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PHYSICOCHEMICAL CHANGES IN BAMBOO SHOOT OF ASSAM DURING PROCESSING AND ITS QUALITY STUDY BY APPLICATION OF ANTIMICROBIAL-ANTIBROWNING EDIBLE COATING

A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

By

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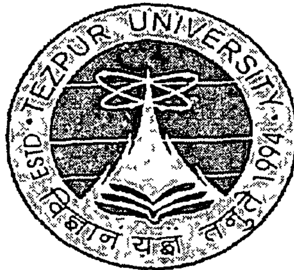
Registration No. 020 of 2012



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TEZPUR UNIVERSITY
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My son Devansh, wife Kanchan
and my parents

Physicochemical changes in bamboo shoot of Assam during processing and its quality study by application of antimicrobial-antibrowning edible coating

ABSTRACT

The thesis includes the nutritional status of some edible bamboo shoot species of Assam and their comparison. The current investigation deals with the effect of various processing treatments viz., blanching, osmotic dehydration and fermentation on physicochemical properties of bamboo shoot of Assam. The study also includes the extraction of antibrowning and antimicrobial agents from different biological sources for their application in edible coating. The thesis describes the process of composite edible film preparation using different polysaccharides. In addition, the study highlights the application of antibrowning and antimicrobial agents in edible coating and its effect on bamboo shoot quality.

The thesis is divided into seven chapters which are briefly discussed below:

Chapter 1 deals with the general introduction about present work. It highlights the gap of study and background behind the selection and planning of current work plan. The application and justification of current work is also highlighted. Finally the scope and objectives of the present investigation are included.

Chapter 2 presents the review of literature for present study. The general introduction about bamboo shoot, its nutritional status and its consumption pattern are included. The effect of different processing parameters on bamboo shoot quality and shelf life is also discussed. Blanching effect on physicochemical properties on fruits and vegetables is highlighted. Application of osmotic dehydration process, its mass transfer kinetics and effect of centrifugal force and vacuum pressure on osmotic dehydration are discussed. This chapter also includes the methods and microbiology of fermentation and its effect on quality of bamboo shoot of different regions/ country. Antimicrobial potential of lactic acid bacteria, its metabolites and their activity on different pathogenic

microorganisms are included. However, the applications of microwave assisted extraction for extraction of different active components from various biological sources are also included. Edible coating and film and its application on different fruits and vegetables are also discussed.

Chapter 3 includes the nutritional analysis of four different species of bamboo shoot of Assam. Further the effects of blanching temperature (75, 85 and 95 °C) and time (5, 10, 15, 20, 25 and 30 min) on proximate composition, vitamin C, total phenols, antioxidant activity, colour and texture of bamboo shoot (*Bambusa balcooa*) are also investigated. All the blanching treatments are compared for their deteriorative effect on bamboo shoot.

Chapter 4 reports the effect of the process time, solution temperature and salt concentration on osmotic dehydration of bamboo shoot and their optimization using response surface methodology (RSM). The mass transfer kinetics as well as water and solute diffusivity during osmotic dehydration of bamboo shoot are also included. The chapter also reports the effects of centrifugal force and pulsed vacuum on the osmotic dehydration process. The changes in hardness, colour and microstructure during osmotic dehydration are included.

Chapter 5 investigates the influence of fermentation on physicochemical and microbial change in bamboo shoot. The changes during fermentation of bamboo shoot with and without addition of *Garcinia pedunculata* Roxb. fruit are also reported. It also includes the organic acids and carbohydrate profile of fresh and fermented bamboo shoots analyzed by HPLC.

Chapter 6 presents the extraction method of biometabolites from *Lactobacillus plantarum* isolated from fermented bamboo shoot. Antimicrobial activity of biometabolites against *Escherichia coli*, *Streptococcus aureus* and *Bacillus cereus* is reported. The organic acid composition of biometabolites and its effect on minimum lethal dose concentration (LD_{min}) and death rate kinetics of test bacterial strains are

included. It also deals with the optimization of microwave assisted extraction process of antioxidant extract using RSM on the basis of maximum DPPH and ABTS radical scavenging activity.

Chapter 7 deals with the method development of composite films with varying composition of alginate, starch and carboxymethyl cellulose. The mechanical, thermal and barrier properties of these films are discussed. The process of incorporation of antibrowning and antimicrobial agents in edible coating and its application on bamboo shoot are included. The effect of coating on weight loss, surface colour and microbial count is discussed.

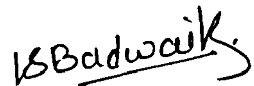
Chapter 8 presents the conclusion, salient findings and future scope of the present investigation. It is concluded that the processing e.g. blanching, osmotic dehydration and fermentation have significant influence on bamboo shoot quality. Low temperature blanching could be better option for retention of micro nutrients. Enhancement of osmotic dehydration process resulted, with application of vacuum pressure and centrifugal force. Addition of *Garcinia pedunculata* Roxb. in bamboo shoot enhanced the fermentation and quality of fermented product (*khorisa*). The application of antimicrobial biometabolites and antioxidant extract in edible coating evinced better retention of bamboo shoot quality.

DECLARATION BY THE CANDIDATE

The thesis entitled **"Physicochemical changes in bamboo shoot of Assam during processing and its quality study by application of antimicrobial-antibrowning edible coating"** is being submitted to School of Engineering, Tezpur University in partial fulfillment for the award of the degree of Doctor of Philosophy in the Department of Food Engineering and Technology is a record of bonafide research work accomplished by me under the supervision of Prof. S. C. Deka.

All helps from various sources have been duly acknowledged.

No part of the thesis has been submitted elsewhere for the award of any other degree.



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All helps received by him from various sources have been duly acknowledged.

