there was no significant difference between stages I and II; on the other hand, stage II, III and IV were found to be significantly different.

Table 3 The pH, browning potential and titrable acidity

Stages	pH	Browning potential (mean abs at 440 nm)	Titrable acidity (g/100g)
Stage I	6.03 ± 0.15^{b}	0.51 ± 0.02^{c}	0.16 ± 0.01^{a}
Stage II	5.01 ± 0.09^{a}	0.47 ± 0.04^{b}	0.19 ± 0.02^{a}
Stage III	5.12 ± 0.17^{a}	0.39 ± 0.07^{a}	0.23 ± 0.03^{b}
Stage IV	5.25 ± 0.37^{a}	0.31 ± 0.09^{a}	0.32 ± 0.02^{c}

Results in mean±SD (n=4), Means in each column followed by different superscript letters were significantly different ($p < 0.05$)

Ascorbic acid, cellulose and lignin contents

An irregular trend was observed in ascorbic acid level which was initially 1.12 mg/100g at 20 DAE then declined to 0.83 mg/100g at 50 DAE and thereafter increased slightly to 0.91 mg/100g at 65 DAE (Table 4) with no significant difference among the four stages. The highest cellulose content of 1.06 g/100g was recorded at stage IV and significant difference was observed among all the four stages (Table 4). The lignin content also increased with fruit growth, registering its maximum value 1.57 mg/100g at stage IV, there was no any significant difference between stage I and II; whereas, significant difference was observed in stage II, III and IV. Both lignin and cellulose being the structural components of cell wall experienced an increasing trend with the advancement of fruit growth.

Table 4 The ascorbic acid, lignin and cellulose content

Stages	Ascorbic acid (mg/100g)	Lignin (mg/100g)	Cellulose (mg/100g)
Stage I	1.12 ± 0.02^{b}	0.56 ± 0.08^{a}	0.04 ± 0.01^{a}
Stage II	0.86 ± 0.02^{a}	0.68 ± 0.10^{a}	0.25 ± 0.01^{b}
Stage III	0.83 ± 0.03^{a}	1.25 ± 0.12^{b}	0.31 ± 0.02^{c}
Stage IV	0.91 ± 0.11^{a}	1.57 ± 0.09^{c}	1.06 ± 0.05^{d}

Results in mean±SD (n=4), Means in each column followed by different superscript letters were significantly different ($p < 0.05$)

Starch, amylose and sugar contents

Starch, amylose and sugar contents of *kachkal* are presented in Table 5. Starch is the main form of carbon storage in unripe bananas. The present study revealed that the starch content increased rapidly with the enhancement of fruit growth. It increased from 11.02 g/100g at stage I to 46.31 g/100g at stage IV with significant difference among the stages. Starch is a polysaccharides made up of two components amylose and amylopectin. The

present study evinced a gradual increase in amylose content with maturity. The amylose content varied from 19.77g/100g at stage I to highest amylose content 32.05 g/100g at stage IV. It was observed that there was significant difference between all the four stages. An increase in total carbohydrate content may be correlated with the active synthesis of starch with fruit growth. The present investigation showed that starch is the major carbohydrate component present in *kachkal*. The increase in starch content might be attributed to the fruit growth. The data presented in Table 5 showed that total soluble sugar content of *kachkal* was highest 2.01 g/100g at stage I which then gradually decreased to 0.64 g/100g at stage IV with significant difference among the stages. The reducing and non-reducing sugar contents (Table 5) also experienced a similar trend of decreasing with maturity. The reducing sugar content varied from 0.54 - 0.16 g/100g while the non-reducing sugar content varied from of 1.57 - 0.37 g/100g from stage I to stage IV. There was significant difference in both reducing and non-reducing sugar. The decrease in total soluble sugar content may be due to the utilization of sugar content for starch biosynthesis till the onset of ripening and consequently the level of both reducing and non-reducing sugars content decrease towards maturity.

Table 5 The starch, amylose, total soluble, reducing and non reducing sugar content

Stages	Starch (g/100g)	Amylose (g/100g)	Total soluble sugar (g/100g)	Reducing sugar (g/100g)	Non-reducing sugar (g/100g)
Stage I	11.02 ± 0.17^{a}	19.77 ± 0.51^{a}	2.01 ± 0.006^{d}	0.54 ± 0.004^{d}	1.57 ± 0.07^{d}
Stage II	17.95 ± 0.10^{b}	22.84 ± 0.48^{b}	1.35 ± 0.03^{c}	0.41 ± 0.01^{c}	0.63 ± 0.01^{c}
Stage III	35.66 ± 0.61^{c}	26.25 ± 0.62^{c}	0.72 ± 0.05^{b}	0.29 ± 0.06^{b}	0.48 ± 0.04^{b}
Stage IV	46.31 ± 0.99^{d}	32.05 ± 0.52^{d}	0.64 ± 0.03^{a}	0.16 ± 0.002^{a}	0.37 ± 0.008^{a}

Results in mean±SD (n=4), Means in each column followed by different superscript letters were significantly different ($p < 0.05$)

Pectin, tannin and phytic acid contents

The pectin content (Table 6) increased from 0.92 g/100g (stage I) to 1.37 g/100g (stage III) which thereafter declined to 1.26 g/100g (stage IV) with significant difference between stage I and II and no significant difference was found among stages II, III and IV (Table 6. The tannin content was found to be highest (0.05 mg/100g) at stage I which then decreased to a minimum value of 0.02 mg/100g at fully matured stage and observed no significant difference in any of the stages studied. The highest phytic acid content of 24.15 mg/100g was recorded at stage II which then reduced to a level of 10.88 mg/100g at stage IV. There was significant difference among all the four stages. The plausible reason for increase

in pectin content with the advancement of fruit growth up to stage III may be due to less interaction between the pectin and the other cellular components. As a consequence the pectin was more available for extraction. On the other hand, decrease at stage IV may be due to the degradation of pectin under the action of enzymes, such as polygalacturonase (PG), pectin methyl esterase (PME) or pectate lyase (PL). The decrease in tannin content with the enhancement of the fruit growth may be attributed to the loss of astringency property as the fruit attains maturity. The *kachkal* in all four stages contained a very low amount of phytic acid (Table 6 compared to other starchy foods like cassava.

Table 6 The pectin, tannin and phytic acid content

Stages	Tannin (mg/100g)	Phytic acid (mg/100g)	Pectin (g/100g)
Stage I	0.05 ± 0.001^{b}	15.50 ± 0.39^{b}	0.92 ± 0.15^{a}
Stage II	0.05 ± 0.002^{b}	24.15 ± 0.95^{d}	1.27 ± 0.10^{b}
Stage III	0.01 ± 0.006^{a}	20.05 ± 1.50^{c}	1.37 ± 0.05^{b}
Stage IV	0.02 ± 0.003^{a}	10.88 ± 0.97^{a}	1.26 ± 0.04^{b}

Results in mean±SD (n=4), Means in each column followed by different superscript letters were significantly different ($p < 0.05$)

Total phenolics, DPPH radical scavenging activity, total carotenoids, vitamin A and thiamine content

The results of the phenol analysis and DPPH radical scavenging activity are presented in Table 7. The total phenolics content varied from 307.99 mg/100g at stage I to 150.96 mg/100g at stage IV. The highest DPPH radical scavenging activity of 59.12 % SA was observed in stage I which thereafter declined to 46.96 % SA at stage IV. In the present investigation the total phenolics content showed a decreasing trend towards maturity. The DPPH radical scavenging activity also showed a decreasing trend with the advancement of fruit growth. This correlates mostly with the total phenolics and ascorbic acid content of the fruits, which were higher during the initial stages of fruit development.

The present study evinced a gradual increase in total carotenoids, vitamin A and thiamine content (Table 7). The thiamine (0.002 - 0.032 mg/100g), vitamin A content (0.029 - 0.033 mg/100g) increased from stage I to stage IV. The results also revealed that *kachkal* is not a rich source of carotenoids. The highest carotenoid content of 0.019 mg/100g was observed at stage IV while the lowest carotenoid content (0.010 mg/100g) was recorded at stage I. It was observed that there was a significant difference among all the stages in case of

total phenolics, DPPH radical scavenging, total carotenoids and thiamin content of the samples (Table 7).

Table 7 Total phenolics, DPPH radical scavenging activity, total carotenoid, vitamin A and thiamine content

Stages	Total phenoilics (mg/100g)	DPPH radical scavenging activity (% SA)	Total carotenoids (mg/100g)	Vitamin A (mg/100g)	Thiamine (mg/100g)
Stage I	307.99 ± 2.86^{d}	59.12 ± 0.73^{c}	0.010 ± 0.001^{a}	0.029 ± 0.001^{a}	0.002 ± 0.001^{a}
Stage II	261.22 ± 2.29^{c}	55.60 ± 1.16^{b}	0.015 ± 0.001^{b}	0.028 ± 0.003^{a}	0.019 ± 0.01^{b}
Stage III	178.72 ± 2.60^{b}	52.66 ± 2.47^{b}	0.017 ± 0.002^{c}	0.030 ± 0.002^{a}	0.027 ± 0.003^{c}
Stage IV	150.96 ± 2.40^{a}	46.96 ± 4.20^{a}	0.019 ± 0.001^{d}	0.033 ± 0.002^{b}	0.032 ± 0.003^{d}

Results in mean±SD (n=4), Means in each column followed by different superscript letters were significantly different ($p < 0.05$)

Minerals content

The results of the mineral contents are shown in Table 8. The results showed that *kachkal* retained maximum concentrations of iron (185.36 ppm), copper (9.19 ppm), magnesium (1620.42 ppm) and calcium (1442.74 ppm) in stage II, whereas the concentrations of zinc increased with the maturity. The most abundant mineral in stage IV was zinc 1177.12 ppm followed by magnesium 940.10 ppm and calcium 274.36 ppm. The toxic minerals like cadmium and chromium were not detected in any of the growth stages. The minerals like Zn, Fe, and Cr are essential trace micronutrients for living organisms. All the eight minerals studied were found to be significantly different among all the stages.

In the present study Zn content of *kachkal* was found to be abundant in stage IV. The observed variation in the mineral concentrations in different growth stages is mainly attributed to the preferential absorbability of the fruit at different stages. The absence of toxic elements like chromium and cadmium makes *kachkal* suitable for human consumption in all stages.

Table 8 Mineral contents

Stages	Various minerals (ppm)							
	Iron	Copper	Manganese	Zinc	Magnesium	Cadmium	Chromium	Calcium
Stage I	30.23 ± 001^{c}	0.84 ± 0.001^{a}	5.67 ± 0.27^{b}	25.70 ± 0.04^{a}	561.22 ± 0.006^{a}	N.D	N.D	432.82 ± 3.11^{c}
Stage II	185.36 ± 1.71^{d}	9.19 ± 0.31^{d}	13.41 ± 0.66^{d}	189.50 ± 1.09^{c}	1620.42 ± 1.7^{d}	N.D	N.D	1442.74 ± 4.92^{d}
Stage III	21.07 ± 0.46^{a}	1.12 ± 0.03^{b}	4.91 ± 0.16^{a}	53.07 ± 1.50^{b}	983.89 ± 0.003^{c}	N.D	N.D	245.08 ± 6.56^{a}
Stage IV	24.08 ± 1.25^{b}	2.14 ± 0.03^{c}	7.35 ± 0.32^{c}	1177.12 ± 1.28^{d}	940.10 ± 0.001^{b}	N.D	N.D	274.36 ± 0.03^{b}

N.D = Not Detected; Results in mean±SD (n=4), Means in each column followed by different superscript letters were significantly different ($p < 0.05$)

Fatty acids composition

Fatty acid compositions of the *kachkal* at various developmental stages are presented in Table 9. The major saturated fatty acids were palmitic acid and stearic acid. The palmitic acid content decreased from 1678.28 ppm at stage I to 422.00 ppm at stage IV while stearic acid decreased from 91.88 – 68.69 ppm from stage I to stage III and was absent totally at stage IV. Lauric and myristic acids were present in minor quantities during early stages of fruit development, which gradually decreased towards maturity. Some other saturated fatty acids like arachidic, behenic acids were not detected throughout the fruit growth. Among the unsaturated fatty acids most dominant were linoleic acid and linolenic acid. These two essential fatty acids were highest 2081.39 ppm and 1208.59 ppm respectively during early stages of fruit development. The results revealed considerable amount of oleic acid 128.95 ppm during initial growth stage which increased to 524.47 ppm at fully matured stage and results evinced that the fatty acid compositions changed noticeably during the fruit growth. This indicates the involvement of lipids to some extent in metabolic changes during fruit development. It was observed that all the fatty acid studied were significantly different among all the four stages. The present study showed that the *kachkal* fruits at their early growth stage were rich in essential fatty acids like linoleic and linolenic. The observed early rise in linoleic acid appeared to be associated with glycolipid which is a unique component in chloroplast of young fruits. Thus presences of these two fatty acids in a reasonable amount enhance the nutritional value of *kachkal* fruit.

Table 9 Fatty acid profile of *kachkal*

Fatty acid (ppm)	Stage I	Stage II	Stage III	Stage IV
Caprylic acid	N.D	N.D	N.D	N.D
Capric acid	N.D	N.D	N.D	N.D
Lauric acid	13.93 ± 1.54^{b}	6.88 ± 1.01^{a}	92.52 ± 1.04^{c}	N.D
Myristic acid	19.04 ± 0.97^{b}	8.69 ± 0.50^{a}	131.62 ± 1.41^{c}	N.D
Palmitic acid	1678.28 ± 1.52^{d}	988.43 ± 1.84^{c}	593.38 ± 1.32^{b}	422 ± 0.003^{a}
Palmitoleic acid	50.98 ± 1.22^{b}	27.22 ± 0.53^{a}	N.D	N.D
Stearic acid	91.88 ± 0.82^{c}	61.99 ± 0.81^{a}	68.69 ± 1.18^{b}	N.D
Oleic acid	128.95 ± 10.51^{a}	651.18 ± 6.1^{c}	524.47 ± 6.76^{b}	N.D
Linoleic acid	2081.39 ± 18.49^{d}	1386.70 ± 12.39^{c}	387.94 ± 6.05^{a}	603.53 ± 7.58^{b}
Linolenic acid	1208.59 ± 10.91^{d}	670.90 ± 11.66^{c}	139.33 ± 5.16^{a}	213.82 ± 6.30^{b}

Arachidic acid	N.D	N.D	N.D	N.D
Behenic acid	N.D	N.D	N.D	N.D
Erucic acid	N.D	N.D	N.D	N.D
Lignoceric acid	N.D	N.D	N.D	N.D

N.D = Not Detected; Results in mean±SD (n=4), Means in each column followed by different superscript letters were significantly different ($p < 0.05$)

Amino acids composition

The amino acid compositions of developing culinary banana are presented in Table 10. The results revealed that 18 amino acids were found in protein of banana and the total amount of the amino acids decreased gradually from 20 DAE to 65 DAE of fruit development. The three most dominant amino acids were glutamic acid (3.12 g/100g), aspartic acid (1.08 g/100g) and alanine (0.45 g/100g) which decline with fruit development. Noteworthy is the presence of high amount of leucine in all four stages. In all stages, the methionine had lower value than other amino acids. Hence this is the limiting amino acid. The results further revealed that, all the essential amino acids were present in *kachkal* in all the growth stages. From the present study it was observed that there was a marked variation in most of the amino acid contents among all the four stages except in few amino acids like proline, leucine, tyrosine and phenylalanine

Table 10 Amino acid composition

Amino acid (g/100g protein)	Stage I	Stage II	Stage III	Stage IV
Tryptophan	0.16 ± 0.004^{a}	0.17 ± 5.22^{a}	0.19 ± 0.002^{b}	0.31 ± 0.006^{c}
Aspartic acid	1.08 ± 0.01^{d}	1.02 ± 0.009^{c}	0.73 ± 0.012^{b}	0.051 ± 0.002^{a}
Threonine	0.29 ± 0.002^{d}	0.26 ± 0.004^{b}	0.28 ± 0.004^{c}	0.23 ± 0.002^{a}
Serine	0.43 ± 0.006^{c}	0.38 ± 0.007^{b}	0.46 ± 0.004^{d}	0.27 ± 0.002^{a}
Glutamic acid	3.12 ± 0.031^{d}	3.01 ± 0.06^{c}	1.95 ± 0.033^{b}	1.22 ± 0.009^{a}
Proline	0.20 ± 0.003^{c}	0.17 ± 0.003^{b}	0.12 ± 0.004^{a}	0.13 ± 0.004^{a}
Glycine	0.37 ± 0.005^{d}	0.33 ± 0.006^{c}	0.31 ± 0.004^{b}	0.30 ± 0.004^{a}
Alanine	0.45 ± 0.006^{d}	0.41 ± 0.005^{b}	0.44 ± 0.006^{c}	0.32 ± 0.006^{a}
Cystine	0.03 ± 0.004^{b}	0.04 ± 0.001^{c}	0.02 ± 0.001^{a}	0.03 ± 0.006^{b}
Valine	0.42 ± 0.007^{c}	0.38 ± 0.007^{b}	0.43 ± 0.004^{d}	0.34 ± 0.006^{a}
Methionine	0.047 ± 0.001^{c}	0.048 ± 0.002^{c}	0.035 ± 0.003^{b}	0.029 ± 0.001^{a}
Isoleucine	0.30 ± 0.005^{d}	0.27 ± 0.005^{b}	0.29 ± 0.009^{c}	0.23 ± 0.004^{a}
Leucine	0.58 ± 0.009^{c}	0.52 ± 0.006^{b}	0.52 ± 0.005^{b}	0.44 ± 0.008^{a}
Tyrosine	0.14 ± 0.003^{c}	0.13 ± 0.002^{b}	0.13 ± 0.002^{b}	0.09 ± 0.002^{a}
Phenylalanine	0.46 ± 0.005^{c}	0.41 ± 0.003^{b}	0.42 ± 0.004^{b}	0.33 ± 0.005^{a}

Histidine	0.31 ± 0.005^{d}	0.24 ± 0.005^{c}	0.19 ± 0.003^{b}	0.12 ± 0.002^{a}
Lysine	0.45 ± 0.008^{d}	0.37 ± 0.004^{c}	0.26 ± 0.005^{a}	0.33 ± 0.005^{b}
Arginine	0.45 ± 0.009^{d}	0.40 ± 0.007^{c}	0.35 ± 0.005^{a}	0.38 ± 0.006^{b}

Results in mean±SD (n=4), Means in each column followed by different superscript letters were significantly different ($p < 0.05$)

Colour measurement

The colour measurement of dried *kachkal* fruit during various developmental stages is presented in Table 11. The results of the present investigation clearly showed that the degree of lightness and the degree of yellowness increased with maturity, while the degree of redness followed a decreasing trend with maturity. The increase in degree of lightness of *kachkal* with maturity may be attributed to the reduction in browning potential of the fruit with the advancement of growth. An increase in degree of yellowness can also be correlated with the increase in total carotenoid content with maturity.