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(Laxmikant S. Badwaik)

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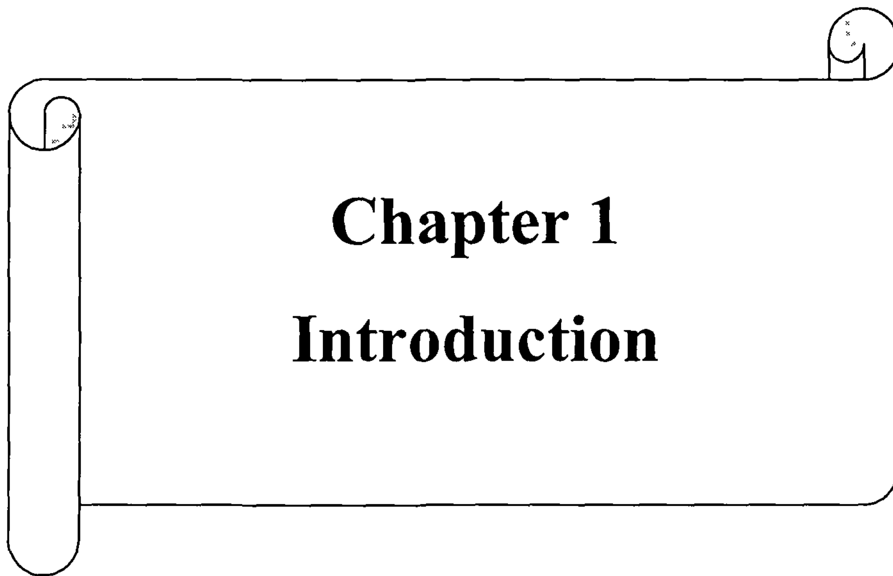
List of Abbreviations

ABTS	2, 2'-azino-bis, 3-ethylbenzo thiazoline-6-sulphonic acid
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
ASTM	American Society for Testing and Materials
BBD	Box–Behnken design
CCRD	Central composite rotatable design
CFCS	Cell-free crude supernatants
CFOD	Centrifugal force + Osmotic Dehydration
CFPS	Cell-free purified supernatants
CFU	Colony forming units
CMC	Carboxymethyl cellulose
dE	Colour change
DPPH	2,2 diphenyl 1-picrylhydrazyl
E_a	Activation energy
FCR	Folin-Ciocalteu reagent
FTIR	Fourier transform infrared spectroscopy
GPR	*Garcinia pedunculata* Robx.
LAB	Lactic acid bacteria
LD_{min}	Minimum lethal dose concentration
MAE	Microwave assisted extraction
OD	Osmotic Dehydration
PAL	Phenylalanine ammonia-lyase
PPO	Polyphenol oxidase
RSA	Radical scavenging activity
RSM	Response surface methodology
SEM	Scanning electron microscope
SG	Solids gain
TGA	Thermo Gravimetric Analysis
TPC	Total plate count
TSM	Total soluble matter
v/w	Volume by weight
VOD	Vacuum pulse + Osmotic Dehydration
WL	Water loss
w/w	Weight by weight
WR	Weight reduction
WVP	Water vapour permeability
WVTR	Water vapour transmission rate



Chapter 1

Introduction

1.1 Introduction

India is one of the rich genetic resources of bamboo with 136 indigenous exotic species and 23 genera under cultivation. Assam is one of the richest biodiversity zones in the world and consists of tropical rainforests, deciduous forest, riverine grasslands and bamboo orchards. Bamboos play an important role in daily life of rural people especially tribals in numerous ways, from house construction, agricultural implements to provide food, fodder etc. The edible parts of bamboo, i.e. shoots are highly nutritious and potentially rich sources of dietary fibers, antioxidants[1], amino acids, minerals, vitamins and low in calories. The protein content of the shoots is also high, and contains fewer amounts of fats; however, it is rich in essential fatty acids[2-4]. Presence of high quality vitamins, carbohydrates, proteins and minerals in bamboo shoot and their easy availability to common man may help in solving nutritional deficiency of rural poor [5]. All this indicate vast potential of bamboo shoot as food resource.

In Assam and other states of Northeast India, bamboo shoot is consumed either raw or processed because of its exotic taste, flavour and medicinal value. The major edible species that are suitable for processing in Assam are *Dendrocalemus hamiltonii, Bambusa balcooa, B tulda,* and *B pallid* [6]. At present bamboo shoots are largely sold in the unprocessed form. They are harvested from homestead in clumps and brought to the local market for sale. The shelf life of bamboo shoot is limited and they have to be sold immediately after harvest.

In regular practices bamboo shoot are boiled before consumption for a particular time. The boiling time depends on locality, traditional practices and use for removal of bitterness of shoot. Heat processing is applied to various vegetables to increase its shelf life, to stop various enzymatic reactions, reduce microbial load in food, soften tissues, eliminate intracellular air to prevent oxidation and reduce the antinutritional components[7-8]. With regards to removal of antinutritional components some valuable nutrients may get loss and the effect of boiling/ blanching on nutritional components is imperative to consider during processing of bamboo shoot.

Potential use of bamboo shoot as nutritionally rich food has created the importance of preserving it to overcome its seasonality. Various drying processes have adverse effect on the nutritional and physical quality of bamboo shoot; however, osmotic dehydration could be the best options for preserving its nutrients. Osmotic dehydration is a semi-drying process applied to high moisture fruits and vegetables to reduce the moisture content to an optimum level so that the shelf life of the product is increased without much deterioration in sensorial and nutritional status. It is a useful treatment of dehydration compared to the other dehydration processes, as it does not bring adverse and irreversible changes in physical and nutritional status of food material like other dehydration processes[9]. Bamboo shoots are tested with different drying methods viz., hot air drying, vacuum drying, freeze drying and microwave drying etc[10-13]. But study of osmotic dehydration of bamboo shoot is not yet reported in any literature or seldom available.

Fermented bamboo shoot is an important part of the traditional foods in the Northeastern states of India. Fermentation of bamboo shoots not only helps to extend storage life but also enhances safety of foods using the natural microflora and their antibacterial compounds. Such traditional fermented food will be a potential source of lactic acid bacteria[14-15]. *Khorisa* is a traditional fermented bamboo shoot product of Assam, India and it is important part of diet of both rural and urban people and is extensively used as a main ingredient in preparation of different food items like meat, fish, pickles etc. Sometime, during *khorisa* fermentation small quantities of dried fruit of *Garcinia pedunculata* Roxb. (Local name: Borthekera) are added along with the shoot as a possible acidifier to enhance fermentation. Fermentation adds specific flavour, aroma and taste to the bamboo shoot. These indigenous traditional knowledge of tribal community needs to be scientifically validated which would provide guidelines for evolving a simple, efficient system for bamboo shoot utilization and preservation for better shelf life of bamboo shoot.

Bamboo shoots deteriorate very rapidly during storage and transportation which is a serious post harvest problem for traders. Enzymatic browning also poses a serious problem during the storage of post harvest bamboo shoots. Edible coatings might have

the potential to stop or inhibit the rapid quality degradation of harvested bamboo shoots. Edible film and coatings could be used to preserve fresh cut fruits and vegetables, providing a selective barrier to moisture, oxygen and carbon dioxide, improving mechanical and textural properties. Further addition of antioxidants and antimicrobial compounds impart microbial barriers, avoiding volatile loss, etc[16].

In the light of the above backgrounds the present study was undertaken to investigate the nutritional potential of edible bamboo shoots of Assam and further determine the effect of blanching temperature and time on physicochemical properties of bamboo shoot. The optimization of osmotic dehydration process of bamboo shoot and its mass transfer kinetics has been studied. In addition, the physicochemical and microbial changes during the fermentation of bamboo shoots were examined in the process of making *khorisa*. The effect of edible coating coupled with antimicrobial and antibrowning agents on bamboo shoot quality was also investigated.

Objectives of the present investigation

- To study the effect of blanching temperature and time on physicochemical properties of bamboo shoot
- To optimize the osmotic dehydration process of bamboo shoot
- To investigate the influence of fermentation on bamboo shoot quality with *Garcinia pedunculata* Roxb.
- To extract antimicrobial biometabolites and antioxidant extract from different biological sources
- To develop antimicrobial and antibrowning coating and its effect on bamboo shoot quality

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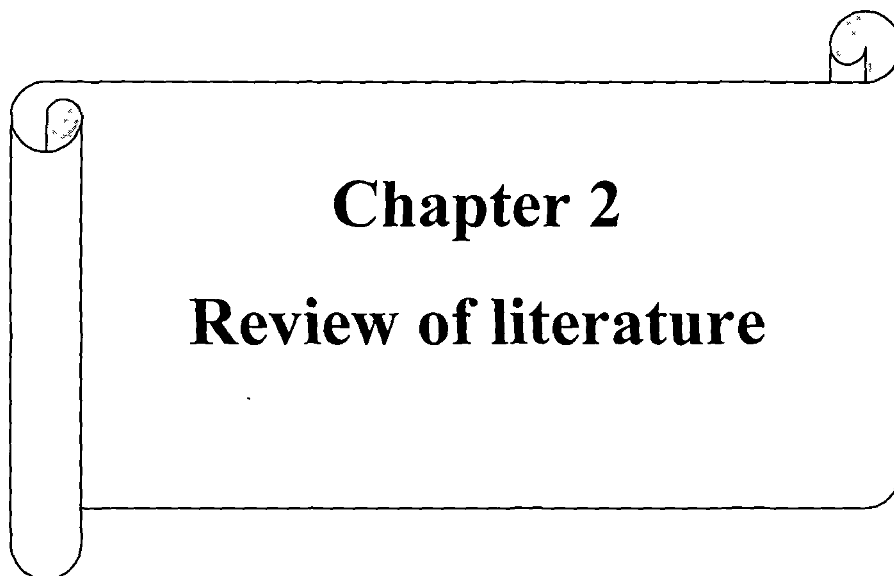

Chapter 2
Review of literature

2.1 Bamboo shoot

2.1.1 Nutritional potential

Bamboo belongs to the subfamily Bambusoideae in the family Poaceae and it includes over more than 1250 species, belonging to 75 genera, to which India has contributed more than 125 species belonging to 23 genera.[1] Bamboo shoots are the immature and edible culms arising from the rhizomes. They are harvested generally at the time of June and July when the shoots are of 30 cm in height (Fig. 2.1). The edible part consists of meristematic cell tissue with regions of rapid cell division and differentiation, which is enveloped in protective, non-edible leaf sheaths.[2] Bamboo shoot is a very traditional food for the tribal people and has been a major part of diet in Asian countries like China, Japan, Korea, Taiwan, Thailand and Philippines and its demand is growing worldwide.[3] Bamboo shoots could be considered as an ideal vegetable as it has high nutritional properties. It is a good source of high dietary fiber and low in fat content. Bamboo shoots are also low in cholesterol content and has high amount of potassium which is a heart-healthy mineral. It is rich in many vitamins like tocopherol, vitamin C, Vitamin B_6, thiamin, riboflavin and niacin. Bamboo shoots are rich in many minerals and have 17 different types of amino acids and have excellent antimicrobial qualities.[1,4] The nutritional potential of various species of bamboo shoots are given in Table 2.1.

Bamboo shoots are also well known for its medicinal values. There are number of literatures showing the medicinal and functional properties of bamboo shoot. [1,5-6] Shoots are rich in lignans and also which have anti-inflammatory, anticancer, antibacterial, antifungal, and antiviral properties. It is very effective in reducing the risk of cancer and also prevents any injury to blood vessels.[7] As bamboo shoots being locally available, could be a good source of nutrition for the poor people. Apart from being used as a source of food, bamboo is also used in different other fields like construction, decoration purpose, making furniture etc.