Table 11 The colour measurement of *kachkal* (*Musa* ABB) at the different growth stages

Sample	L	a	b
Stage I	47.04 ± 1.24^{a}	2.87 ± 0.04^{d}	6.49 ± 0.05^{a}
Stage II	60.62 ± 1.12^{b}	2.19 ± 0.03^{c}	7.79 ± 0.12^{b}
Stage III	70.31 ± 1.35^{d}	2.04 ± 0.03^{b}	8.29 ± 0.08^{c}
Stage IV	69.06 ± 2.28^{c}	1.52 ± 0.02^{a}	9.26 ± 0.52^{d}

Results in mean±SD (n=4), Means in each column followed by different superscript letters were significantly different ($p < 0.05$)

(ii) Effect of Three Conventional Cooking Methods on the Biochemical Compositions of '*Kachkal*'

Kachkal samples were washed thoroughly under running water followed by distilled water wiped out the surface moisture. Processing of *Kachkal* involved three conventional household cooking methods, namely blanching (75°C for 10 min), boiling (100°C for 8 mins) and microwave cooking (600Hz, 40°C for 4 min). The processed samples were dried in a tray drier at 40°C for 12 hrs and grounded and taken for analysis. The various biochemical compositions of the samples were studied following standard procedures.

Proximate Compositions

The proximate compositions of *kachkal* subjected to different cooking methods are given in Table 12. The results showed that moisture content increased from 8.07% in untreated sample to 13.97% in boiled sample. This increase in moisture content may be attributed to the absorption of water during boiling. Ash content showed significant decrease during all three cooking treatments. Accordingly, the lowest ash content (1.97g/100g) was recorded in the boiled sample whereas the highest ash content (5.38 g/100g) was recorded in the untreated sample. Reduction in ash content might due to the absorption of water during processing leading to dilution. Maximum protein content (4.99 g/100g) was recorded in the untreated sample while the lowest protein content (3.32 g/100g) was recorded during blanching. However, in the present study fiber content decreased significantly from 2.66 g/100g in the untreated sample to 1.03 g/100g in the microwave cooked sample. The 80.03 g/100g of carbohydrate content in untreated *Kachkal* makes it one of the non-oil calorie rich foods. According to the results different carbohydrate fractions reduced during all three cooking treatments. Total carbohydrate content decreased from 80.03g/100g to 68.01 g/100g during boiling. These reductions are mainly due to their diffusion into the cooking water. Maximum loss of these components during boiling occurred as higher temperature enhances the rate of solubilisation.

Table 12 Effect of three conventional cooking treatments on the proximate compositions (g/100g)

Sample	Moisture	Ash	Fat	Protein	Fiber	Carbohydrate
Raw	8.07 ± 0.97^{a}	5.38 ± 1.34^{a}	$0.58\pm1.87^{a,b}$	$4.99\pm2.09^{a,c}$	$2.66\pm1.76^{a,b,c}$	80.031 ± 1.23^{a}
Blanched	13.45 ± 1.87^{b}	$3.35\pm1.99^{b,c}$	0.71 ± 2.08^{a}	3.32 ± 0.98^{b}	2.00 ± 0.85^{a}	72.004 ± 1.76^{b}
Microwave	13.14 ± 2.01^{b}	2.46 ± 1.78^{b}	1.52 ± 1.06^{b}	3.51 ± 1.33^{b}	1.03 ± 1.49^{b}	74.001 ± 2.43^{c}
Boiled	13.97 ± 0.93^{c}	$1.97\pm0.60^{b,c}$	1.03 ± 0.03^{b}	3.67 ± 1.74^{c}	$1.16\pm2.06^{b,c}$	68.015 ± 1.87^{d}

Results in mean±SD (n=4), Means in each column followed by different superscript letters were significantly different ($p < 0.05$)

Table 13 shows the carbohydrate fractions of *kachkal* undergoing different cooking treatments. The cellulose and lignin contents were also reduced to various extents during different cooking treatments (Table 13). As a result of boiling 39-59 g/100g of cellulose content was reduced. This might be due to the conversion of cellulose into simple carbohydrates like glucose. However, in the present study lignin content was reduced to maximum (0.421 g/100g) during microwave cooking. In the present study pectin content evinced a gradual decrease during all three cooking methods. This can be explained as during processing and storage

operations, pectin can be demethylated and depolymerized by both enzymatic and non enzymatic reactions.

Table 13 Effect of three conventional cooking treatments on the carbohydrate components (g/100g)

Sample	Starch	Amylose	Reducing Sugar	Total soluble sugar	Cellulose	Lignin	Pectin
Raw	46.81±2.39	31.61±2.88	0.16±2.68	0.53±1.54	1.06±1.99	1.57±0.55	1.28±0.93
Blanched	46.75±2.88	31.10±3.09	0.05±2.90	0.35±0.93	0.65±1.58	0.75±0.38	1.24±0.79
Microwave	46.70±3.07	30.86±3.14	0.07±2.83	0.37±2.85	0.63±1.64	0.42±0.86	1.17±1.09
Boiled	46.49±2.76	30.84±2.77	0.03±2.76	0.23±1.79	0.44±1.43	0.51±0.47	1.15±0.67

Results in mean±SD (n=4), Means in each column followed by different superscript letters were significantly different ($p < 0.05$)

Total phenolics, vitamins, carotenoids and antioxidant activity

It has been observed in the study that the raw *kachkal* contained 150.33 mg/100g total phenol content which decreased to 64.73 mg/100g when subjected to boiling. This could be due to phenolics breakdown during cooking. Ascorbic acid, thiamine and vitamin A contents were higher (Table 14) in raw sample and were destroyed considerably during cooking treatments. The losses of vitamins were due to a combination of leaching and thermal destructions of vitamins. But microwave cooking and autoclaving improved the retention of these vitamins compared to boiling. The carotenoid content showed an increasing trend with the degree of processing. Maximum carotenoid content (0.02 mg/100g) was recorded during boiling. Raw *kachkal* contained 0.04 mg/100g tannin which was then reduced to 0.04 mg/100g during boiling. This reduction in tannin content during different cooking treatments are as a result of the fact that tannins are polyphenols and polyphenolic compounds are water soluble in nature.

Anti nutritional factor like phytic acid content was higher (12.15 mg/100g) in the untreated sample. Boiling and blanching reduced the phytic acid content to a considerable level (3.60 and 4.05 mg/100g respectively). The present study clearly revealed that loss of phytic acid content is positively correlated with cooking time. Antioxidant activity was measured as the DPPH radical scavenging activity. The antioxidant activity increased during all the three cooking treatments. Highest antioxidant activity (50.14 % SA) was recorded in boiled *kachkal* samples. It was reported that the antioxidant activity of the vegetables as increased by boiling.

Table 14 Effect of three conventional cooking treatments on the phenolic, ascorbic acid, thiamine, vitamin A, carotenoid, tannin, phytic acid content and antioxidant activity (mg/100g)

Sample	Phenol	Ascorbic	Thiamine	Vit A	Carotenoid	Tanin	Phytic	Antioxidant Activity (%)
Raw	150.3± 3.08	0.91±0 .76	0.02± 0.09	0.03± 0.12	0.02± 0.08	0.04± 0.79	12.15± 1.27	45.86± 3.78
Blanched	78.13± 4.27	0.58± 0.81	0.01± 0.06	0.02± 0.93	0.02± 0.07	0.04± 0.65	4.05± 1.76	48.40± 3.88
Microwave	98.46± 4.43	0.61± 0.94	0.02± 0.08	0.02± 0.83	0.02± 0.12	0.043± 0.99	11.12± 1.82	49.10± 2.70
Boiled	64.73± 3.51	0.38± 0.59	0.01± 0.11	0.02± 0.09	0.02± 0.18	0.04± 0.86	3.60± 0.96	50.1± 3.71

Objective 2: To develop Resistance starch (RS) from *Kachkal* by use of heat processing and enzymatic methods

(i) Isolation of Starch from *Kachkal*

Materials and Methods

Kachkal at matured edible stage was taken for isolation of starch. Using the procedure of Bello-Pérez et al.(1999). In brief, the fruits were peeled and cut into 5–6 cm cubes and immediately rinsed in sodium sulfite solution (1.22 g/l) and then macerated at low speed in a blender (500 g fruit : 500 g solution) for 2 min. The homogenate was consecutively sieved through screens number 50 and 100 US mesh, until the washing water was clean; then the starch milk was centrifuged at 10800 g for 30 min. The white-starch sediments were dried in a convection oven at 40 °C for 24 hours and ground with mechanical grinder and passed through 100 mesh screen sieve and stored at room temperature in sealed containers.

Chemical analysis of *Kachkal* starch

Moisture content was determined as weight loss after vacuum drying at 70 °C until a constant weight is reached. Ash, protein, fat content were determined according to AOAC (2010) methods.The pH of starch dispersion (8% w/v) was measured by using a pH meter (Systronics micro pH system 301).Total amylose content was determined as per the method of Sowbhagya and Bhattacharya (1979). The carbohydrate content was measured as per the method of Sadasivam and Manickam (2008), by hydrolyzing the polysaccharides into simple sugars by acid hydrolysis and estimating the resulting monosaccharide by anthrone method. Starch content was determined according to the method of Hodge and Hofreiter (1962). Soluble sugars were extracted with 80% ethanol (hot) and were quantified by phenol-sulphuric acid method (Dubois et al., 1956). The reducing sugar content was estimated by the Nelson- Somogyi method(1952).

Functional properties of *Kachkal* starch

Water holding capacity, starch swelling power and solubility

Water holding capacity was determined as described by Hallgren (1985) Starch pastes 5% (w/v) were heated to 60, 70, 80, and 90 °C for 15 min with shaking every 5 min period. Tubes were centrifuged at 3000 g for 15 min, the supernatant was decanted, and the tubes were then weighed, and the gain in weight was used to calculate the water holding capacity.

Starch suspension (40 ml of a 1%w/v) was taken in previously weighed 50 ml flask and was heated from 50 to 90°C at 5 ° intervals for 30 min .The flask was removed and left for cooling to room temperature and centrifuged for 15 min at 3000g; the supernatant

decanted and the swollen granules weighed. A 10 ml sample was taken from the supernatant, placed in a crucible and dried in a convection oven at 120 °C for 4 hour to constant weight. Percentage solubility and swelling power were calculated using the formulas:

% Solubility = dry weight at 120°C × 400/ sample weight

Swelling Power = weight of swollen granules ×100/sample weight× (100- %solubility)

Freeze-thaw stability

Freeze-thaw stability of the starch paste was studied by four alternate freezing and thawing of 5 ml of 5% starch pastes (freezing for 18 hour at -20°C and 6 hour thawing at room temperature respectively) followed by centrifugation at 3000 g for 10 min. The percentage of water separated after the freeze thaw cycle was measured.

Paste clarity

Stability and clarity of starch pastes were determined at room temperature and at 24, 48 and 72 h. Starch sample (0.2 g) was suspended in 5 ml of water in screw cap tubes and placed in a boiling water bath for 30 min. The tubes were thoroughly shaken every 5 min. After cooling to room temperature (14 min), the % T at 650 nm was determined against a water blank in a spectrophotometer (CECIL Aquarius 6000).

Morphological analysis

Starch granules were observed under a scanning electron microscope (JEOL JSM 6390 LV) operating at an electron voltage of 15 kv. Starch sample was assembled on metallic stubs with double sided tape and coated with a thin layer of gold. Magnification was taken at 500X and shape and size of the starch granules were observed.

Pasting properties

Pasting properties of starches were evaluated in Rapid Visco-Analyser (RVA-4, Newport Scientific, Sydney, Australia). An 8% slurry was given a programmed heating and cooling cycle set for 23 min, where the sample was held at 30°C for 1 min, heated to 95°C in7.5 min, further held at 95°C for 5 min before cooling to 50°C within 7.5 min, and holding at 50°C for 2 min. The speed was 960 rpm for the first 10 sec, then 160 rpm for the remainder of the experiment. Peak viscosity, final viscosity and pasting temperature of starches were measured. All measurements were replicated four times.

Structural analysis of *Kachkal* starch

X- Ray diffraction

The X-ray diffraction was obtained from a D/max 2500 X-ray difractometer (Rigaku Miniflex), a conventional X-ray tube set to 30 kv acceleration potential and 15 mA current. The X-ray source was Cu Kα radiation. Data were collected from 2θ of 2 to 30°C (θ being the angle of diffraction) with a scan speed of 8° 2θ/min. The XRD patterns were evaluated according to Zobel (1984). The starch sample was dried at 50°C to constant moisture (10%) in a vacuum oven, then 50 mg samples were added into the slide for packing prior to X-ray scanning.

Fourier transforms infrared (FTIR) spectra

IR spectra of *Kachkal* starch was measured using KBr's method of Pushpamalar et al. (2006). The dry sample was blended with KBr in a ratio starch/KBr 1:4. The blend was pressed to obtain a pellet and introduced in the spectrometer (Nicolet Instruments 410 FT-IR equipped with KBr optics and a DTGS detector). Each spectrum was analyzed in the range of resolution from 400– 4000 cm^{-1} and 16 scans were collected.

Statistical analysis

The means (at least four replicates) and standard deviations were determined for all data.

Results

Yield and chemical analysis of *Kachkal* starch

The moisture content of the *kachkal* starch (11.90%) The data of the present research (Table 15) revealed that the *kachkal* starch is low in protein content (1.35%). The higher (0.50%) fat content of *kachkal* starch is perhaps the reason for its resistance to amylosis due to the formation of amylose-lipid complex. The ash content of *kachkal* starch was found to be 0.35%. The high ash content of *kachkal* starch is indicative of presence of more minerals. The pH value obtained (Table 15) for the *kachkal* starch (6.70) was within the pH range of 3-9 obtained for most starches used in the pharmaceutical, cosmetics, and food industries. The carbohydrate content of *kachkal* starch was 96.15%.

Table 16 showed that the yield of starch from *kachkal* was 34.00% with a purity of 96.00%. Amylose content of 34.10% and 61.90 % amylopectin content of *kachkal* starch revealed a non-waxy starch type. The soluble sugar content was 0.15% and reducing sugar was found in the range of 0.02 % which was comparatively low.

Table 15 proximate compositions (g/100g) of *kachkal* starch

Moisture (%)	pH	Protein	Fat	Ash	Crude fibre	Total Carbohydrate
11.90±0.03	6.70±0.03	1.35± 0.03	0.50± 0.02	0.35± 0.07	0.87±0.97	96.15±0.03

Table 16 Chemical compositions (g/100g) of *kachkal* starch

Starch Yield	Starch content	Amylose	Amylopectin	Soluble sugar	Reducing Sugar	Non-reducing sugar
34.00±2.34	96.00±0.08	34.10±0.02	61.90±0.02	0.15±0.01	0.02±0.0	0.13±0.00

Functional properties of *kachkal* starch

The present study revealed that water holding capacity of *kachkal* starch (Table 17) increased with rise in temperature. The maximum water holding capacity (42.24%) was observed at 90°C. The swelling behavior of starch is an indication of the water absorption characteristics of the granules during heating. Generally, the solubility and swelling profiles show a general trend of increase with increase in temperature. The swelling and solubility profile of *kachkal* starch is presented in Table 17. The *kachkal* starch swelled slowly up to 70°C and above 70°C the starch granules swelled rapidly due to the breakage of inter-molecular hydrogen bonds in amorphous region. The maximum solubility (9.00%) of *kachkal* starch was observed at 90°C.

Table 17 Water holding capacity, swelling and solubility profile of *kachkal* starch.

Parameters	**Temperature (°C)**			
	60	70	80	90
Water holding capacity	12.64±0.09	20.42±0.07	36.26±0.01	42.24±0.01
Swelling (gH_2O/g dry samples)	2.30±0.12	4.50±0.04	8.90±0.00	12.80±0.06
Solubility	1..55±0.01	3..51±0.52	7.11±0.08	9.00±0.17

Kachkal starch gel was unstable during different freezing and thawing cycles releasing 24.13 % -42.58 % of the water (Fig. 1). The amount of water separated from the gels during freezing increased with storage time. This result suggests that banana starch is not desirable for frozen products.

Starch gel clarity is a much desirable functionality of starches for its utilization in food industries since it directly influences brightness and opacity in foods that contain it as thickeners. The *kachkal* starch experienced low paste clarity (3.2 - 1.2 % light transmittance)

during various hours of storage. Since the paste clarity of *Kachkal* starch is very low, therefore it could be used in food products that do not required transparency.

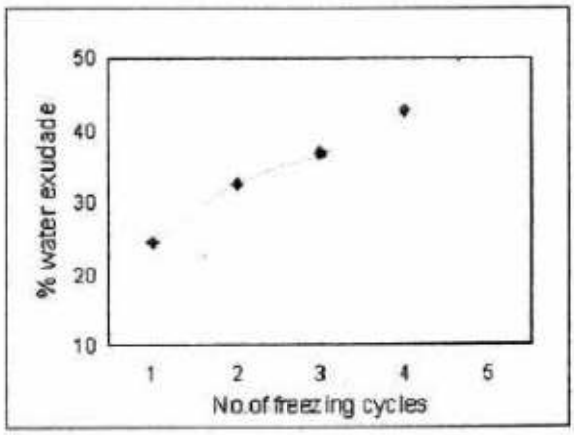


Fig. 1. Freeze-thaw stability of *kachkal* starch

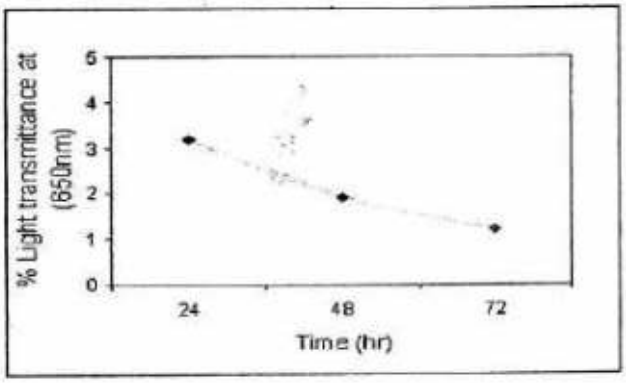


Fig. 2. Paste-Clarity of *kachkal* starch

Morphological analysis

The scanning electron micrographs (Fig. 3) of the starch revealed that the *kachkal* starch granules appeared as a mixture of spherical and elliptical shaped with granule size ranged from 7.55μm-68.00μm. The surface of the *kachkal* starch appeared to be smooth therefore, it could be indicated that the isolation process was efficient and it did not cause damage to starch granules.