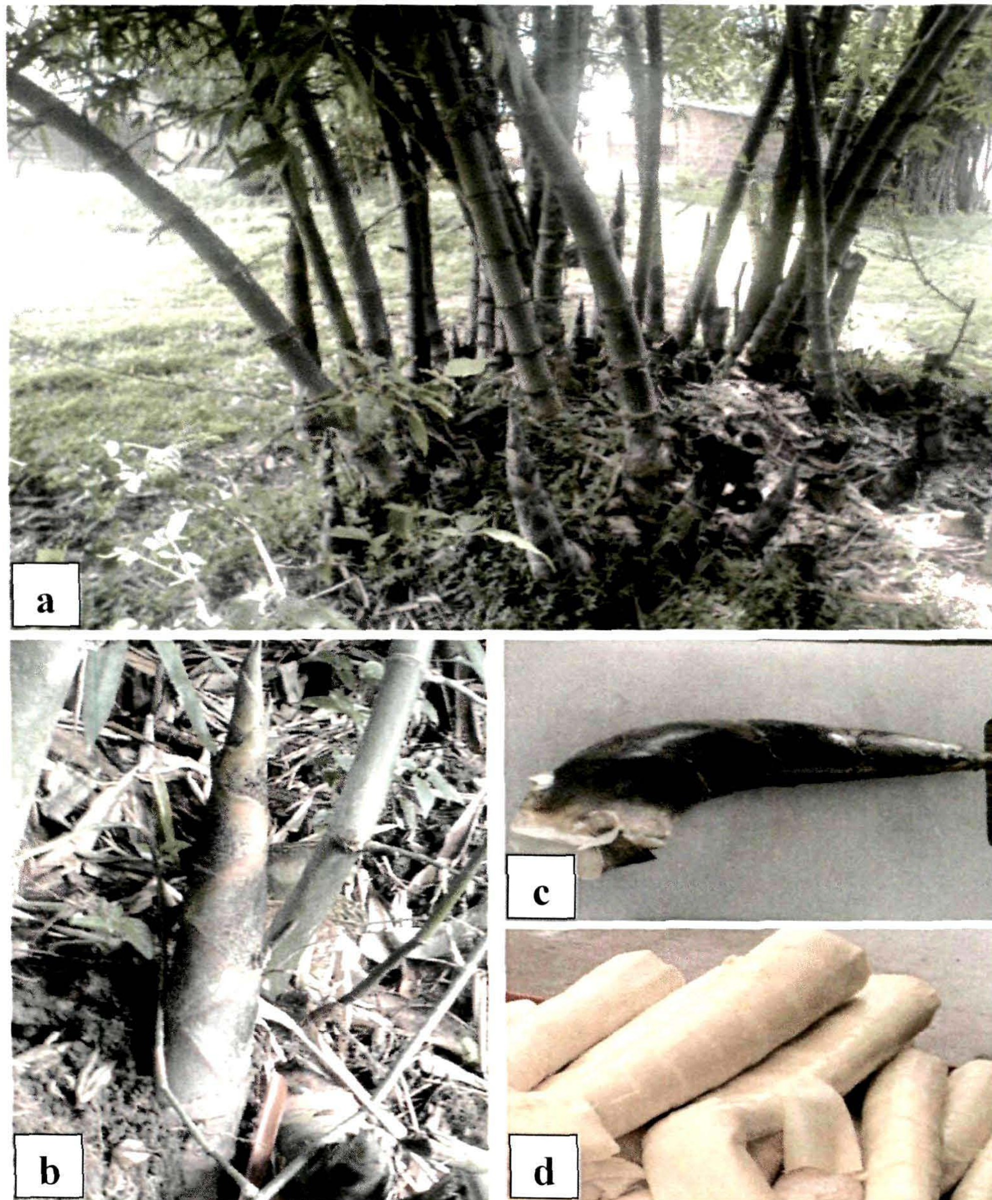

Fig. 2.1. Bamboo shoot (a) & (b) growing on bamboo plant, (c) harvested shoot and (d) peeled shoot

Table 2.1. Nutritional potential of various species of bamboo shoots

Name of species	Amino acids (g/100g)	Proteins (g/100g)	Carbohydrates (g/100g)	Starch (g/100g)	Fat (g/100g)	Vitamin C (mg/100g)	Vitamin E (mg/100g)	Ash (g/100g)	Moisture (g/100g)	Dietary fiber (g/100g)
B. bambos	3.98±0.02	3.57±0.03	5.42±0.02	0.25±0.04	0.50±0.02	1.90±0.08	0.61±0.05	1.38±0.03	89.83±0.08	3.54±0.02
B. kingiana	3.701± 095	3.57±0.08	5.45±0.12	0.34±0.03	0.35±0.03	2.10±0.12	0.50±0.10	1.38±0.23	90.00±1.02	4.490±0.06
B. nutans	3.89 ± 0.04	2.84±0.12	5.47±0.05	0.21±0.02	0.40±0.02	1.19±0.10	0.47±0.06	0.68±0.11	92.00±0.23	2.28±0.01
B. polymorpha	3.42±0.02	3.64±0.02	5.44±0.05	0.38±0.04	0.46±0.03	2.60±0.13	0.49±0.12	0.76±0.22	90.26±1.68	3.815±0.06
B. tulda	3.65±0.03	3.69±0.03	6.92±0.04	0.59±0.12	0.48±0.07	1.42±0.06	0.61±0.14	0.85±0.13	83.60±1.26	3.97±0.02
B. vulgaris	3.57±0.04	3.64±0.03	6.51±0.05	0.27±0.05	0.50±0.01	4.80±0.11	0.52±0.10	1.01±0.21	90.60±0.82	4.24±0.01
D. asper	3.12 ± 0.07	3.59±0.06	4.90±0.11	0.36±0.08	0.40±0.06	3.20±0.06	0.91±0.13	0.95±0.03	89.40±0.98	3.54±0.07
D. brandisii	3.01±0.11	2.31±0.12	4.90±0.10	0.49±0.04	0.24±0.10	1.59±0.10	0.42±0.10	0.61±0.11	89.80±0.15	4.03±0.09
D. giganteus	3.86 ± 0.13	3.11±0.17	5.10±0.04	0.51±0.06	0.39±0.03	3.28±0.02	0.69±0.03	0.89±0.13	90.70±0.12	2.65±0.03
D. hamiltonii	3.18±0.05	3.72±0.12	5.50±0.08	0.47±0.03	0.41±0.02	2.45±0.08	0.71±0.03	0.86±0.20	92.51±0.51	3.90±0.03
D.membranaceus	3.46±0.02	3.38±0.10	5.40±0.03	0.23±0.04	0.43±0.05	1.58±0.06	0.65±0.10	0.63±0.04	89.30±1.34	2.91±0.06
D. strictus	3.07±0.03	2.60±0.07	6.17±0.02	0.31±0.05	0.33±0.04	2.43±0.11	0.58±0.03	0.71±0.10	90.10±0.93	2.26±0.01
G. albociliata	3.52±0.11	3.05±0.11	4.59±0.09	0.31±0.04	0.51±0.10	1.00±0.08	0.60±0.04	0.73±0.04	89.23±0.30	4.15±0.11
G. rostrata	3.17±0.08	3.56±0.11	4.32±0.11	0.22±0.03	0.56±0.12	3.20±0.10	0.49±0.05	0.68±0.05	90.56±1.02	4.20±0.09