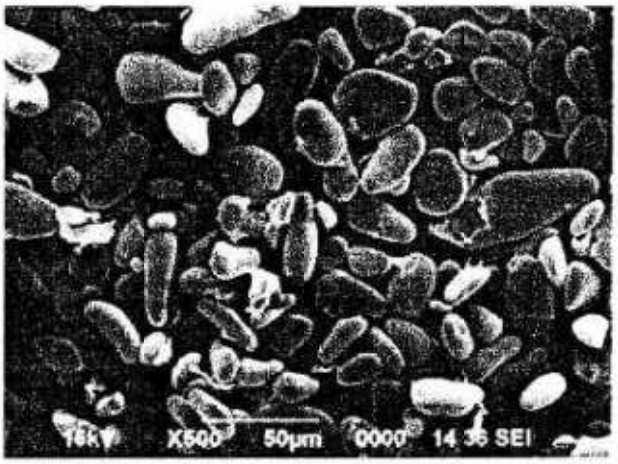


Fig. 3. Scanning electron micrographs of *kachkal* starch

Pasting Properties

Pasting properties of *kachkal* starch are presented in Table 18. The *kachkal* starch exhibited a high (81.80° C) paste temperature which indicates that the starch is highly resistant towards swelling. The *kachkal* starch had a moderate peak viscosity and the maximum of the peak viscosity reflects the ability of starch granules to swell freely before their physical breakdown. The starch exhibited high setback viscosity (Table 18) during cooling indicating that they were highly retrograded, which might due to the effect of amylose and amylopectin contents. During the holding temperature at 95° C accompanied with shear, a decrease in the viscosity of the starch pastes was shown, resulting from the breakdown of some swollen starch granules. The *kachkal* starch also experienced a low breakdown (447cP) viscosity which is also indicative of lower degree of swelling and subsequent disintegration.

Table 18 Pasting profile of *Kachkal* starch

Pasting Temp. (°C)	Peak Viscosity (cP)[a]	Hold Viscosity (cP) [a]	Final Viscosity (cP)[a]	Breakdown Viscosity (cP)[a]	Setback Viscosity(cP)[a]
81.80°C±0.04	4507 ±1.24	4060± 1.24	6214 ± 1.69	447±0.47	2154± 1.63

(Results are mean of four replicates and ±SD), [a]: Centiposie

X-Ray Diffraction

The wide-angle X-ray (Fig. 4) diffractogram revealed that *kachkal* starch is a mixture between the A and B-type polymorphs. The starch exhibited strong diffraction peaks at 15.01 and 17.23 (2θ/θ) and one very broad peak at 23 (2θ/θ).

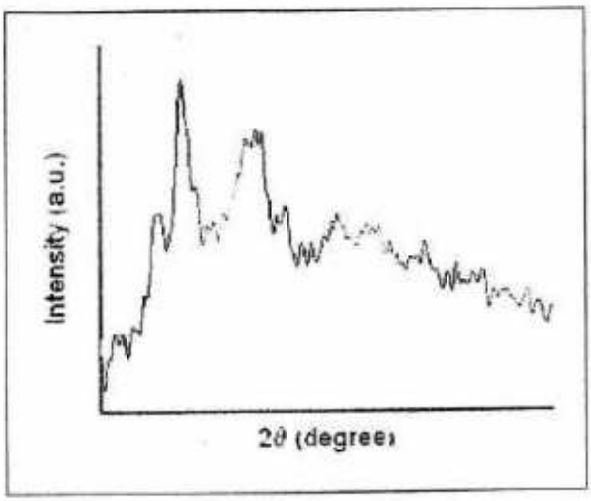


Fig 4: X-ray diffractogram of *kachkal* starch

FTIR spectral analysis

In the FTIR spectra (Fig. 5), spectral bands were observed at 999.25, 1161cm^{-1}. Additional characteristics absorption bands appeared at 575,523,432,708 and 856cm-1. The band at 1649 is related to O-H def and is directly related to the moisture content of the sample. The sharp band at 2929 cm^{-1} is characteristics of C-H stretches associated with the ring methine hydrogen atom. Broad band at 3431 cm^{-1} appeared due to hydrogen bonded hydroxyl groups.

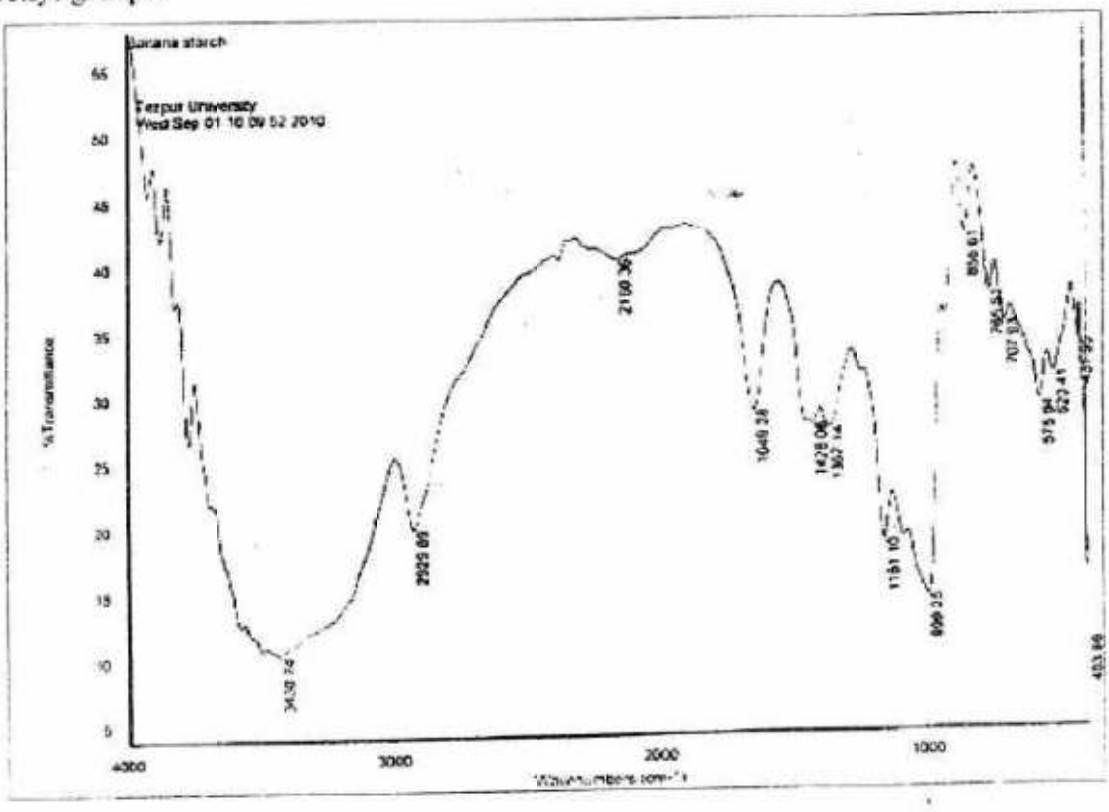


Fig 5. FTIR spectrum of *Kachkal* starch

(ii) Resistant starch (RS) production from *kachkal* starch

a) By autoclaving and cooling method

The samples were prepared following the method of Berry (1986) with slight modification by suspending 60gm of *kachkal* starch in 250 ml of water and autoclaved using 15 psi pressure at 120°C for 30 min followed by cooling at 4°C for 24 hour. After three repetitions of the autoclaving and cooling cycles, the sample was freeze-dried and ground into fine particles by using mechanical grinder and passed through 100 mesh screen sieve.

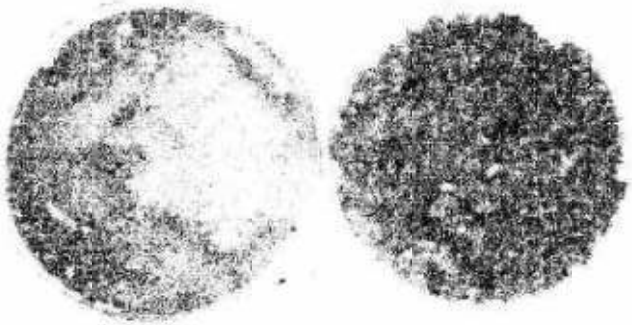


Fig. 6: Kachkal RS

Optimization of enzyme concentration for enzymatic debranching

Prior to starch debranching, the optimal concentration of pullulanase was determined. A *kachkal* starch suspension (20 %, w/v) was gelatinized on a boiling water bath for 15 min under stirring. This gel was autoclaved at 120 °C for 30 min and then the gel was re-dissolved in distilled water to obtain a 10% (w/v) gel solution. The gel was cooled to 50 °C and pullulanase at different concentrations (0.25, 0.5, 1.0 and 2.0 %) calculated on dry starch weight) was added. The mixture was incubated at 50°C with constant stirring for 1hour and resistant starch content was determined as per the method of AOAC, 2010.

b) RS production by enzyme debranching

Kachkal starch was debranched using the enzyme concentration determined previously. Starch gel was prepared as described above and the reaction temperature (40, 50 and 60°C) and time were varied (1, 9, 16 and 24 hour); after these times, the samples were autoclaved at 120°C for 30 min, cooled down and stored for 24 h at 4°C or -20 °C. The samples were dried at 40°C for 12 hrs and stored in closed glass containers.

Table 19 Effect of starch concentration and number of autoclaving & cooling cycles on RS content

Starch concentration (%)	No. of cycles	RS content (%)
10% (w/v)	I	9.45±0.00
10% (w/v)	II	10.23±0.06
10% (w/v)	III	12.30±0.00
20 % (w/v)	I	10.14±0.02
20 % (w/v)	II	11.98±0.14
20 % (w/v)	III	14.00±0.01

(Results are mean of three replicates and ±SD)

Table 20 Effect of storage temperature on resistant starch content

Sample	Storage	RS (%)
Autoclaved (10%)	4°C	12.30±0.05
Autoclaved (10%)	-20 °C	15.00±0.00
Autoclaved (20%)	4°C	13.03±0.03
Autoclaved (20%)	-20 °C	16.42±0.12
Enzyme debranched(24hrs)	4°C	30.20±0.01
Enzyme debranched(24 hrs)	-20 °C	31.17±0.01

(Results are mean of three replicates and ±SD)

Fig.7. *Kachkal* RS prepared using different temperature n storage condition

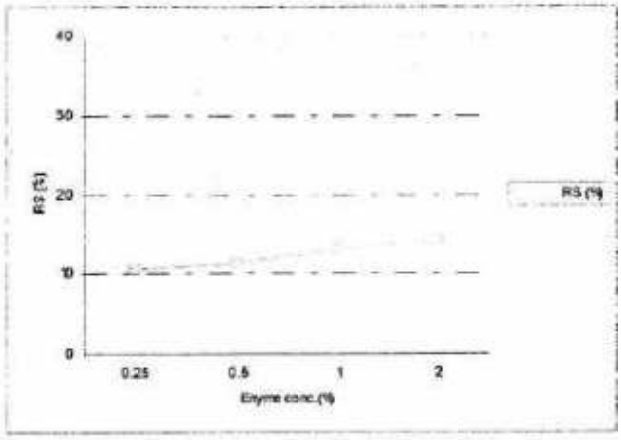


Fig 8. Effect of pullulanase concentration on RS content

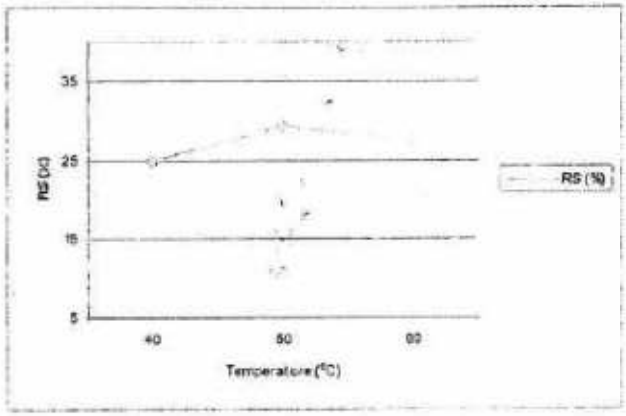


Fig 9. Effect of incubation temperature on RS content

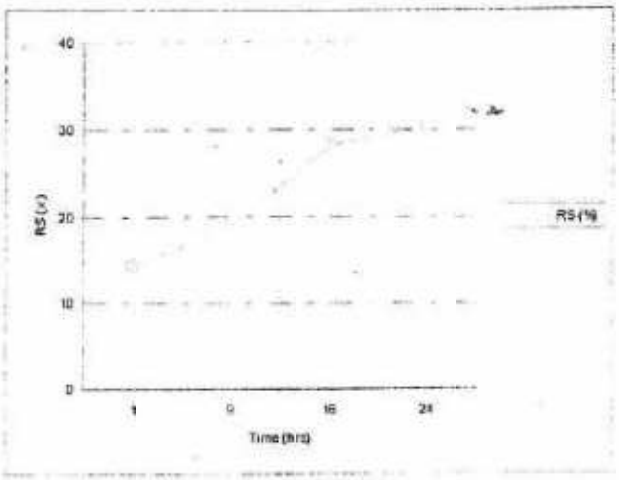


Fig. 10. Effect of incubation time on RS content

Resistant starch determination

The RS content analysis was carried out using the method of Onyango et al. (2006), with a slight modification. Resistant starch sample prepared from *kachkal* starch (0.4 g on a dry basis) was weighed in a pre-weighed centrifuge tubes; 20 ml phosphate buffer (pH 6.0,

55.6 mM) and 0.16 g α-amylase (Sigma A-3176) were added and incubated at 37°C for 16 hour. The sample was cooled to ambient temperature before adjusting to pH 4.5 using phosphoric acid solution (2 ml/100 ml). Then, 0.4 ml amyloglucosidase (Sigma A-7095) was added and the sample was incubated at 60°C for 30 min. After digestion, the *kachkal* resistant starch samples were centrifuged at 4,000×g for 15 min by a high speed micro centrifuge and the residue re-suspended in 20 ml phosphate buffer (pH 7.5, 0.08 M). 0.4 ml protease (Sigma P-2143) was added and incubated at 42°C for 4 h. The sample was centrifuged at 6,000×g for 15 min then dried at 60°C to constant weight in a constant-temperature oven and weighed to determine the RS. The RS content was calculated using the following formula

$$\text{Resistant starch content (\% dry basis)} \frac{\text{Resistant starch weight}}{\text{sample weight}} X\ 100 \qquad (1)$$

(iii)Preparation of resistant starch bread (RSB)

The RS prepared was further incorporated in whole wheat flour and RSB was prepared following the standard procedure of bread making (Olaoye, et al., 2006). The ingredients for RSB were 50% wheat flour mixed with 50 % RS, 36% luke warm water, 3.4% sugar, 1% table salt and 1% yeast. Another set of bread was also prepared without adding RS which was considered as standard bread (BS).

Flow chart for preparation of RSB

Wheat flour + RS + sugar + salt + yeast mix thoroughly

↓

Addition of luke warm water n mixed properly

↓

Continuous kneading for 20 - 25 min for proper dough formation

↓

Transfer the dough in bread mold n cover with clean polyethylene bag

↓

Proofing for 90 min at room temperature (30±2^0 C), RH (85±2 %)

↓

Remove the bread mold from proofing cabinet and remove polyethylene bag

↓

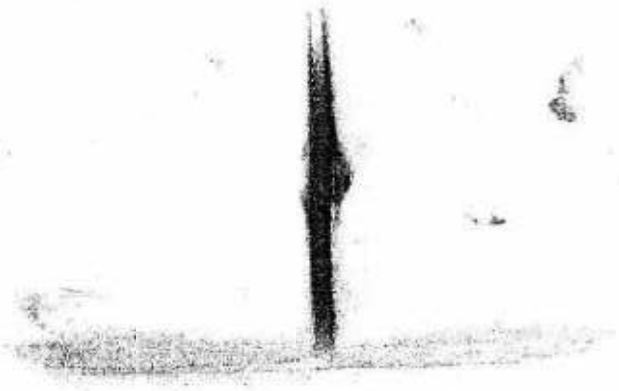
Spray small amount of water inside pre-heated oven at 190^0 C and place bread mold inside the oven

↓

Baking at 190^0 C for 90 min

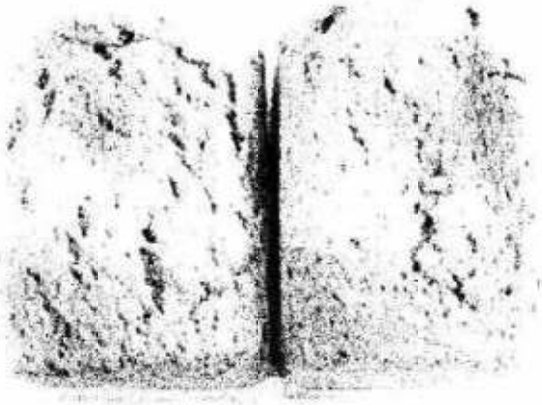
↓

After 90 min take the bread molds out from oven and cool it in room temperature



Brown Bread (Control)

Fig. 11. Bread standard sample (BS)



Brown Bread
Prepared by Incorporating
***Kachkal* Resistant Starch**

Fig. 12. Brown bread prepared by incorporating kachkal resistant starch (RSB)

(iv) Determination of Glycaemic Index (GI)

Subjects

Sixteen subjects (male and female) were recruited for the study; out of which ten were healthy (i.e. disease free) subjects (age 20-50 years) and six were diabetics (aged 40-50 years). All subjects were moderately active, non-smoking and non-alcoholics. On the day prior to a test, subjects were asked to restrict their activities and not to eat or drink after 21:00 hours the night before a test, although water was allowed. The blood samples were collected to determine the fasting blood glucose (FBG) levels and postprandial blood sugar level of the subjects at 8 am on the following day. Blood samples were collected from the subjects using a disposable syringe before and after the meals were administered and analysis was done using the Enzymatic (GOD/POD) Method kit (Mediclone Biotech Pvt. Ltd., India) in the Health Centre of Tezpur University, Assam.