B = Bambusa; D = Dendrocalamus; G = Gigantochloa

Source: Chongtham, et al.[8]

2.1.2 Bamboo shoot processing

Bamboo shoots have high water content and thus are highly perishable. Moreover, since it is a seasonal product so it requires preservation to make the product available throughout the year. Bamboo shoots are tried to be preserved by using different methods like fermentation, canning, pickling, blanching, drying etc. It is consumed in the form of boiled, cooked, fermented and sometime roasted whole shoot.[1] These processing methods are applied basically for the preservation of the shoots so that shoots could be used for a longer time than usual. But many researchers such as Kumbhare and Bhargava,[9] Nirmala et al.[10] have reported out that all such kind of processing methods lead to the substantial decrease in nutritional values. While decrease in carbohydrate was noticed in case of fermented and canned products, decrease in reducing sugar has been noticed on boiling. The ash content decreased on boiling, fermentation and canning of bamboo shoots. Kumbhare and Bhargava[9] reported the decrease in crude protein level on various processing while decrease in amino acids has also been studied. Loss of free amino acids also takes place through leaching or may react with sugars to form complexes.[11] Loss of vitamins during processing is also obvious, while loss of trace elements like Cd, Co, Cu, Mg, Mn, Ca, Fe, K, P, Na and Se were also observed.

Bamboo shoots could be dried as it reduces the water content and make it unavailable for the microbes. Study on preservation of bamboo shoots by drying has been done by many researchers. Drying decreases water activity and moisture content which plays important role towards the activity of microorganisms, which is the prime cause of food deterioration. Muchtadi and Adawiyah[12] dried bamboo shoot (*D. asper*) in a cabinet dryer at 60^0C for 7-8 h and there was significant decrease in starch and ascorbic acid recorded. However, the colour of shoots dried using superheated steam gets darker than shoots dried using hot air even at same drying temperature.[13] Vacuum freeze drying is applied for preserving colour, aroma, taste and shape of foods; and product quality is comparatively good compared to convective hot airflow drying.[14] Madamba[15] reported a linear relationship between dimensionless volume change and moisture content during hot air drying of bamboo shoot. Shrinkage of bamboo shoot

parallel to its fibers was different from perpendicular to its fibers. Xu et al.[16] applied a two-stage hybrid drying techniques i.e. hot airflow drying followed by vacuum freeze drying and reverse of the process i.e. vacuum freeze drying followed by hot airflow drying on bamboo shoot for producing high quality product.

Bal et al.[3] studied the effect of microwave drying on the colour of bamboo slices. They found that the total colour change (dE) of bamboo shoot slices increased significantly during microwave drying with drying time. Increased in microwave power level the drying rate and the effective moisture diffusivity also increased.[17] Cheng[18] found that the combination of vacuum drying, hydro cooling and vacuum cooling have the advantages of lower number of bacteria, a higher stability, longer preservation period and better appearance of bamboo shoot. However, combination of these methods helps to increase the cooling speed and prolong the preservation length in low temperature storage of bamboo shoot. Other kind of preservation techniques viz., modified atmospheric packaging and packaging with other packaging material such as LDPE, PVC also tried on bamboo shoot.[2,19]

2.2 Blanching and its effect

Blanching is a unit operation and a method of preservation used widely in the agro-food sector and particularly important in the processing of vegetables. Its main objective is to inactivate the various enzymes involved in the spoilage of fresh vegetables. Blanching also softens the vegetable tissues to facilitate filling into containers and removes intracellular air which increases the density of food and prevents the oxidation of canned food.[20] Blanching is a short term heat treatment which serves a variety of function applied to vegetables prior to the processing. It imparts benefits such as to reduce the contaminating microorganism on the surface of vegetables and enhances the colour, texture and keeping quality of product. Despite its preserving advantage, it leads to nutrient degradation, particularly of vitamins and loss of colour. Duration and temperature of blanching inactivate particular enzymes; but over blanching might result in an undesirable loss of colour, flavour, texture and nutrient quality in addition to excessive energy requirement and water disposal.

Blanching at 95±3 °C significantly degrades the ascorbic acid and *β*-carotene in leafy vegetables. However, controlled blanching could contribute to retention of vitamins and nutrients in processed foods.[21]

The quality of blanched product depends significantly on the time and temperature of blanching. Industrial blanching process involves temperature ranging from 70^{0}C to 95^{0}C and time usually not higher than 10 min.[22] Inactivation of peroxidase was the best indicator to assess the efficiency of blanching of vegetables. It is more resistant to the heat as compared to the other microorganism. Inactivation of enzymes responsible for off-flavor and odour of the vegetables is very important to increase their shelf life. Blanching is done to achieve the stabilization of texture and mixture quality and destruction of microbial load. However, since blanching is a heat treatment that might affect the structural and integrity of the plant tissue as well as the nutritional components.[23]

The improvement in texture by the changes occurred in cell wall and middle lamella by the increase in enzyme activity (pectin methylesterase) during low temperature and long duration hot water blanching. It also helps in the wilting of leafy vegetables.[24] Some researchers have found that the blanching conducted at temperature higher than 80^{0}C catalyze the degradation of pectin due to elimination reaction and their solubilization from the cell wall and the middle lamella between adjacent cell wall.[25] The two most widespread and suitable initial operation involve passing food through an atmosphere of saturated steam or hot water bath. Blanching at different time and temperature combinations has different impact on antioxidant, total phenolic content and other nutrients.[26] Different studies have showed that steam blanching have advantages over hot water blanching as most of the soluble material leached out into the water during hot water blanching. Higher temperatures for shorter time intervals have minimal loss of nutrients including total solid, vitamin, amino acid etc.[27]

Green leafy vegetable reduces their antioxidant properties drastically during hot water blanching.[28] Gonçalves et al.[29] studied the kinetics of peroxidase thermal inactivation, total phenolic content degradation, colour and texture changes during blanching of carrot in a temperature range of 70–90 °C. Most of the changes were

described by a first-order reaction model. Song et al.[30] recommended that blanching at a high temperature and short time is significant, as they found minimal losses of nutritive components including sugars, vitamins B_1, B_2, and C, were minimal at 100 °C for 10 min compared to blanching at 80 °C for 30 min, 90 °C for 20 min. Blanching at 80 and 100 °C caused a significant reduction in firmness and colour of cabbage. However, the temperature effect followed the Arrhenius law, with activation energies for polyphenolic content, antioxidant capacity, colour and texture 9.22–11.5, 9.05–35.05, 15.73 and 33.8 kJ/mol K, respectively.[22]

2.3 Osmotic dehydration

2.3.1 Mechanism and importance

Osmotic dehydration is a process that is generally used for the partial removal of water of materials like fruits and vegetables and water diffusion takes place through a semi-permeable membrane. The main phenomenon observed during osmosis is mass transfer between food and surrounding solution.[31] During osmotic dehydration water is removed from fruits and vegetables with the help of hypertonic solution of sugar, salt or any other osmotic agent. The higher osmotic pressure of the hypertonic solution, force the water to move from the tissue into the solution by creating driving force. The two major counter-current flows occur during osmotic dehydration. In one flow water flow from the tissue into the solution and in another solute is transfered from the solution into the food.[15] Osmotic dehydration is an energy efficient method of partial dehydration, since there is no need for a phase change.[32] Osmotic dehydration is useful as a treatment for drying, preventing colour changes due to enzymatic oxidation and the loss of volatile compounds, and reducing the acidity and damage caused by the heat[33-34]

Osmotic dehydration could also be used as a pre-treatment to many processes like freezing, freeze drying, vacuum drying or air drying and it has been established as one of the most useful pretreatments for drying of fruit and vegetables. It could be carried out at ambient temperature, which helps to improve nutritional, sensorial and functional properties of food and minimum damage to texture, colour and flavour.[35]

Osmotic dehydration also helps to reduce the water activity of many food materials so that microbial growth will be inhibited.[36] The rate of water loss (WL) and solids gain (SG) during osmotic dehydration is depends on several factors such as solution concentration, temperature, contact time, level of agitation, sample size, solution to sample ratio etc.[37] However, the choice of solutes and its concentration depend on several factors, namely the effect on organoleptic quality properties, solute solubility, cell membrane permeability, its stabilizing effect and cost.[38]

2.3.2 Selection of solutes

The solutes used as osmotic agent affects the final product's taste, its organoleptic qualities, the preservative effect and the cost. Sugars and salts are two most widely used solute types for osmotic dehydration, with relevance for sucrose and sodium chloride.[39] Sometimes their mixture is also used but the concentrations used depend on the type of food material to be dehydrated. It has been reported earlier that combined solutions of these two substances could be used to enhance water removal with low solids gain by the products.[39-40] Generally salt is preferred for dehydration of vegetables and meat as sugar gives a candying effect which is not acceptable in case of meat and vegetables. Similarly sugar is used for the dehydration of fruits. Addition of small quantities of sodium chloride to osmotic solutions increased the driving force of the drying process and synergistic effects between sucrose and sodium chloride.[41] Apart from sugar and salt, other agents like corn syrup, glucose and fructose are also used.

2.3.3 Factors affecting osmotic dehydration

Osmotic dehydration is mainly used for the purpose of water removal and so the efficiency of the process depends on the rate and extent of water removed from the material during the process. The rate of diffusion of water from any material made up of such tissues depends upon factors such as: temperature and concentration of the osmotic solution, the size and geometry of the material, the solution-to-material mass ratio and the level of agitation of the solution.[42-43] These factors determine the extent to which the

different mass transport mechanisms act in the tissue and their influence on the overall mass transfer rate, solids gain, water loss, and also the structural changes.

The size and shape of the food sample also plays a major role in mass transfer due to different surface area or surface to thickness ratio. Agitation has a positive effect on the rate of diffusion also it helps to speed up the water removal process since agitation provides better contact between the sample and the solution. Agitation was used to reduce the mass transfer resistance at the surface of the carrots and to ensure good mixing and close temperature uniformity and control in the osmotic medium.[44] The solute used and its molecular weight also affects the process. The osmotic solute to be used generally should be of low molecular weight and it should have high dissolving property in water. Smaller the size of the osmotic solute, larger will be its penetrating property. Generally salt and sucrose are used. The salt presence promotes a faster osmotic dehydration of tissues than sucrose, due to its greater water activity depression power.[45-46] On the other hand sugar even though it is considered to be a good agent, because of its bigger size, it has less diffusivity and thus in comparison to salt, its effect is less.