Determination of Blood Glucose (B-Glucose) by Enzymatic method

Measurement of glucose concentration in serum or plasma is mainly used in diagnosis and monitoring of treatment in diatetes mellitus. The analysis was done using the Enzymatic (GOD/POD) Method. Determination of glucose after enzymatic oxidation by glucose oxidase. The colorimetric indicator is quinoneimine, which is generated from 4-aminoantiipyrine and phenol by hydrogen peroxide under the catalytic action of peroxidise (Trinder, 1969).

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{GOD}} \text{Quinoneimine} + 4H_2O$$

The glycaemic index (GI) was calculated using the method described by FAO/WHO (1998) as the incremental area under the blood glucose response curve of a 50 g portion of the test food expressed as a per cent of the response to the same amount of carbohydrate from a standard food taken by the same subject. After intake of the test food, they were allowed to take some water and their blood samples collected at 30 min interval for a period of 2 hrs. The tasted foods were resistant starch (RS) powder, resistant starch bread (RSB) and bread standard sample (BS).

In each volunteer, the GI (%) was calculated using the following formula:

$$GI\ (\%) = \frac{IAUC}{IAUCS} \times 100 \qquad (2)$$

IAUC – Incremental Area Under the blood glucose response Curve for the tested meal.
IAUCS – Incremental Area Under the blood glucose response Curve for the Standard meal.

Statistical analysis

The results were expressed by means of values ± standard deviations of three separate determinat

Results

Chemical Compositions of RS, RSB, BS

The proximate compositions of RS, RSB and BS sample used in this study are given in Table 19. The moisture content of *kachkal* RS was 7.72%. Whereas, moisture content of RSB and BS were found to be 58.50 % and 62.77 % respectively. The protein content of RS, RSB and BS were 1.08 %, 1.67 % and 0.74 % and fat content as 0.16 %, 0.46 % and 1.68 % respectively. Higher fat content of banana starch have technological and nutritional importance due to the amylose-lipid complexes formed during the processing of this starch. The amylose and resistant starch content of RS, RSB and BS are given in Table 20. The

amylose content of the RS obtained in the present study was quite high (34.10 %). All these factors revealed that *kachkal* starch is an excellent source for RS type III production.

Table 21 Proximate compositions (%) of *kachkal* RS, RSB, BS

Test foods	Moisture	Protein	Fat	Crude fibre	Ash	Carbohydrate
RS	7.72±0.93	1.08±0.96	0.16±0.62	2.94±0.63	1.64±0.90	12.86±0.78
RSB	58.50±0.38	1.67±0.77	0.45±0.85	2.35±0.86	1.23±0.21	14.54±1.09
BS	62.77±0.56	0.74±0.88	1.68±0.60	1.09±0.84	1.29±0.56	50.76±1.34

(Results are mean of three replicates and ±SD)

(RS= resistant starch; RSB = resistant starch bread; BS = bread standard)

Table 22 Amylose (%) and resistant starch content (%) of *kachkal* RS, RSB, BS

Test foods	Amylose content	RS content
RS	34.10±0.75	19.32±0.79
RSB	23.32±0.71	8.88±0.69
BS	11.08±0.79	0.56±0.68

(Results are mean of three replicates and ±SD)

(RS= resistant starch; RSB = resistant starch bread; BS = bread standard)

Glycaemic Index (GI) and Blood Glucose (B-Glucose) Curve

A total of 720 tests (240 tests for each food) were performed. The mean incremental area under blood glucose responses of both tested and standard foods are shown in Fig. 13, 14 and Table 21. From the study it was concluded that there was a significant elevation in the postprandial blood glucose levels in both healthy volunteers and diabetics upon the consumption of BS ($p<0.01$). Further the postprandial B-glucose levels were lower in different time intervals with the administration of RS ($p<0.05$) followed by RSB ($p< 0.05$). The mean values of GI (Table 22) of BS, RSB and RS in healthy volunteers were found to be 83.15, 80.77 and 55.87 respectively. On the other hand, GI of BS, RSB and RS in diabetics were found to be 120.98, 104.53 and 91.76 respectively (Fig. 15). For practical measures, GI values are often grouped into categories as producing either a low, medium or high glycaemic response (Brand-Miller et al., 2003). Thus from the present study it can be concluded that the *kachkal* RS can be grouped under low glycaemic food. RS and RSB contain 12.86 and 14.54 % of carbohydrates per 50g of serving size. International table of glycaemic index (GI) and glycaemic load (GL) values mentioned wide variety of foods with different GI values (Foster-Powell et al., 2002). A comparison of the test product with a standard bread sample

proved that the *kachkal* RS and bread prepared by incorporating it exhibits low glycaemic response which can be recommended for use in the diet of diabetic patients.

Considering the widespread consumption of breakfast foods and snacks in India, it is useful to have information regarding the glycaemic response of different foods. Identification of foods with lower glycaemic responses will have practical applications because such information will be useful for diabetic patients.

Table 23 Mean B-glucose level (mg/dl) of diabetic and non-diabetic persons

Time (min)	Bread standard (BS)		Resistant starch (RS)		Resistant starch bread (RSB)	
	NDP	DP	NDP	DP	NDP	DP
AOF	91	142	81	146	90	149
0	95	150	83	152	94	153
30	128	165	96	161	118	165
60	137	178	112	169	128	172
90	111	169	100	158	95	166
120	104	161	82	147	91	156

*AOF: After Overnight Fasting (NDP = non-diabetic persons; DP = diabetic persons

Table 24 Glycaemic index (GI) value of disease free subjects and diabetic persons

	GI of Disease free subjects	GI of Diabetic person
RS	55.87±3.50	91.76±4.07
RSB	80.77±2.91	104.53±3.72
BS	83.15±3.45	120.98±6.63

(RS= resistant starch; RSB = resistant starch bread; BS = bread standard)
(Results are mean of three replicates and ±SD)

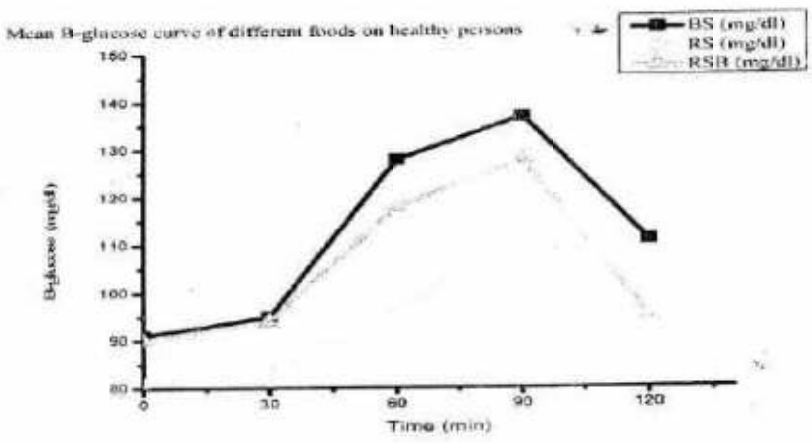


Fig. 13. Mean B-Glucose curve of different foods on healthy persons

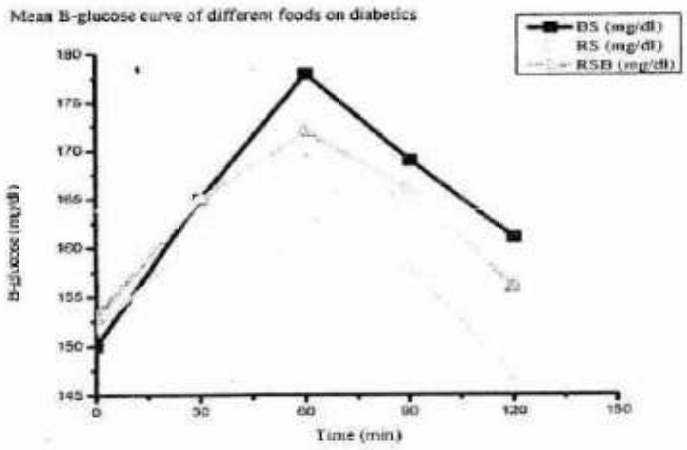


Fig. 14. Mean B-Glucose curve of different foods on diabetics

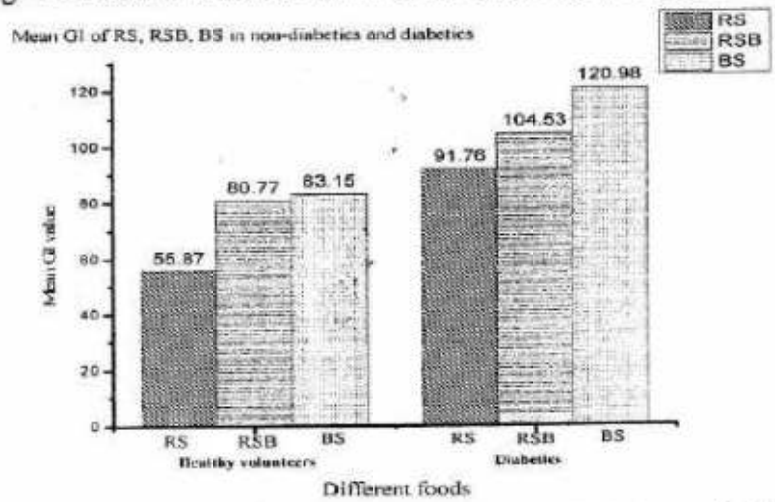


Fig. 15. Mean GI values of different foods on non-diabetics and diabetics

Objective 3: To develop Nutraceuticals from *kachkal* blossom

Study of biochemical composition of banana blossom

The flower of the banana plant is known as banana blossom or banana heart which is used traditionally in North Eastern parts of India and Assam. It is treated as cuisines in NE India and as vegetable in other part of the world as it has good nutritional value. Freshly harvested male flower of banana were collected from local vegetable market, washed under running tap water after removing 3-4 layers of outermost fibrous bracts followed by distilled water washing and wiped with tissue paper. The blossom was then sliced with a sharp knife and dried in a tray drier at 50^0C for 5-6 hours. The dried blossoms were then ground in a mechanical grinder to powdery form sieved and stored in air tight container for further analysis. Study on biochemical composition and nutritional aspects of the above sample were carried out following the standard procedures.

RESULTS

Table 25 Proximate compositions (g/100g) of the *Kachkal* blossom

Moisture content (% wb)	Ash	Protein	Fat	Crude fiber	Carbohydrates
89.98±3.97	1.59±0.76	2.64±0.09	1.68±0.06	5.29±0.96	88.77±2.89

Table 26 pH, titrable acidity, ascorbic acid, lignin, tannin and pectin content

pH	Tritable acidity (g/100g)	Accorbic acid (g/100g)	Ligning (mg/100g)	Tanin (mg/100g)	Pectin (g/100g)
5.035±1.06	1.873±0.06	3.3±0.06	1.429±0.04	6.825±1.03	0.281±0.08

Table 27 Starch, amylose, total soluble, reducing and non reducing sugar content (g/100g)

Starch	Amylose	Total soluble sugar	Reducing sugar	Non-reducing sugar
11.02±0.07	1.072±0.04	1.415±0.03	0.465±0.07	0.097±0.02

Table 28 Thiamine, total carotenoid, phenolics content and DPPH radical scavenging activity

Thiamine (mg/100g)	Total Carotenoid (g/100g)	Total Phenoilics (g/100g)	DPPH Radical scavenging activity(%SA)
0.080±0.01	0.0193±0.03	0.631±0.01	99.876±3.09

Table 29 The colour measurement

L	a	b
42.06±248	2.46±0.08	6.89±1.23

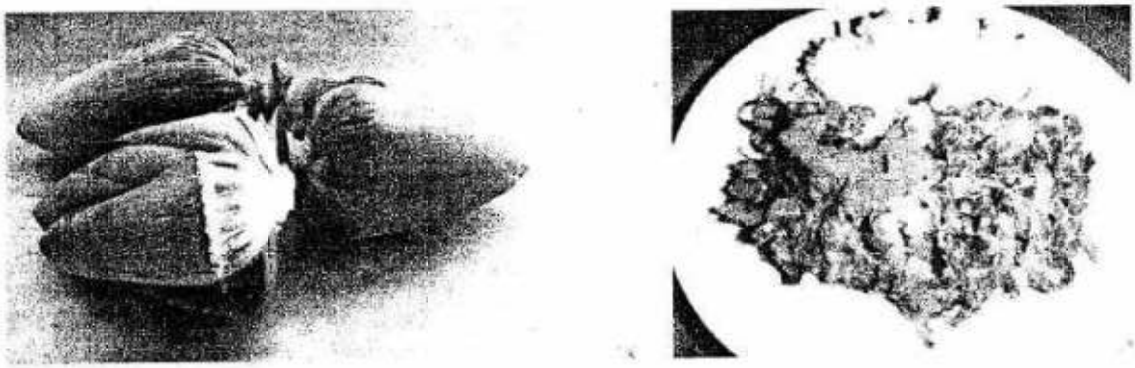


Fig. 16. *Kachkal* blossom

Preparation of Nutraceuticals and its biochemical composition study

The nutraceutical has been prepared from *kachkal* blossom which is used as traditional medicine in North East India. The blossom was dried to moisture content of 10% wet basis, grounded in a mechanical grinder, passed through 72 Mesh screen and the powder was mixed with the different concentration of turmeric powder. The sensory analysis of the prepared banana blossom nutraceutical was done and the final product which was accepted by the experts was taken for analysis. The concentration of banana blossom: turmeric was 18:1, 20:1 and 25:1. The biochemical compositions were studied following standard procedures.

Table 30 Proximate compositions (g/100g) of the *Kachkal* blossom

Sample	M. C (% wb)	Ash	Protein	Fat	Crude fiber	Carbohydrates
Nutraceutical (18:1)	10.32±0.87	2.09±0.08	2.09±0.82	0.98±0.05	4.87±0.94	89.95±3.92
Nutraceutical (20:1)	11.09±0.92	2.63±0.05	2.43±0.09	0.92±0.07	4.20±0.96	89.80±3.61
Nutraceutical (25:1)	10.65±0.75	2.87±0.04	2.13±0.27	0.76±0.09	5.21±0.88	89.00±2.97

Table 31 pH, titrable acidity, ascorbic acid, lignin, tannin and pectin content

Sample	pH	Tritable acidity (g/100g)	Accorbic acid (g/100g)	Ligning (mg/100g)	Tanin (mg/100g)	Pectin (g/100g)
Nutraceutical (18:1)	5.87±1.24	2.87±0.67	2.98±0.83	0.56±0.07	0.09±0.01	0.03±0.01
Nutraceutical (20:1)	5.27±0.97	2.29±0.38	2.43±0.61	0.34±0.03	0.06±0.03	0.01±0.01
Nutraceutical (25:1)	5.76±0.94	1.97±0.09	3.76±0.74	0.49±0.05	0.10±0.01	0.01±0.01

Table 31 Starch, amylose, total soluble, reducing and non reducing sugar content (g/100g)

Sample	Starch	Amylose	Total soluble sugar	Reducing sugar	Non-reducing sugar
Nutraceutical (18:1)	8.98±2.08	0.07±0.07	0.06±0.01	0.03±0.01	0.02±0.02
Nutraceutical (20:1)	8.63±1.76	0.12±0.06	0.02±0.03	0.02±0.01	0.12±0.06
Nutraceutical (25:1)	8.28±1.54	0.20±0.09	0.02±0.01	0.02±0.01	0.17±0.04

Table 33 Thiamine, vitamin A, total carotenoid, phenolics content and DPPH radical scavenging activity

Sample	Thiamine (mg/100g)	Total Carotenoids (g/100g)	Total Phenoilics (g/100g)	DPPH Radical scavenging activity(%SA)
Nutraceutical (18:1)	0.08±0.02	0.019±0.05	0.63±0.07	98.65±3.78
Nutraceutical (20:1)	0.12±0.08	0.078±0.08	0.97±0.06	99.18±3.98
Nutraceutical (25:1)	0.16±0.08	0.18±0.09	1.09±0.08	99.87±3.08

Table 34 The micronutrients content (µg/ml)

Sample	Na	Fe	Cu	Mn	Zn	Mg	Cd	Cr	Ca	Al	K	Ni
Nutraceutical (18:1)	50.37± 4.78	70.86± 4.99	15.61± 1.89	263.60± 2.67	24.01± 1.06	4410.05± 1.58	0.06± 0.02	3.09± 0.03	3711.00±1.89	28.36± 1.49	59345.00± 2.89	1.50± 0.07
Nutraceutical (20:1)	38.51± 3.59	72.77± 2.90	14.64± 2.07	259.55± 2.89	25.53± 0.94	4323.55± 2.67	0.04± 0.04	3.34± 0.06	3922.00±2.08	22.69± 1.78	55345.00± 3.08	1.52± 0.02
Nutraceutical (25:1)	41.38± 4.08	73.23± 4.85	13.26± 1.37	271.35± 1.96	27.36± 0.98	4459.05± 1.90	0.04± 0.02	3.25± 0.03	3704.50±2.04	23.61± 1.04	56845.00± 2.86	1.47± 0.02

Test for biogenic amines, phytates and sugars in nutraceutical:

Test for the presence of biogenic amines:

The test for the presence of biogenic amines in the nutraceutical developed was done according to the method of Nishikawa et al. 2012. The detection was carried out for the presence of histamine, putrescine, spermine, spermidine, agmatine, cadaverine, trimethylamine, *O*-phthalaldehyde and 4-(1-py-rene) butyric acid N-hydroxysuccinimide ester (PSE). No biogenic amines were detected in the functional food developed.

(Ref: Nishikawa H., Tabata T. and Kitani S. . Simple Detection Method of Biogenic Amines in Decomposed Fish by Intramolecular Excimer Fluorescence. *Food and Nutrition Sciences*, 2012, 3, 1020-1026.)

Estimation of phytates:

Phytate was determined using anion exchange method following Ma et al. (2005). The content of phytates in the nutraceutical developed was found to be 0.048%. This was well within the acceptable range (Nititham et al, 2004; John et al., 2000) and hence the product can be considered as safe in terms of phytate content.

Ref: Ma G. Jin Y, Piao J. Kok F. Guusje B & Jacobsen E (2005). Phytate. Calcium, Iron, and Zinc contents and their molar ratios in foods commonly consumed in China. J Agric Food Chem 53: 10285-10290.

Nititham S, Komindr S, Nichachotsalid A. Phytate and fiber content in Thai fruits commonly consumed by diabetic patients. J Med Assoc Thai. 2004 87(12):1444-1446.

John N.A. Lott1, Irene Ockenden, Victor Raboy and Graeme D. Batten (2000). Phytic acid and phosphorus in crop seeds and fruits: a global estimate. Seed Science Research 10: 11-33.

Test for the presence of oligosaccharides:

Oligosaccharides are generally defined as carbohydrates from 2 to 20 monomeric units long. Oligosaccharides have been dietary staples since antiquity but have received much less attention than other carbohydrates such as simple sugars or dietary fiber. Recently, interest in oligosaccharides has increased not only because of properties that include sweetening ability and fat replacement, but also because of resistance to digestion in the upper gastrointestinal tract and fermentation in the large bowel. Thus, some oligosaccharides have functional effects similar to soluble dietary fiber such as enhancement of a healthy gastrointestinal tract, improvement of glucose control, and modulation of the metabolism of triglycerides. These oligosaccharides are the nondigestible oligosaccharides. These compounds are easily incorporated into processed foods and hold much promise as functional ingredients in nutraceutical products (Roberfroid and Slavin, 2000)

Estimation of simple sugars and oligosaccharides by HPLC

The samples were first degassed for 15 minutes in an ultrasonic bath (RZ 08892-26, Cole Parmer, USA). They were then passed through 0.22 µm pore size organic syringe filter of 30 mm diameter (SF2-1, Himedia) and then through a Sep-Pak® C18 cartridge which had previously been activated with 10 ml of methanol, followed by 10 ml of Type I water. Analytical conditions: The analysis of carbohydrates was carried out in a HPLC system (Ultimate 3000 Dionex, Germany) equipped with an autosampler. The injection volume was 20 µl and the detector used was Shodex RI-101 Refractive Index Detector with Plus polarity at 512 µRIU recorder range and 500 µRIU/V integrator range. The column used was a Hamilton HC-75 Ca++ column. The mobile phase used was Type I water with an isocratic flow rate of 0.6 ml min^{-1} at a temperature of 80^0C.

Results of sugars and oligosaccharides:

Sugar/Oligosaccharide	Concentration (in ppm)
Glucose	2675.88±17.08
Fructose	1318.84±32.51
Sucrose	234.98±14.51
Raffinose	230.16±22.50
Maltose	145.98±13.98
Trehalose	0.0
Arabinose	423.80±9.01
Xylose	0.0
Rhamnose	19.45±1.97
Melibiose	0.0

Sugars and oligosaccharides tested and detected were of very useful.

(**Ref:** Roberfroid M, Slavin J. (2000). Nondigestible oligosaccharides. Crit Rev Food Sci Nutr. 40(6):461-80.)

Objective 4: To use banana powder as a substitute in different foods and developing value added products from *Kachkal*

Development of value added products from *Kachkal*

Harvested green matured *kachkal* was cleaned in a bowl containing water and washed to remove dirt and possible chemical residue. Peeled the fruit manually with the aid of stainless kitchen knife and keep in a bowl containing water, and allow remaining in water until the peeling process is complete to prevent browning of the resultant flour. Slice the pulps longitudinally to about 5 mm thickness with stainless steel knife to further prevent discoloration and to enhance dehydration. The slices were dried in a try dryer at 50°C for about 6 hours. Milled the resultant dried pulp slices and sieved through 72 Mesh screen to obtain a fine powder or flour. Banana flour can be used in many different ways wholly or partially for making bread, cakes, noodles, extruded products etc. Therefore, in order to achieve the objectives various value added products such as *kachkal* beer, *kachkal* chips and noodles were prepared following standard procedure.

Fig. 17. Flour prepared from *kachkal*

i) Preparation of *kachkal* beer

Unripe matured *kachkal*

↓

Cleaning, washing and peeling

↓

Cutting into slices of 0.5-1mm thickness

↓

Drying at mechanical dryer (temp 50^0 C for 6 hours)

↓

Boiling

↓

Cooling to lukewarm

↓

Addition of starter culture (5g per 200 g of sample)

↓

Mixing thoroughly

↓

Allow fermentation at 30^0c for 9, 6 and 3 days

↓

Taken for analysis

Fig. 18. Fermented *Kachkal* beer

ii) Preparation of *kachkal* chips

Chips or crisps are hard, brittle fried products abruptly releasing energy that gives rise to characteristic sound effects when they are bitten. They are the most popular post harvest processed products of cooking bananas. Deep-fried plantain and banana chips may potentially be used in intervention programmes to combat micronutrient deficiencies, by virtue of their iron, zinc and total carotenoid content. The fat content of chips may increase the carotenoid bioavailability though, may also reduce product shelf life due to lipid oxidation Min and production and marketing of plantain chips in

Cooking bananas chips are produced by deep-frying green unripe pulp slices. Washed the fruit, peel neatly and slice using a slicer (kitchen wonder) into disc of approximately 2mm thickness. The fruit was salted before or after slicing and then deep-fry in vegetable oil until crisp. Drained the chips and allowed to cool at room temperature, then packed in a polyethylene bag and sealed and taken for analysis. The shelf life of plantain chips is greatly reduced when exposed to air and light.

Unripe matured *kachkal*

↓

Cleaning, washing and peeling

↓

Blanching at 85^0 C for 3-4 minutes

↓

Cutting into 1mm thickness slices

↓

Addition of salt and turmeric powder

↓

Frying in oil

↓

Packing and taken for analysis

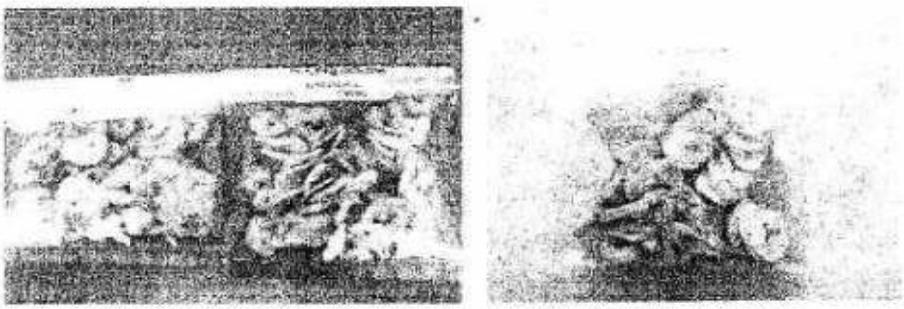


Fig. 19. *Kachkal* Chips

iii) Preparation of *kachkal* noodles

Kachkal flour is an ideal fibre ingredient in noodles as the source of fibre as well as it can be a good source of combat food due to high nutritional value of *kachkal*. *Kachkal* noodle *was* prepared by incorporating Maida flour with *kachkal* flour in 1:1 ratio in noodle making machine (LB ITALIA SRL, FLORIDA 30). The prepared noodle was dried, packed and taken for analysis.

Maida flour: Kachkal flour (1:1)

↓

Mixing with water in machine chamber (150ml water for 500g flour)

↓

Mixing for 10-15 minutes

↓

Switch on the extruder and collect the noodle strands

↓

Drying and packaging and taken for analysis

Fig. 12. *Kachkal* Noodles

RESULTS

Table 35 Proximate compositions (g/100g) of the value added products from *kachkal*

Sample	M. C (% wb)	pH	Ash	Protein	Fat	Crude fiber	Carbohydrates
Rice beer (3 Days)	-	5.15±0.46	0.39±0.02	0.284±0.02	0.290±0.04	0.492±0.02	98.554±1.30
Rice beer (6 Days)	-	4.47±0.45	0.482±0.01	0.519±0.04	1.30±0.36	0.333±0.03	97.366±1.30
Rice beer (9 Days)	-	4.90±0.47	0.327±0.02	0.878±0.04	2.9±0.40	0.497±0.02	95.398±1.01
Kachkal beer (3 Days)	-	4.02±0.28	2.071±0.19	0.884±0.03	0.083±0.46	0.26±0.01	96.703±0.65
Kachkal beer (6 Days)	-	3.56±0.13	2.305±0.13	1.23±0.17	0.466±0.02	0.623±0.04	95.376±0.99
Kachkal beer (9 Days)	-	3.24±0.49	2.213±0.22	0.928±0.40	1.31±0.29	0.347±0.03	95.77±0.94
Kachkal: Rice beer (3 Days)	-	3.57±0.32	2.314±0.22	1.552±0.16	0.27±0.04	0.206±0.01	95.658±1.41
Kachkal : Rice beer (6 Days)	-	3.07±0.28	1.651±0.13	1.218±0.34	0.755±0.11	0.325±0.03	96.051±0.77
Kachkal: Rice beer (9 Days)	-	3.23±0.15	2.164±0.36	1.186±0.21	0.03±0.50	0.211±0.03	96.409±0.67
Kachkal chips	10.062±0.96	5.65±0.32	1.1004±0.24	0.534±0.06	0.19±0.02	0.136±0.04	98.036±0.22
Kachkal Noodle	13.236±1.18	5.43±0.34	1.0069±0.12	0.574±0.03	1.17±0.47	0.994±0.31	96.255±0.36

Microbial Count of fermented *kachkal* beer

The Colony Forming Unit (CFU) count of the fermented samples was done for yeast, moulds, *Lactobacillus* species and general aerobes. The media used were PDA for yeast and moulds, MRS for *Lactobacillus* species and PCA for general aerobes. The samples were serially diluted and the volume of inoculums used was 100 micro litres.

Table 36 Microbial Count of fermented *kachkal* beer

***Kachkal* Beer**				
Samples	0 Hour	3 Days	6 Days	9 days
General Aerobes	$1.63X10^4$	$1.46X10^7$	$1.5X10^5$	$1.01X10^7$
Lactobacillus Species	$2X10^4$	$1.1X10^6$	$2.4X10^5$	$2.86X10^7$
Yeast	$6.3X10^3$	$3.79X10^7$	$1.03X10^7$	$3.56X10^7$
Mould	$8.7X10^4$	$5.32X10^4$	0	0

Table 37 Microbial Count of fermented *kachkal*: Rice beer

***Kachkal*: Rice Beer (1:1)**				
Samples	0 Hour	3 Days	6 Days	9 days
General Aerobes	$1.63X10^4$	$3.33X10^7$	$0.8X10^7$	$0.9X10^6$
Lactobacillus Species	$2X10^4$	$1.96X10^7$	$1.06X10^7$	$0.7X10^6$
Yeast	$6.3X10^3$	$1.86X10^8$	$2.26X10^7$	$7.9X10^6$
Mould	$8.7X10^4$	4.1X104	0	0

Table 38 Microbial Count of fermented *Rice* beer

Rice Beer				
Samples	0 Hour	3 Days	6 Days	9 days
General Aerobes	$1.63X10^4$	$0.2X10^6$	$1.5X10^6$	$1.3X10^6$
Lactobacillus Species	$2X10^4$	$0.1X10^5$	$1.2X10^6$	$0.1X10^6$
Yeast	$6.3X10^3$	$0.2X10^5$	$2.7X10^6$	$4.9X10^6$
Mould	$8.7X10^4$	$3.87X10^4$	0	0

Work done over and above the approved objectives

(i) Study on Thin-layer Drying kinetics of *Kachkal* pulp

As *kachkal* being unique variety of culinary banana found only in Assam the main challenge has been to preserve it with desired moisture content as it is responsible for most of the deteriorative microbial reactions, for safe storage life and rehydration ratio during dehydration process. Generally, in fruits and vegetables moisture removal process is controlled by diffusion mechanism. Comprising drying process where moisture is removed from the food because of concomitant heat transfer from the surrounding to the foods and mass transfer from the food is provided them with large shelf life because of decrease of water activity achieved in the product at the end of the process (Reis, Lenzi, & Masson, 2012), drying also affects food sensory properties, providing unique taste, colour and texture (Ruiz-Lopez, Martinez-Sanchez, Cobos-Vivaldo, & Herman-Lara, 2008).

Though *kachkal* being the only culinary banana found in the entire Assam and North East India, there has been no any information available in literature on drying characteristics of *kachkal*. Proper investigation is prerequisite to improve the efficiency of the drying process and drying system. This study therefore investigates the thin layer drying characteristics of *kachkal* slices in a convective tray dryer and fit the experimental data to nine popular drying models to identify the best fit model and to obtain the diffusivity at different temperatures.

Materials and methods

Sample preparation

Fresh *kachkal* sample at matured edible stage were taken for the study. They were thoroughly cleaned before peeling to remove any dirt or dust particles attached to the surface. The initial moisture content of *kachkal* slices was measured by convectional hot air oven drying method described by Rangana (2008). The fresh and dry weight were measured with electronic weighing balance (CPA225D, Sartorius AG, Germany) having 0.001g accuracy.

Experimental procedure

The sorted cleaned *kachkal* were peeled and sliced into 8 mm thickness manually with stainless steel knife. The uniform thickness of *kachkal* sample 8 mm (±0.01 mm) was prepared using vernier calliper having the least count of 0.01 mm. A laboratory scale convective tray dryer (Model No.IK-112, Make IKON Instruments, Delhi) was set at desired temperature of 40, 50, 60 and 70^0 C by circulating hot air through blower fan. The samples were placed in stainless steel trays in single layers and drying of samples was carried out at fixed temperature of hot air. Initial weight of trays and the samples were noted and trays were

placed inside the drying chamber after steady state condition was achieved. *Kachkal* sample was loaded into pre-heated drying chamber and the sample trays were taken out for weighing at every 30 min interval till constant weight was achieved. Each experiment was replicated thrice and average values were taken for analysis.

Drying kinetics

The most frequently used model for thin layer drying is the lumped parameter type such as the Newton Equation (Tunde-Akintunde & Afon, 2010; Kingsly et al., 2007). As drying proceeds, the moisture content of the material decreases and the mechanism of drying changes, which is controlled by liquid diffusion mechanism described by Fick's second law. The solution of the Fick's Equation, with the assumptions of diffusion based moisture migration, negligible shrinkage, constant diffusion coefficients and temperature, is simplified to get the single exponential model (Lewis, 1921) as:

$$MR = Mt - Me/Mi - Me \quad (3)$$

Where, MR = Moisture Ratio

M_i = initial moisture content

M_t = moisture content at time t

M_e = equilibrium moisture content

Since equilibrium moisture content is zero, moisture ratio can be reduced to $MR = M_t / M_i$ (Jena & Das, 2007).

Drying curves were obtained from the experimental data using nine different Equations (Table 33). In mathematical modeling, thin layer drying Equations listed in Table 33 were tested to select the best model for describing the drying curve of *kachkal* slices. Moisture ratio was used to interpret the drying kinetics by fitting some of the selected thin layer drying models listed in Table 33.

Table 39 Mathematical models used for thin-layer drying of *kachkal* peel

Model	Mathematical Equation	References
Lewis	$MR = \exp(-kt)$	Doymaz (2005)
Page	$MR = \exp(-kt^n)$	Page (1949)
Modified Page	$MR = \exp(-kt)^n$	Yaldiz et al. (2001)
Henderson & Pabis	$MR = a \exp(-kt)$	Doymaz (2004)
Logarithmic	$MR = a \exp(-kt) + c$	Togrul & Pehlivan (2002)
Two-Term Model	$MR = a \exp(-k_0t) + b \exp(-k_1t)$	Rahman et al. (1998)
Approximation of Diffusion	$MR = a \exp(-kt) + (1-a) \exp(-kat)$	Lahsasni et al. (2004)

Wang & Singh	$MR = 1 + at + bt^2$	Wang & Singh (1978)
Modified Page Equation II	$MR = \exp[-c\,(t/L_c^2)^n]$	Doymaz (2005)

The design of the experiment was done by using statistical software Origin 8.5. The reduced chi-square value (χ^2) and adjusted coefficient of determination value (R^2) were used as the primary criteria to select the best Equation to account for variation in the drying curve of the dried samples. The reduced chi-square value (χ^2) is the mean square of the deviations between the experimental and calculated values for the models and used to determine the goodness of fit. For quality fit, R^2 values should be higher and χ^2 values should be lower (Goyal, Kingsly, Manikantan, & Ilyas 2006).

Effective moisture diffusivity

Shrinkage of agricultural products during drying may have a significant effect on the mass diffusivity and the moisture removal rate and it is necessary to take into account the effect of shrinkage. The drying material is considered as a thin slab of thickness L = 2b at uniform initial temperature T_0 and moisture content M_0 because the two sides are exposed to an air flow at temperature T_a and relative Humidity (RH). Assuming uniform initial content and negligible external resistance the Fick's diffusion Equation for object with slab geometry was used for calculation of effective moisture diffusivity (Crank, 1975).

By using Fick's second law and considering following assumptions, proposed Eq. (3) for the effective moisture diffusivity for an infinite slab (Crank, 1975):

(i) Moisture is initially distributed uniformly throughout the mass of a sample.

(ii) Mass transfer is symmetric with respect to the centre.

(iii) Surface moisture content of the sample instantaneously reaches equilibrium with the condition of surrounding air.

(iv) Resistance to the mass transfer at the surface is negligible compared to internal resistance of the sample.

(v) Mass transfer is by diffusion only.

(vi) Diffusion coefficient is constant and shrinkage is negligible.

$$MR = M - Me/Mo - Me \qquad (4)$$

$$= 8/\pi^2 \{\exp[(2n-1)^2 \pi^2/4\, Deff/(T_1/L^2)]\} \qquad (5)$$

Where MR is moisture ratio, M is the moisture content at any time (% wet basis), M_0 is the initial moisture content (% wet basis), n = 1, 2, 3, . . the number of terms taken into consideration, D_{eff} is the effective moisture diffusivity (m^2/s), L is the sample thickness (mm) and T is the drying time (min). The moisture diffusivity was estimated by plotting the natural logarithm of moisture ratio (ln MR) with respect to drying time (t) at various temperatures. Linear regression analysis was used to obtain values of diffusion coefficients for different drying conditions.