Another factor is the immersion time. It has been seen from many studies that the water loss is linearly affected by immersion time.[39] Time has a significant effect on solids gain (sugar uptake) and moisture loss; while for the mass gain, time has no effect.[47] The sample to solution ratio also has significant effect on the osmotic dehydration process. Higher osmotic solution/fruit ratio favored higher moisture removal. The sample to solution ratio generally varies depending on the sample size. In osmotic dehydration of sugar beet in combined aqueous solutions of sucrose and sodium chloride in order to avoid dilution of osmotic solution and subsequent decrease of driving force for osmotic dehydration, the weight ratio between sample and osmotic solution was 1:10.[39,48]

2.3.4 Mass transfer during osmotic dehydration

During osmotic dehydration the dominant resistance to the mass transfer is semi-permeable cell membranes of fruits and vegetables. During this process the

material is soaked in different osmotic solution as per requirement. The difference of chemical potential between the components in the solution and the material leads to three mass transfer flows:

- Transfer of water from the material to the osmotic solution.
- Transfer of solutes from the solution into the material.
- Transfer of soluble solids from the material to the osmotic solution.

The last flux is often considered negligible, but can affect the organoleptic and nutritional characteristics of the product.[49]

Due to the difference of osmotic pressure inside and outside of the cells, part of mass transfer process takes place through the cell membranes. However, other mass transfer phenomena take place during the process is convective movement and diffusion of substances in the soaking solution and in the intercellular spaces filled with liquid in the material, liquid movement through the pores due to capillary forces, symplastic transport between cells, etc.[50-51]

Modelling of mass transfer process during osmotic dehydration is important for an adequate control of composition of the resulting dehydrated material and to reduce the experimental work. Various authors have used different approaches for modelling the osmotic dehydration process. Panagiotou et al.[52] used an empirical approach, in which the mathematical equations fitted to experimental data to obtain mass transfer coefficients. However, by considering only internal resistance to mass transfer, and using Fick's second law of diffusion, effective diffusion coefficients could be obtained for the diffusing substances.[46] The rate of diffusion of water from any material depends upon various factors such as temperature, concentration of the osmotic solution, size and geometry of the material, solution to material ratio and the level of agitation of the solution.[53]

Sometime during mass transfer modelling different assumptions are considered, like any finite food geometry as infinite flat plate configuration, neglecting the diffusion in the other directions and out of these only a few have considered unsteady state mass transfer during osmotic dehydration. Such assumptions hold good when thickness is

very small as compared to sides indicating negligible peripheral diffusion, but practically it is impossible. Therefore it is important to take the peripheral diffusion by considering food piece as rectangular parallelepiped rather than infinite plate. Such work is done on pineapple and carrot.[54-56]

The following unsteady state Fickian diffusion model can be applied to describe the osmosis mechanism for infinite flat plate (Eq. 2.1).

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial Z^2} \qquad (2.1)$$

With the following assumptions and boundary conditions,

$C = C_0$ at $t = 0$; $-l < x < +l$ and $C = C_1$ at $t > 0$; $x = l$

Where, C_0 and C_1 are the initial and bulk concentrations, respectively.

The solution of Fick's second law for diffusion from a rectangular parallelepiped of sides 2a, 2b and 2c (cube is a special case when all the sides are equal) results in the following well-known equations for the transfer of water and solute (Eq. 2.2 & 2.3), respectively.[57]

$$M_r = [(m_t - m_\infty)/(m_o - m_\infty)] = \sum_{n=1}^{\infty} C_n^3 \exp\{-D_{cw} t q_n^2[(1/a^2)+(1/b^2)+(1/c^2)]\} \quad \ldots \quad (2.2)$$

and

$$S_r = [(s_t - s_\infty)/(s_o - s_\infty)] = \sum_{n=1}^{\infty} C_n^3 \exp\{-D_{es} t q_n^2[(1/a^2)+(1/b^2)+(1/c^2)]\} \qquad (2.3)$$

Where, M_r and S_r are the moisture and solute ratio; the subscripts o, ∞ and t represent relevant initial concentrations, at equilibrium, and at any time; D_{ew} and D_{es} are the effective diffusivity of water and solute, respectively, and C_n is equal to $2\alpha(1+\alpha)/(1+\alpha+\alpha^2 q_n^2)$ where, q_n's are the nonzero positive roots of the equation $\tan q_n = -\alpha q_n$. Here α is the ratio of volume of solution to that of each piece.

Considering only the first term of the above equations to be significant and other terms to be negligible (which can be done when the Fourier number = $D_e t/A^2$ value is

more than 0.1, and A being defined below), the equations reduces to the following equations (Eq. 2.4 & 2.5).[54,58]

$$-\ln (M_r/C_1^3) = q_1^2[(D_{ew}t)/A^2] \quad (2.4)$$

$$-\ln (S_r/C_1^3) = q_1^2[(D_{es}t)/A^2] \quad (2.5)$$

Where, $1/A^2 = [(1/a^2) + (1/b^2) + (1/c^2)]$. The values of D_{ew} and D_{es} can be calculated from the slopes of the regression lines obtained by plotting $-\ln (M_r/C_1^3)$ and $-\ln (S_r/C_1^3)$ against t.

Calculated D_{ew} and D_{es} values can be fitted to Arrhenius type of equation to calculate activation energy (Eq. 2.6 & 2.7).

$$D_{ew} = D_{ow} \exp(-E_{aw}/RT) \quad (2.6)$$

$$D_{es} = D_{os} \exp(-E_{as}/RT) \quad (2.7)$$

Where, D_{ow} and D_{es} is the reference diffusivity of water and solute at infinitely high temperature, R is the ideal gas constant (J/mol K), T is temperature (K), E_{aw} and E_{as} are the respective activation energy (J/mol).