Energy of activation

The energy of activation was calculated by using an Arrhenius type Equation (Akpinar et al., 2003)

$$Deff = Do\ exp[-Ea/R(T - 273.15)] \quad (6)$$

Where D_0 is the Arrhenius factor, E_a is the activation energy (kJ/g mol), R is the ideal gas constant (8.314 J/g mol K) and T is the drying temperature. The above Equation can be written as

$$ln\ (Deff) = Ln\ (Do)[-Ea/R(T - 273.15)] \quad (7)$$

The activation energy can be obtained from the slope of the Arrhenius plot, ln (D_{eff}) versus $1/T_{abs}$, from Equation (4) a plot of ln (D_{eff}) versus $1/T_{abs}$ gives a straight slope of k

$$K = Ea/R \quad (8)$$

Results

Drying characteristics

The thin-layer drying of *kachkal* pulp slices exhibited falling rate period. The slice thickness of the pulp was 8 mm and the initial moisture content was 87.80 % (wet basis) which was reduced to 9.42 – 11.94 %. The change in moisture content elapsed time of drying was studied at different temperatures of 40, 50, 60 and 70^0 C. The drying rate of sample was calculated from the drying data by estimating the change in moisture content at specific time interval. The moisture content data was expressed in % wet basis. The value of moisture ratio was obtained using Equation 4.

Fig. 20 shows the relation between moisture ratio and drying time for all the four drying temperature. As the drying process progressed, it was observed that the moisture ratio decreased non-linearly with increase in drying time. It can be observed from the Fig.20 that sample at 40^0 C took nearly 20 hours of drying time to reduce moisture content of the sample

from 87.80 % to 9.42 – 11.94 %. The result showed with increase in drying temperature the drying time decreases. As the temperature was increased to 70^0 C the same thickness of slice took only 5 hours to reduce the same moisture content. Therefore, it can be observed from the Fig. 20 that air drying temperature has significant effect on drying time.

It is observed that all drying process occurred in falling rate period (Fig. 20) and during this period; the drying process of *kachkal* slices was mainly controlled by diffusion mechanism. The moisture ratio at each drying air temperature rapidly dropped at the initial state and then it gradually reached an equilibrium state. As surface moisture of sample reduced, removal of moisture from inside the material became slower thereby requiring more energy to detach water molecules from the solid matrix.

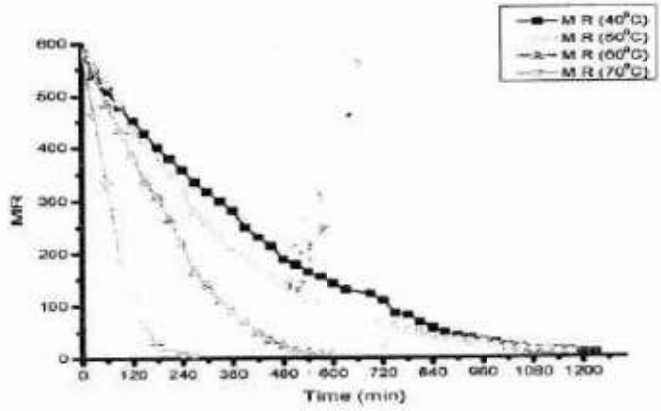


Fig. 20. Moisture Ratio vs drying time of *kachkal* pulp slices

Mathematical modeling for fitting drying curves

The moisture content data obtained by different air drying temperature was converted to moisture ratio and were fitted to different thin-layer drying models which are presented in Table 34. The models were evaluated on the basis of coefficient of determination (R^2) and the reduced chi-square (χ^2). The selection of best model to describe the drying behaviour of *kachkal* slices was based on the highest R^2 and lowest χ^2 values. The drying model constants and coefficients from the results of statistical analysis undertaken by regression of all models are given in Table 34. The average value of χ^2 varied from 2.589×10^{-4} to 3.897×10^{-3} and value of R^2 varied between 0.83 to 0.99. Consequently from Table 34, it was clear that modified Page model obtained least χ^2 (2.58×10^{-4}) and highest R^2 (0.99) values. This indicates that modified Page model gave a better correlation between M.R and drying time than other models fitted. Therefore, it is considered that modified Page model was more precise to describe thin-layer drying of *kachkal* pulp slices. The experimental and predicted

moisture ratio (MR) values with drying time of different drying models are shown in Fig. 21 to Fig. 29

Table 40 Values of model constants for thin-layer drying of *kachkal* peel

Drying temperature 40° C			
Model name	**Coefficients and constants**	**□²**	**R²**
Lewis/Newton	k = 0.0029	3.7×10^{-2}	0.9640
Page			0.9063
Modified Page	**k = 0.0028, n =1.4390**	**2.236×10^{-4}**	**0.9986**
Henderson & Pabis	a =1.1060, k = 0.0032	2.61×10^{-2}	0.9748
Logarithmic	a = 1.2293, c = -0.1735, k = 0.0022	8.659×10^{-4}	0.9917
Two-Term	a = 0.5530, b = 0.5530,m = 0.0032, n = 0.0032	2.842×10^{-2}	0.973
Approximation of Diffusion	a = 5.47×10^{-8}, k = -53798.89, m =2.212	4.0×10^{-3}	0.9615
Wang & Singh	a = -0.0021 b = 1.175×10^{-6}	3.431×10^{-4}	0.9967
Modified Page Equation II	c = 0.0146, m = -4.3223, n = 1.4354	2.815×10^{-4}	0.9947
Drying temperature 50° C			
Model name	**Coefficients and constants**	**□²**	**R²**
Lewis/Newton	k = 0.0051	5.95×10^{-2}	0.9489
Page			0.9248
Modified Page	**k = 0.0049, n = 1.5612**	**2.654×10^{-4}**	**0.9982**
Henderson & Pabis	a = 1.1020, k = 0.0056	4.9×10^{-2}	0.9580
Logarithmic	a = 1.3291, c = -0.2825, k =0.0034	1.675×10^{-2}	0.9855
Two-Term	a = 0.5510, b = 0.5510, m = 0.0056, n = 0.0056	5.6×10^{-2}	0.9520
Approximation of Diffusion	a = -0.2020, k = 0.0304, m= 2.2683	3.8×10^{-3}	0.9667
Wang & Singh	a = -0.0037 , b = 3.421×10^{-6} ,	9.929×10^{-4}	0.9914
Modified Page Equation II	c = 0.0049, m = -2.5886, n = 1.5567	7.10×10^{-4}	0.9939
Drying temperature 60° C			
Model name	**Coefficients and constants**	**□²**	**R²**
Lewis/Newton	k = 0.0053	7.30×10^{-2}	0.9387
Page			0.9500
Modified Page	**k = 0.0051, n = 1.5781**	**2.657×10^{-4}**	**0.9966**
Henderson & Pabis	a = 1.1006, k = 0.0059	6.354×10^{-2}	0.947
Logarithmic	a = 1.4944, c =-0.45974, k = 0.0029	1.7×10^{-2}	0.9850
Two-Term	a = 0.5502, b = 0.8761, m = 0.0059, n = 0.0453	7.31×10^{-4}	0.9382
Approximation of Diffusion	a = 1.262×10^{-8}, k = -426903.57, m = 2.1312	8.422×10^{-2}	0.9292
Wang & Singh	a = -0.0037, b = 3.281×10^{-6}	1.38×10^{-3}	0.9885
Modified Page Equation II	c = 0.0051, m = -2.6155, n = 1.5730	1.7×10^{-2}	0.9856
Drying temperature 70° C			
Model name	**Coefficients and constants**	**□²**	**R²**
Lewis/Newton	k = 0.0136	9.305×10^{-4}	0.9919

Page			0.8068
Modified Page	**k = 0.0131, n = 1.1895**	**2.642×10^{-4}**	**0.9977**
Henderson & Pabis	a = 1.0246, k = 0.0138	9.495×10^{-4}	0.9917
Logarithmic	a = 1.0659, c = -0.0553, k = 0.0120	4.435×10^{-4}	0.9961
Two-Term	a = 0.5123, b = 0.5123, m = 0.0138, n = 0.0138	1.272×10^{-3}	0.9890
Approximation of Diffusion	a = -0.1302, k = 0.1165, m = 3.0879	6.404×10^{-4}	0.9944
Wang & Singh	a = -0.0093, b = 2.159×10^{-5}	8.991×10^{-4}	0.9922
Modified Page Equation II	c = 0.0032, m = 0.7797, n = 1.1841	3.026×10^{-4}	0.9973

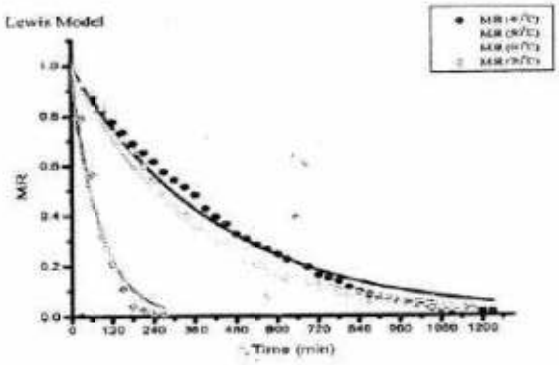


Fig. 21. Moisture ratio vs drying time in Lewis model

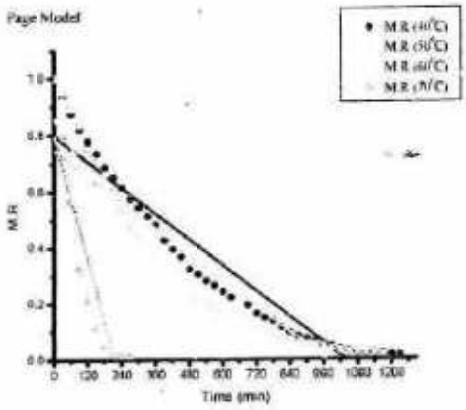


Fig. 22. Moisture ratio vs drying time in Page model

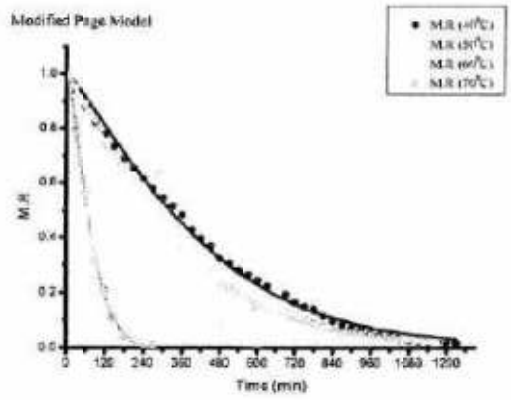


Fig. 23. Moisture ratio vs drying time in modified Page model

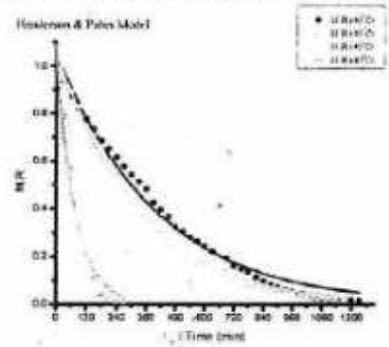


Fig. 24. Moisture ratio vs drying time in Henderson & Pabis model

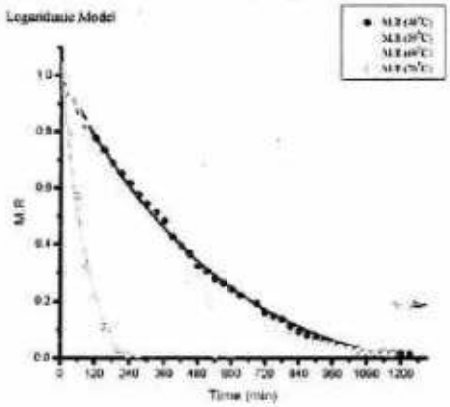


Fig. 25. Moisture ratio vs drying time in Logarithmic model

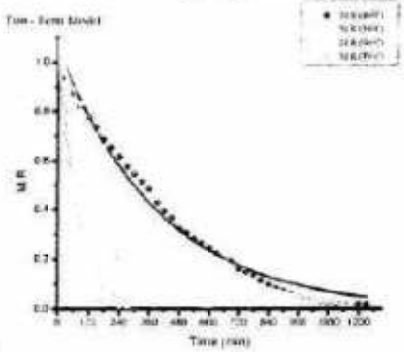


Fig. 26. Moisture ratio vs drying time in Two-Term model

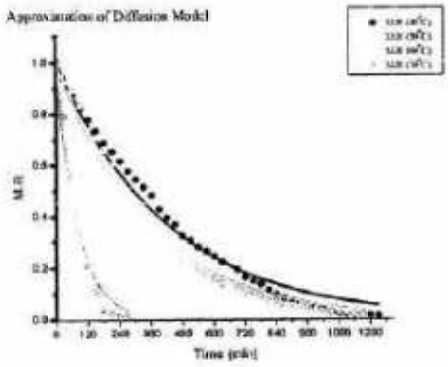


Fig. 27. Moisture ratio vs drying time in Approximation of Diffusion model

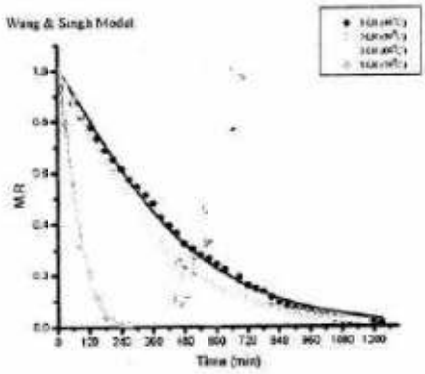


Fig. 28. Moisture ratio vs drying time in Wang & Singh model

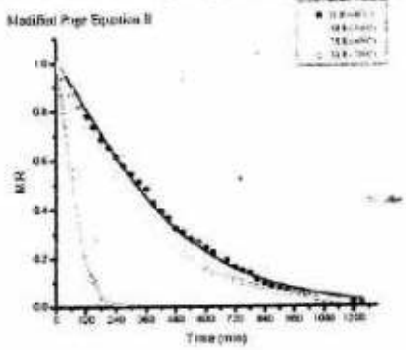


Fig. 29. Moisture ratio vs drying time in modified Page Equation II

Effective moisture diffusivity

An analysis of falling rate period was carried out to understand the drying kinetics by determination of effective moisture diffusivity (D_{eff}). To determine the effective moisture diffusivity, slope method was used (Equation 6). The relationship between logarithmic of MR with respect to drying time has been shown in Fig. 30. The calculated values of D_{eff} for all the four temperatures are shown in Table 35. The highest diffusivity (D_{eff}) value of 8.38×10^{-9} m^2/s was observed at 70^0 C and the lowest D_{eff} value of 5.6×10^{-9} m^2/s was observed at 40^0 C.

Table 41 Effective moisture diffusivity, activation energy of *kachkal* peel

Temp (^{0}C)	D_{eff} (m^2/s)	1/Tabs
40	2.05×10^{-9}	0.003195
50	3.85×10^{-9}	0.003096
60	4.05×10^{-9}	0.003003
70	7.80×10^{-9}	0.002915

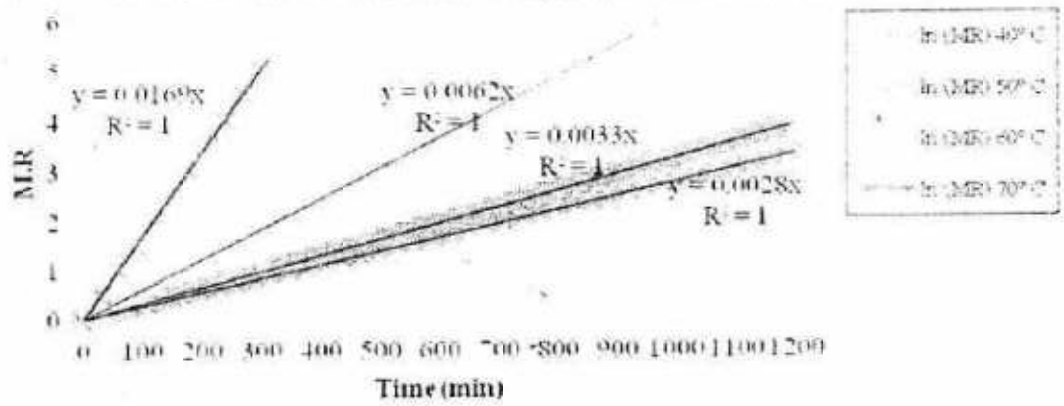


Fig. 30. Variation in ln (MR) with time (T) of *kachkal* pulp

Activation energy

Activation energy is the minimum energy required to initiate moisture diffusion from the food products. The logarithmic of effective moisture diffusivity values (D_{eff}) obtained at different temperatures plotted against the corresponding absolute temperature [1/(T+273)] to obtain the constants of Arrhenius Equation (Table 36; Fig. 31). The plots in Fig 24 were found to be the straight line in the temperature range investigated indicating the Arrhenius Equation dependence. The slope of the straight line (Fig. 31) described by the Arrhenius Equation the activation energy was found to be 53.279 kJ/mol (Table 24) which was calculated by using Equation 7.

Table 42 Progressive parameters of Arrhenius relationship between affective diffusivity and absolute temperature

Regression parameters	Values
Slope (E_a/R)	327.48
Activation energy (E_a)	27.22 kJ/mol
Intercept (ln D_0)	8.9×10^{-2}
R^2	0.9998

Fig. 31. Logarithmic of effective moisture diffusivity vs function of the inverse of absolute temperature in K

(ii) Study on Thin-layer Drying kinetics of *Kachkal* pulp

The peel of *kachkal* represents 40 % of the total weight of fresh banana which has always been underutilized and discarded as waste. Like *kachkal* pulp counterpart, its peel can also be potentially be used in new products with standardized composition for various industrial and domestic uses Peels are the major by-products of all fruits and vegetables obtained during processing; some studies show that these are good sources of polyphenols, carotenoids and other bioactive compounds which possess various beneficial effects on human health. Many researchers have proved that banana peels are good source of nutrients can be used for medicinal purpose (Rodriguez-De-Sotillo, et al., 1994; Zhang, et al., 2005). Keeping in view the bright side of *kachkal* peel, effort was made to investigate thin layer convective drying behaviour of *kachkal* peel at different drying air temperatures to select the suitable mathematical model for describing the drying kinetics of *kachkal* peel and also to calculate the effective diffusivity and activation energy by adopting appropriate mathematical model to the experimental data. The drying experiment of *kachkal* peel was conducted similarly as drying of *kachkal* pulp.

Results

Drying characteristics

Kachkal peel paste approximately 4 mm thickness was dried in a convective dryer at 40, 50. 60 and 70^0 C in a thin-layer. The initial moisture content of the sample was 85.47% wet basis which was reduced to 7.02 – 10.59 %. The decrease in weight was measured at each 30 min interval. The moisture ratio of *kachkal* peel paste during the thin-layer drying is shown in Fig. 32. At 40^0 C it took approximately 15 hours to reach the safe final moisture content and the same sample required only 3 hours at 70^0 C to reach the same moisture value.

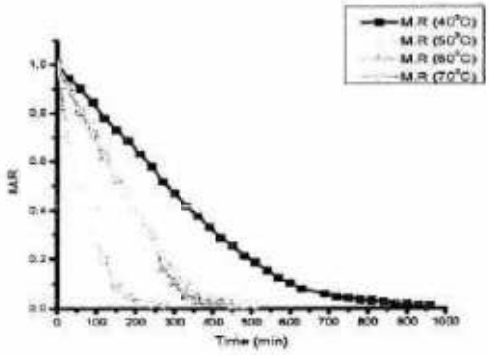


Fig. 32. Moisture ratio vs drying time of *kachkal* peel

Mathematical modelling for fitting drying curves

The drying model constants and coefficients from the results of statistical analysis undertaken by regression of all models are given in Table 37. The average value of χ^2 varied from 2.23×10^{-4} to 8.44×10^{-2} and value of R^2 varied between 0.80 to 0.99, for all mentioned thin-layer drying models, R^2 values were greater than 0.924 except Page model, which showed the minimum R^2 value of 0.8069. Therefore, from Table 37 it can be concluded that modified Page model was found to be the best fitted model with least χ^2 value of 2.236×10^{-4} and highest R^2 value of 0.99, thus modified Page model was selected as suitable model to represent the thin-layer drying behaviour of *kachkal* peel. The coefficients of all the models applied and fitted are given in Table 37.

Table 43 Values of model constants for thin-layer drying of *kachkal* peel

Drying temperature 40° C			
Model name	**Coefficients and constants**	χ^2	R^2
Lewis/Newton	k = 0.0029	3.7×10^{-2}	0.9640
Page			0.9063
Modified Page	**k = 0.0028, n =1.4390**	**2.236×10^{-4}**	**0.9986**
Henderson & Pabis	a =1.1060, k = 0.0032	2.61×10^{-2}	0.9748
Logarithmic	a = 1.2293, c = -0.1735, k = 0.0022	8.659×10^{-4}	0.9917
Two-Term	a = 0.5530, b = 0.5530,m = 0.0032, n = 0.0032	2.842×10^{-2}	0.973
Approximation of Diffusion	a = 5.47×10^{-8}, k = -53798.89, m =2.212	4.0×10^{-3}	0.9615
Wang & Singh	a = -0.0021 b = 1.175×10^{-6}	3.431×10^{-4}	0.9967
Modified Page Equation II	c = 0.0146, m = -4.3223, n = 1.4354	2.815×10^{-4}	0.9947
Drying temperature 50° C			
Model name	**Coefficients and constants**	χ^2	R^2
Lewis/Newton	k = 0.0051	5.95×10^{-2}	0.9489
Page			0.9248
Modified Page	**k = 0.0049, n = 1.5612**	**2.654×10^{-4}**	**0.9982**

Henderson & Pabis	a = 1.1020, k = 0.0056	4.9×10^{-2}	0.9580
Logarithmic	a = 1.3291, c = -0.2825, k =0.0034	1.675×10^{-2}	0.9855
Two-Term	a = 0.5510, b = 0.5510, m = 0.0056, n = 0.0056	5.6×10^{-2}	0.9520
Approximation of Diffusion	a = -0.2020, k = 0.0304, m= 2.2683	3.8×10^{-3}	0.9667
Wang & Singh	a = -0.0037 , b = 3.421×10^{-6} ,	9.929×10^{-4}	0.9914
Modified Page Equation II	c = 0.0049, m = -2.5886, n = 1.5567	7.10×10^{-4}	0.9939
Drying temperature 60^0 C			
Model name	**Coefficients and constants**	**□²**	**R^2**
Lewis/Newton	k = 0.0053	7.30×10^{-2}	0.9387
Page			0.9500
Modified Page	**k = 0.0051, n = 1.5781**	**2.657×10^{-4}**	**0.9966**
Henderson & Pabis	a = 1.1006, k = 0.0059	6.354×10^{-3}	0.947
Logarithmic	a = 1.4944, c =-0.45974, k = 0.0029	1.7×10^{-2}	0.9850
Two-Term	a = 0.5502, b = 0.8761, m = 0.0059, n = 0.0453	7.31×10^{-4}	0.9382
Approximation of Diffusion	a = 1.262×10^{-8}, k = -426903.57, m = 2.1312	8.422×10^{-2}	0.9292
Wang & Singh	a = -0.0037, b = 3.281×10^{-6}	1.38×10^{-3}	0.9885
Modified Page Equation II	c = 0.0051, m = -2.6155, n = 1.5730	1.7×10^{-2}	0.9856
Drying temperature 70^0 C			
Model name	**Coefficients and constants**	**□²**	**R^2**
Lewis/Newton	k = 0.0136	9.305×10^{-4}	0.9919
Page			0.8068
Modified Page	**k = 0.0131, n = 1.1895**	**2.642×10^{-4}**	0.9977
Henderson & Pabis	a = 1.0246, k = 0.0138	9.495×10^{-4}	0.9917
Logarithmic	a = 1.0659, c = -0.0553, k = 0.0120	4.435×10^{-4}	0.9961
Two-Term	a = 0.5123, b = 0.5123, m = 0.0138, n = 0.0138	1.272×10^{-3}	0.9890
Approximation of Diffusion	a = -0.1302, k = 0.1165, m = 3.0879	6.404×10^{-4}	0.9944
Wang & Singh	a = -0.0093, b = 2.159×10^{-5}	8.991×10^{-4}	0.9922
Modified Page Equation II	c = 0.0032, m = 0.7797, n = 1.1841	3.026×10^{-4}	0.9973

Effective moisture diffusivity

The highest diffusivity value of 7.80 $\times 10^{-9}$ m^2/s was observed at 70^0 C and the lowest D_{eff} value of 2.05 $\times 10^{-9}$ m^2/s was observed at 40^0 C. From the study it can be concluded that effective moisture diffusivity declined sharply with moisture content in the first falling rate period and when drying entered into the second falling rate period the diffusivity changed slightly with moisture content. The values of D_{eff} found in this study was in the range of 10^{-9} to 10^{-8} m^2/s which is typical value for drying of agricultural products (Maskan, et al., 2002).

Activation energy

Fig. 33 shows the logarithmic of effective moisture diffusivity values (D_{eff}) obtained at different temperatures plotted against the corresponding absolute temperature [1/ (T+273)] to obtain the constants of Arrhenius Equation. The values of activation energy lie from 12.7 to 110 kJ/mol for most food material (Zogzaz et al., 1996). The plots in Fig. 33 showed the straight line in the temperature range investigated, indication Arrhenius dependence. From the slope of the straight line described by the Arrhenius Elation the activation energy was found to be 27.22 kJ/mol which was calculated by using Equation 8. The value of activation energy compared with literature values of different agricultural products are given in Table 38.

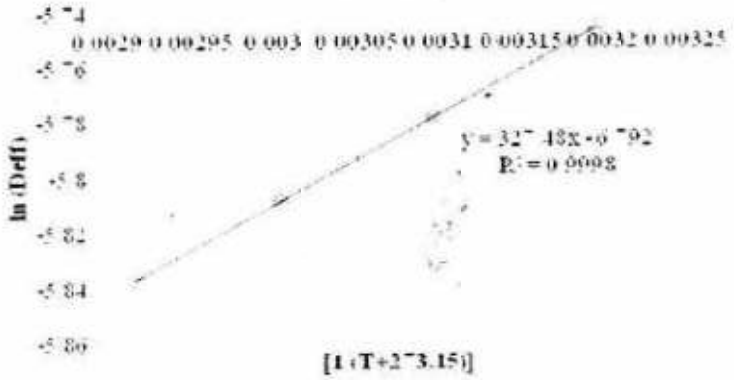


Fig. 33. Logarithmic of effective moisture diffusivity vs function of the inverse of absolute Temperature in K

Table 44 Effective moisture diffusivity, activation energy of *kachkal* peel

Temp (^{0}C)	D_{eff} (m^2/s)	1/Tabs
40	2.05×10^{-9}	0.003195
50	3.85×10^{-9}	0.003096
60	4.05×10^{-9}	0.003003
70	7.80×10^{-9}	0.002915

Table 45 Progressive parameters of Arrhenius relationship between affective diffusivity and absolute temperature

Regression parameters	Values
Slope (E_a/R)	327.48
Activation energy (E_a)	27.22 kJ/mol
Intercept (ln D_0)	8.9×10^{-2}
R^2	0.9998

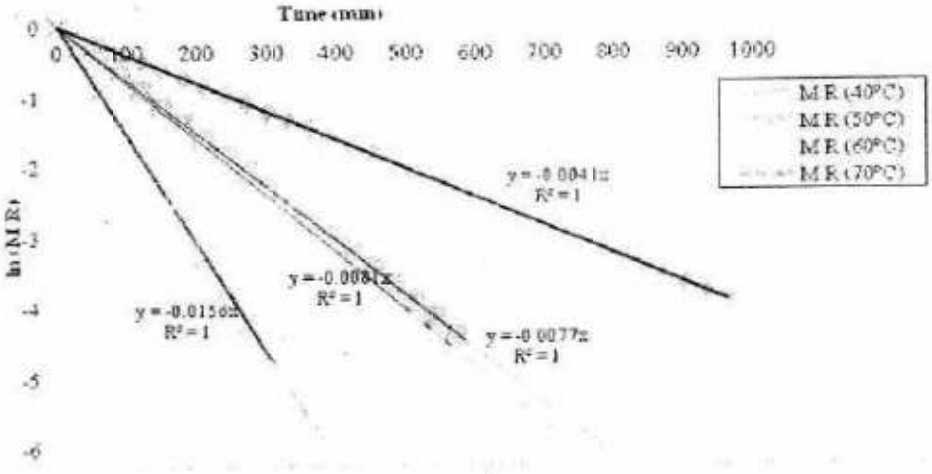


Fig. 34. Variation in ln (MR) with time (T) of *kachkal* peel

Major Outcome and Achievements

The present research focused on the effect of maturity on biochemical composition of *kachkal*, the study clearly revealed that growth stages have a profound influence on the biochemical and nutritional compositions. Since potential application of fruits depends on its biochemical compositions, the present piece of work will be helpful for selecting the best stage for industrial exploitation. The nutrients like protein, fat, ash, total phenolics and soluble sugar contents were higher during early stages of growth. Maximum antioxidant activity was also recorded during initial stages of fruit development. It makes *kachkal* an excellent ingredient for different functional and convenience foods like cookies, biscuits, chips etc. An increase accumulation of starch with maturity was observed, which makes matured *kachkal* a potential source for starch extractions. Presence of considerable amount of amylose also makes it convenient for using in products subjected to high temperature. *Kachkal* harvested at fully matured stage was rich in crude fiber, lignin, vitamin A, thiamine and carotenoid contents. Thus incorporation of matured *kachkal* in different food products will enrich its fiber and vitamin contents. The amino acid compositions revealed that all the essential amino acids were present in reasonable amount throughout the fruit growth which adds more nutritional value in the fruit. The important fatty acid like oleic, palmitic, linoleic and linolenic acids known for health benefits were maximum at stage I (20 DAE), which thereafter declined gradually with maturity.

It was also investigated that the biochemical properties of *kachkal* changed considerably with the different processing treatments. The three cooking methods employed in this study proved to be of great importance as the loss of nutritional components on processing was low with each of the treatment. Among all the three cooking method employed, microwave cooking was found to retained maximum nutrients. Improvement of microwave cooking over blanching and boiling may be attributed to the low temperature used in microwave cooking. Moreover, some of the important health properties like fat content, antioxidant activity, and carotenoid content increased during processing. Looking at the backdrop of immense potential for exploiting the only culinary banana *Kachkal* of the entire Northeast India, the findings of the present study can be used for the exploitation of culinary banana and the information gained through the present study will be useful in determining the best conditions for preparation along with being capable of minimizing losses in nutrients.

Resistant starch studied in present study revealed its potential health benefits and functional properties. The present study evinced that *kachkal* (*Musa* ABB) can be a potential

source for production of resistant starch type III. It was found that *kachkal* RS and bread prepared by incorporating it gave the lowest glycaemic index (GI). There was no difference in B-glucose incremental area under the curve (IAUC) for RS and RSB. The knowledge of an effective processing method for dietary staples to control and reduce hyper-glycaemia is essential in the treatment of diabetes. This is because diet management is crucial to control spikes in blood glucose levels. Our present result give a scientific basis of the glycaemic response of *kachkal* RS and RSB which showed low postprandial rise of blood glucose and may be recommended for use in the diet of diabetic patients.

A nutraceutical or health benefit food was developed in the laboratory from the male bud of *kachkal*. The study evinced that it *kachkal* buds are good source of minerals, such as magnesium, iron and copper. It contains high quality protein because of its well balanced essential amino acid in addition to high dietary fibre and flavonoid concentrations. The utilization of kachkal bud could provide additional benefits in reducing the banana waste, and increasing the use in food science. Dried *kachkal* bud powder was further incorporated with different concentration of turmeric powder and the product showed high antioxidant activity which was a very much desired trait in a nutraceutical product.

As part of the research objectives some value added products like noodles, beer, chips and were prepared from *kachkal* flour. Bread was also prepared by incorporating resistant starch prepared form kachkal starch. The sensory evaluation and biochemical results showed that these value added products can be consumed by diabetic persons.

Some experiments on thin layer drying kinetics of *kachkal* peel and pulp were also investigated to study the drying behaviour and effect of temperature on samples. From the study it was concluded that moisture removal process followed falling rate stage which was governed by the diffusion process. In order to explain the drying behaviour of *kachkal* pulp, nine different models were applied and fitted to the experimental data. According to the statistical analysis applied to all the models, modified Page model gave the best result with minimum χ^2 value of 2.589×10^{-4} and maximum R^2 value of 0.9993. The highest effective diffusion coefficient found was 8.38×10^{-9} m^2/s at 70^0 C and lowest value of 5.6×10^{-9} at 40^0 C. The activation energy required to initiate moisture diffusion from the *kachkal* pulp slice was found to be 53.27 kJ/mol. The effect of temperature on moisture diffusivity was expressed by Arrhenius Equation. It can be concluded that with increase in air drying temperature the drying rate increases. According to the result it can be stated that modified Page model could describe the thin-layer drying characteristics of *kachkal* pulp in the drying process at temperature range from 40 to 70^0 C.

Similarly, in case of kachkal peel also nine different models were applied and fitted to the experimental data. According to the statistical analysis applied to all the models, modified Page model gave the best result with minimum $\square^2$ value of 2.23×10^{-4} and maximum R^2 value of 0.99. The highest effective diffusion coefficient found was 7.80×10^{-9} m^2/s at 70^0 C and lowest value of 2.05×10^{-9} at 40^0 C. The activation energy required to initiate moisture diffusion from the *kachkal* peel was found to be 27.22 kJ/mol. Temperature dependence of diffusivity followed an Arrhenius model with high correlation factor ($R^2 = 0.99$). From the present study it can be concluded that the drying of *kachkal* peel can be accurately predicted using modified Page model. Therefore, this model may be useful in the description of the drying process of *kachkal* characterized by variable properties and drying condition.

Inferences

- Growth stages of *kachkal* have profound influence on the biochemical and nutritional compositions.
- *Kachkal* at 65 days after emergence (DAE) of bunch found to be the best stage for culinary purpose as well as for further processing and value addition.
- *Kachkal* flour may be considered as excellent ingredients in preparation of different functional and convenience foods.
- *Kachkal* starch is the potential source for development of type III resistant starch due to its high amylose content.
- Foods prepared by incorporating by *kachkal* resistant starch gives low glycaemic index therefore may be recommended in the diet of diabetic patient.
- *Kachkal* sap (male bud) is a good source of antioxidant and flavonoids which could be considered for development of nutraceutical foods.
- Value added products prepared from *kachkal* flour are nutritionally rich.

Photographs of *Kachkal* at different developmental stages

Fig. 35. 20 days after emergence (DAE) matured *kachkal*

Fig. 36. 35 days after emergence (DAE) matured *kachkal*

Fig. 37. 50 days after emergence (DAE) matured *kachkal*

Fig. 38. 65 days after emergence (DAE) matured *kachkal*

Fig.39. 80 days after emergence (DAE) matured *kachkal*

Fig. 40. *Kachkal* at growing stage

Publications from the project

P. Khawas, S. C. Deka, and A. J. Das. Biochemical and Nutritional Composition of culinary *Kachkal* (*Musa* ABB) of Assam. Presented on BIOFOODS2011. National Seminar at Dept. of Food Engineering Technology, Tezpur University, Napaam-784028, Assam, India Dated: 14th – 16th November 2011

P. Khawas, S. C. Deka A. J. Das and N. Sit. Isolation and Partial Characterization of Starch from Culinary Banana "*Kachkal*" (*Musa* ABB) of Assam. Presented on SASNET-FF-2011, 5th International Conference at CFTRI, Mysore, India Dated: 15th – 16th December 2011

P. Khawas, S. C. Deka and A.J. Das. Effect of Three Conventional Cooking Methods on the Biochemical Compositions of '*kachkal*' (*Musa* ABB) of North-East India. Presented on 18th International Conference (POST ISCBC) Perspective and Challenges in Chemical and Biological Sciences, Innovation Cross Roads, Held at IASST Guwahati from 28th - 30th January, 2012.

P. Khawas, S. C. Deka, A. J. Das, N. Sit and L. S. Badwaik. Development of Type III Resistant Starch from Culinary Banana (*Musa* ABB) of Assam and Determination of Glycaemic Index in Disease Free Humans. 1st International Conference on "Inovations in Food Precessing, value Chain Management and Food Safety, NIFTEM, Sonepat, Haryana Date: 10th – 11th January, 2013.

P. Khawas, K. K. Dash, A. J. Das and S. C. Deka. Drying kinetics and moisture diffusivity of *kachkal* banana (*Musa* ABB) of Assam. Communicated for publication in journal.

P. Khawas, K. K. Dash, A. J. Das and S. C. Deka. Thin-layer drying characteristics of *kachkal* banana peel (*Musa* ABB) of Assam. Communicated for publication in journal.

P. Khawas, S. C. Deka*, A. J. Das, N. Sit & L. S. Badwaik. Nutritional composition of *kachkal* (*Musa* ABB): A culinary banana of Assam. Communicated for publication in journal.

P. Khawas, S. C. Deka*and A. J. Das.Development of type III resistant starch from culinary banana (Musa ABB) of Assam and determination of glycaemic index in disease free humans. Communicated for publication in journal.

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Major Equipments Used during the Project Works

Sl. No	Equipment	Model
1.	P.C based Double beam UV-visible spectrophotometer	Spetrascan UV-2600
2.	Kjeldahl apparatus	(KelPlus, Pelican Equipment, India)
3.	Soxhlet extractor for fat estimation	(SocsPlus, Pelican Equipment, India)
4.	Gas Liquid Chromatograph	(Varian, CP - 3800).
5.	Amino Acid Analyzer	Beckman 119 CL
6.	Hunter Lab Color Quest	(Model Ultrascan Vis- Model, USA)
7.	AAS	
8.	Rapid Visco-Analyser	(RVA-4, Newport Scientific, Sydney, Australia)
9.	X-ray difractometer	Rigaku Miniflex
	scanning electron microscope	(JEOL JSM 6390 LV)
	FT-IR	(Nicolet Instruments 410 FT-IR equipped with KBr optics and a DTGS detector)
	electronic weighing balance	(CPA225D, Sartorius AG, Germany)
	convective tray dryer	(IK-112, Make IKON Instruments, Delhi)

Nomenclature

%	Percentage
% SA	Percentage scavenging activity
µg	Micro gram
µm	Micro metre
^0C	Degree centigrade
a, b, c, k, m, n	drying model coefficient
AAA	Amino Acid Analyzer
abs	absolute temperature (K)
ANOVA	Analysis of variance
AOF	After overnight fasting
B-glucose	Blood glucose
BS	Bread standard
CD	Critical difference
CFU	Colony forming unit
cP	Centi Pose
D_0	Arrhenius factor (m^2/s)
DAE	Days after emergence
db	dry basis
D_{eff}	effective moisture diffusivity (m^2/s)
E_a	activation energy (kJ/mol)
FBG	Fasting blood glucose
Fig.	Figure
g	gram
g/l	Gram per litre
GI	Glycaemic index
GLC	Gel Liquid Chromatography
GOD	Glucose oxidase
Hz	Hertz
IAUC	Incremental Area Under the blood glucose response Curve for the tested meal.
IAUCS	IAUCS – Incremental Area Under the blood glucose response Curve for the Standard meal.

kv	Kilo volt
L	slab thickness (mm)
LSD	Least significant difference
M	average moisture content ((% wet basis)
M_e	equilibrium moisture content (% wet basis)
mg	miligram
M_i	initial moisture content ((% wet basis)
ml	Mili-litre
mM	Mili molar
MR	moisture ratio
MRS	deMan Rogossa & Smith
M_t	moisture content at time t ((% wet basis)
N	Normal
ND	Not detected
NE	North east
nm	Nano-metre
PCA	Plate count agar
PDA	Potato dextrose
pH	Negative log of hydrogen ions
pmol	Pico mol
POD	peroxidase
ppm	Parts per million
R	ideal gas constant (8.314 J/mol K)
R^2	coefficient of determination
rpm	Revolution per minute
RS	Resistant starch
RSB	Resistant starch bread
SD	Standard deviation
t	drying time (min)
T	drying air temperature (^{0}C)
UV	Ultra violet
w/v	Weight by volume
wb	wet basis

X	independent variable
Y	dependent variable
$\square^2$	chi-square

Appendices

Sensory Evaluation Format

Name: _____
Designation: _____
Sample: _____

Sample	Appearance	Colour	Texture	Taste	Flavour	Mouth feel appeal (after taste)	Overall Acceptability
Control							
Sample 1							
Sample 2							
Sample 3							
Sample 4							
Sample 5							

9 = Extremely like
8 = Like very much
7 = Like moderately
6 = Like slightly
5 = Neither like nor dislike
4 = Dislike slightly
3 = Dislike moderately
2 = Dislike very much
1 = Dislike extremely

Remarks and Suggestions:

Signature

Professor
Deptt. of Food Engineering & Technology
Tezpur University, Napaam-784028
Dist-Sonitpur (Assam)

AUDITED STATEMENT OF EXPENDITURE ACCOUNTS
FOR THE FINANCIAL YEAR 2012-2013 (From 01.04.2012 to 31.03.2013)

a) Title of the Project: Effect of processing on biochemical compositions and production of resistant starch (RS), nutraceuticals and value added products from culinary banana (Musa ABB) "*Kachkel*" of north east India

b) Sanctioned letter No. & Date: ERIP/ER/0803747/M/01/1196, Dated 28th January, 2010

c) Principal Investigator: Prof. Sankar Chandra Deka

d) Date of Start of the Project: 30th April, 2010

e) Total Sanctioned cost of the Project: Rs.14.53 Lakhs

f) Grant received (Rs. Lakhs) in 1st Year – 7.61 Lakhs. 2nd Year – 3.61 Lakhs. 3rd Year – 2.81 Lakhs

g) Total grant received so far: Rs. 14.03 Lakhs

Sl. No.	Sanctioned Heads	Funds sanctioned for the year (Rs)			Total funds sanctioned (Rs) (iii+iv+v)	Carried forward from previous (2nd)yr (Rs)	Funds available (Rs) (v+vii)	Expenditure incurred during the FY (Rs)			Total (Rs) (ix+x+xi)	Balance (Rs) (vi-xii)
		1st yr. (2010-11)	2nd yr. (2011-12)	3rd yr. (2012-13)				1st yr. (2010-11)	2nd yr. (2011-12)	3rd yr. (2012-13)		
i	ii	iii	iv	v	vi	vii	viii	ix	x	xi	xii	xiii
(a)	Staff	1,44,000/-	1,44,000/-	1,68,000/-	4,56,000/-	61,548/-	2,29,548/-	1,20,000/-	1,06,452/-	1,44,000/-	3,70,452/-	85,548/-
(b)	Equipments	4,00,000/-	-	-	4,00,000/-	-	-	4,00,000/-	-	-	4,00,000/-	NIL
(c)	Operation & Maintenance	-	-	-	-	-		-	-		-	
(d)	Expendables	50,000/-	50,000/-	50,000/-	1,50,000/-	12,016/-	62,016/-	38,336/-	49,648 -	50,000/-	1,37,984/-	12,016/-
(e)	Travel	50,000/-	1,00,000/-	-	1,50,000/-	59,786/-	59,786/-	27,128/-	63,086/-	59,786/-	1,50,000/-	NIL
(f)	Contingencies	20,000/-	20,000/-	20,000/-	60,000/-	(-)9,410/-	10,590/-	29,268/-	20,142/-	21,036/-	70,446/-	(-) 10,446/-
(g)	Research consultants	-	-	-	-			-			-	
(h)	Procured service	-	-	-	-			-			-	
(i)	Institutional over head	97,000 -	47,000 -	43,000/-	1,87,000/-	23,711	66,711/-	96,789/-	23,500	66,441 -	1,86,730 -	270 -
(j)	Interest earned, if any	-	-	-				-				
	TOTAL	7,61,000/-	3,61,000/-	2,81,000/-	14,03,000/-	1,47,651	4,28,651/-	7,11,521/-	2,62,828 -	3,41,263 -	13,15,612 -	87,388 -

Name & Signature
of Principal Investigator
Date

Name & Signature of
OSD (Finance)
Date

Signature of Registrar
Date

Internal Audit Officer
Tezpur University

APPENDIX "F"

UTILIZATION CERTIFICATE
FOR THE FINANCIAL YEAR 2012-13 (from 01.04.2012 to 31.03.2013)

1.	Title of the Project	Effect of processing on biochemical compositions and production of resistant starch (RS), nutraceuticals and value added products from culinary banana (*Musa* ABB) "*Kachkal*" of North East India
2.	Name of the Institute	Tezpur University, Napaam,, Tezpur 784028, Assam
3.	Principal Investigator	Prof. Sankar Chandra Deka
4.	DRDO Letter No. and date of sanctioning the project Date of Start of the Project	ERIP/ER/0803747/M/01/1196 Dated 28th January, 2010 30th April, 2010
5.	Head of account as given in the original sanction letter	Major Head 2080 - Defence Services Research & Development, Minor Head 004- Research/Research and Development , Sub-Head (C) -- Extramural Research (EMIR) Code Head 852/06
6.	Amount brought forward from the previous financial year quoting DRDO letter No. & date in which the authority to carry forward the said amount was given	Rs. 1,47,651/-
7.	Amount received during the financial year (Please give No. & Date of DRDO sanction letter for the amount	Rs. 2,81,000/- **(Letter No. ERIP/ER/00803747/M/01/1196 dated 23 July 2012)**
8.	Amount of interest earned, in any from the grants	NIL
9.	Total amount that was available for expenditure (excluding commitments) during the financial year (Sl. No 6+7)	Rs. 4,28,651/-
10.	Actual expenditure (excluding commitments) incurred during the financial year (up to 31st March, 2013)	Rs. 3,41,263/-
11.	Balance amount available at the end of the financial year	Rs. 87,388/-
12.	Unspent balance refunded, if any (Please give details of Cheque No. etc)	
13.	Amount allowed to be carried forward to the next financial year	

APPENDIX "F" (Contd.....)

UTILIZATION CERTIFICATE
FY 2012-2013 (From (From 01.04.2012 to 31.03.2013)

Certified that the sum of **Rs 2.81Lakhs** sanctioned during the year **2012-2013** of grants-in-aid amount in favour of **Tezpur University, Napaam, Tezpur, Assam DRDO letter No. ERIP/ER/0803747/M/01/1196 Dated 23rd July 2012** and Rs. 1,47,651/- on account of unspent balance of the previous year, a sum of Rs. Rs. 3,41,263/-has been utilized for the purpose for which it was sanctioned and that the balance of Rs. 87,388/-remaining unutilized at the end of the year will be refunded.

Signature of Principal Investigator

ernal Audit Officer
Tezpur University

Signature of OSD (Finance)

Certified that I have satisfied myself that the condition on which the grants-in-aid was sanctioned have been fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilized for the purpose for which it was sanctioned.

Kinds of checks exercised

1. Accounts audited by qualified Chartered Accountant appointed by this University as Internal Auditor.
2. The A. G. (Audit), Guwahati has already audited the account.
3. All the instrument (s), chemicals, consumables etc purchased from the grant are entered in the Log Book.

Counter signed by
Registrar
(Designation with seal)

AUDITED/PROVISION STATEMENT OF EXPENDITURE ACCOUNTS
Only for the period 01.04.2013 to 29.04.2013(Project closing date)

a) Title of the Project: Effect of processing on biochemical compositions and production of resistant starch (RS), nutraceuticals and value added products from culinary banana (Musa ABB) "*Kachkal*" of north east India

b) Sanctioned letter No. & Date: ERIP/ER/0803747/M/01/1196, Dated 28th January, 2010

c) Principal Investigator: Prof. Sankar Chandra Deka

d) Date of Start of the Project: 30th April, 2010

e) Total Sanctioned cost of the Project: Rs.14.53 Lakhs

f) Grant received (Rs. Lakhs) in 1st Year – 7.61 Lakhs. 2nd Year – 3.61 Lakhs. 3rd Year – 2.81 Lakhs

g) Total grant received so far: Rs. 14.03 Lakhs

Sl. No.	Sanctioned Heads	Balance carried forward from 3rd year (Rs)	Expenditure incurred from 01.04.2013 to 29.04.2013 (Rs)	Excess amount to be refund (Rs) (iii-iv) (Rs)
i	ii	iii	iv	v
(a)	Staff	85,548/-	11,600/-	73,948/-
(b)	Equipments	NIL	NIL	NIL
(c)	Operation & Maintenance			-
(d)	Expendables	12016/-	1570/-	10,446/-
(e)	Travel	NIL	NIL	NIL
(f)	Contingencies	(-) 10,446/-	-	(-)10,446/-
(g)	Research consultants			
(h)	Procured service			
(i)	Institutional over head	270/-	270/-	NIL
(j)	Interest earned, if any			
	TOTAL	87,388/-	13,440/-	73,948/-

Name & Signature
of Principal Investigator
Date

Name & Signature of
OSD (Finance)
Date

Signature of Registrar
Date

Internal Audit Officer
Tezpur University

UTILIZATION CERTIFICATE
From 01.04.2013 to 29.04.2013

1.	Title of the Project	Effect of processing on biochemical compositions and production of resistant starch (RS), nutraceuticals and value added products from culinary banana (*Musa* ABB) "*Kachkal*" of North East India
2.	Name of the Institute	Tezpur University, Napaam,. Tezpur 784028, Assam
3.	Principal Investigator	Prof. Sankar Chandra Deka
4.	DRDO Letter No. and date of sanctioning the project Date of Start of the Project	ERIP/ER/0803747/M/01/1196 Dated 28th January, 2010 30th April, 2010
5.	Head of account as given in the original sanction letter	Major Head 2080 Defence Services Research & Development, Minor Head 004- Research/Research and Development . Sub-Head (C) – Extramural Research (EMR) Code Head 852/06
6.	Amount brought forward from the previous financial year quoting DRDO letter No. & date in which the authority to carry forward the said amount was given	Rs. 87,388/-
7.	Amount received during the financial year (Please give No. & Date of DRDO sanction letter for the amount	NIL
8.	Amount of interest earned, in any from the grants	NIL
9.	Total amount that was available for expenditure (excluding commitments) during the financial year (Sl. No 6+7)	Rs. 87,388/-
10.	Actual expenditure incurred up to 29th April, 2013 (from 01.04.2013 to 29.04.2013)	Rs. 13,440/-
11.	Balance amount available at the end of the financial year	Rs. 73,948/-
12.	Unspent balance refunded, if any (Please give details of Cheque No. etc)	Rs 73,948/- (DD No. 892438 Dt. 18.6.2013)
13.	Amount allowed to be carried forward to the next financial year	Not Applicable

APPENDIX "F" (Contd.....)

UTILIZATION CERTIFICATE
From 01.04.2013 to 29.04.2013

Certified that the sum of Rs. 87,388/-on account of unspent balance of the previous year, a sum of Rs. 13,440/-has been utilized till 29.4.2013, for the purpose for which it was sanctioned and that the balance of Rs. 73,948/-remaining unutilized (up to 29.4.203) has been refunded.

Signature of Principal Investigator

Internal Audit Officer
Tezpur University

Signature of OSD (Finance)

Certified that I have satisfied myself that the condition on which the grants-in-aid was sanctioned have been fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilized for the purpose for which it was sanctioned

Kinds of checks exercised

1. Accounts audited by qualified Chartered Accountant appointed by this University as Internal Auditor.
2. The A. G. (Audit), Guwahati has already audited the account.
3. All the instrument (s), chemicals, consumables etc purchased from the grant are entered in the Log Book.

Counter signed by
Registrar
(Designation with seal)

Tezpur University

APPENDIX "G"

FORMAT FOR EQUIPMENT PURCHASED

Assets acquired wholly for substantially out of government grants register maintained by grantee institution
block account maintained by sanctioning authorities

Name of Sanctioning Authority: Directorate of Extramural Research & Intellectual Property Rights, DRDO, Ministry of Defence, Government of India, New Delhi

Sl. No	Name of Grantee Institution	No. & Date of sanction	Amount of sanctioned grant	Brief purpose of the grant	Whether any condition regarding the right of ownership of Govt. in the property assets acquired out of the grant was incorporated in the grant-in-aid sanction	Particulars of assets actually created or acquired
(1)	(2)	(3)	(4)	(5)	(6)	(7)
	Tezpur University, Napaam, Tezpur-784028. Assam.	ERPI/ER/08037/47/M/01/1196, Dated: 28th Jan, 2010.	Rs. 14.53 Lakhs (Rupees fourteen lakhs fifty three thousand only)	For pursuing research on the subject titled "Effect of processing on biochemical compositions and production of resistant starch (RS), nutraceuticals and value added products from culinary banana (Musa ABB) "*Kochkal*" of North east India.	Assets of a capital nature acquired wholly or substantially out of this grant are the property of DRDO	PC based double beam scanning UV-vis spectrophotometer, Make: Thermo Fosher Scientific Pvt. Limited. Model: Spectrascan UV-2600

Contd.....2

Value of the assets as on 5.07.2011 (Date of submission)	Purpose for which utilised at present	Encumbered or not	Reasons if encumbered	Disposed if or not	Reasons & authority if any, for disposal	Amount realized on disposal	Remarks
(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
Rs. 4.0 Lakhs (Rupees four lakhs only)	For carrying our R&D works related to the project	NA	NA	NA	NA	NA	Instrument is currently used for R&D activities.

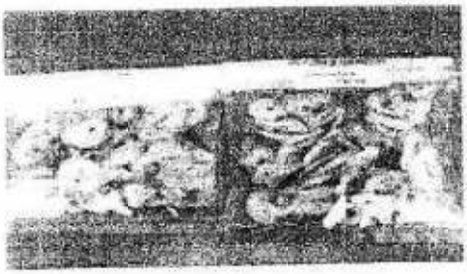
Name & Signature
of Principal Investigator
Date

Name & Signature of
OSD (Finance)
Date

Signature of Registrar
Date

Kachkal

Kachkal Noodles



Kachkal Chips

Kachkal Beer

Kachkal blossom

Fig: Some of the value added products from Kachkal