Above equations could be represented in a linear form and activation energy could be obtained from the slope of the resulting straight line.[59]

$$\ln D_{ew} = \ln D_{ow} + (-E_{aw}/RT) \quad (2.8)$$

$$\ln D_{es} = \ln D_{os} + (-E_{as}/RT) \quad (2.9)$$

2.3.5 Effect of centrifugal force and vacuum pressure on osmotic dehydration

Application of centrifugal force and vacuum pressure to enhance osmotic dehydration process is a new trend in food processing. Azaura et al.[60] showed that application of centrifugal force enhances the water loss from potatoes and apples and limits the uptake of solids undergoing osmotic dehydration. Amami et al.[45] reported that the centrifugal force permits the better dehydration (higher WL) and limits the solids uptake (smaller SG) compared to general osmotic dehydration. However, centrifugal force, in combination with pulsed electric field and salt addition, significantly enhances

water loss during osmotic dehydration of carrots, but decreases SG, rehydration capacity, and firmness of the rehydrated tissue.

The osmotic process can be performed at atmospheric pressure or with vacuum pulse application for a small period at the beginning of the process. Due to application of vacuum the water loss and solid gain are higher during start of the process.[61-62] By applying vacuum pressure, an outflow of internal gas or liquid from the tissue and the entrance of external solution are established that promotes water loss and the uptake of external solutes.[63-64] The kinetics of osmotic dehydration under vacuum are reported to be quicker than at atmospheric pressure.[65-66] Rastogi and Raghavarao[67] also found the same result and concluded that vacuum affects only the rate at which the equilibrium is attained and not the equilibrium osmotic pressure as such.

Corrêa et al.[68] stated that the effects of pressure conditions on the mass transfer kinetics were clearly observed only with the application of the vacuum pulse during 15 min at the beginning of the process. Several studies have shown that when a vacuum pulse is applied for 5 to 10 min the mass transfer rate can increase when atmospheric conditions are re-established.[69-71] However, Fermín and Corzo[72] found that a vacuum pulse of less than 300 mbars applied during osmotic dehydration had no significant effect on solid gain (SG) and weight loss (WL) of melon (*Cucumis melo* L.). Shi et al.[65] performed same kind of experiment to check the diffusivity and porosity of the sample and concluded that Osmotic dehydration under vacuum makes it possible to obtain a higher diffusional rate of water transfer at lower solution temperatures, and that fruits with higher porosity are more suitable for treatment with vacuum treatment.

According to Maneepan and Yuenyongputtakal,[73] the operation is carried out in two steps after product immersion in a container during the liquid phase. In the first step, vacuum pressure is imposed on the system for a short time in the closed container, thus promoting the expansion and outflow of internal gases in the product. In the second step, atmospheric pressure is restored in the container leading to a great volume reduction of the gas remaining in the pores, and thus to the subsequent influx of external liquid into the porous structure.[74] The application of vacuum osmotic dehydration (VOD) can reduce the process time and energy costs. Pulsed-vacuum osmotic dehydration (PVOD),

a variation of VOD, consists of the use of an initial VOD process for different periods followed by the application of osmotic dehydration at atmospheric pressure.[75] Moreover, the application of a vacuum in osmotic dehydration requires an understanding of how the mass transfer, physical properties and cell structure are affected by varying the vacuum pressure level and the vacuum pre-treatment method. A sound understanding of these factors is important for the successful application of the osmotic dehydration process, for efficient treatment and it can be beneficial to the food industry.

2.4 Fermentation of bamboo shoots

2.4.1 Methods of fermentation and its microbiology

Fermented foods are not only attractive and palatable in terms of flavour, aroma, texture, and appearance but are also rich in nutrients and good for digestion.[76] Fermented bamboo shoots are popularly consumed by ethnic people living in Himalayan regions, Nepal and Bhutan.[77] In India, the fermentation of bamboo shoots has extensively been carried out in the states of Manipur, Meghalaya, Sikkim, Mizoram, etc. since ancient times.[78-79] Traditionally bamboo shoots are fermented by grating the shoot and keeping in earthen pot for few days. At the end of fermentation, shoot becomes brown in colour and develops characteristics flavour and taste. In Manipur sliced shoots are dried in sunlight for 10 to 15 min and kept in earthen pot for 2 to 3 months for fermentation by adding small amount of water and salt. Then shoots are taken out and dried in sunlight to about 50% moisture content. In Arunachal Pradesh shoots are chopped and put in bamboo basket, covered tightly with banana leaves for 6-8 days fermentation. In another process, sliced shoots are kept in bamboo basket containing *ekkam* leaves at bottom. The slices covered tightly with *ekkam* leaves and basket is kept for 5-6 days in summer and 8-10 days in winter for fermentation.[80] However, method and length of fermentation depends on native communities, tribes, country and product desired. In Nepal, bamboo shoots are fermented with oil and turmeric and then cooked with potato.[4] *Jiang-sun* is fermented bamboo shoots product, which is a widely used traditional food in Taiwan. Some popular ethnic fermented bamboo shoots products of India are given in Table 2.2.

Lactic acid bacteria are mainly responsible for fermentation of bamboo shoots. Tamang and Sarkar[81] investigated the dominant microorganism viz. *Lactobacillus plantarum, L. brevis* and *Pediococcus pentosaceus* in mesu. The mesu fermentation is initiated by *P. pentosaceus*, followed by *L. brevis*, and finally succeeded by *L. plantarum* species. *L. plantarum* is the main LAB present during the fermentation of jiang-sun.[82] The population distribution of dominant species in Soidon were *Bacillus subtilis* 29.3%, *Bacillus cereus* 35.7%, *Bacillus pumilus* 2.6%, *Lactobacillus brevis* 9.6%, *Lactobacillus plantarum* 5.1%, *Carnobacterium sp.* 11.9%, *Enterococcus faecium* 1.2% and *Pseudomonas.*[83] However, predominant functional LAB strains associated with the fermented bamboo shoot products of Northeast India (viz. mesu, soidon, soibum and soijim) were identified as *Lactobacillus brevis, L. plantarum, L. curvatus, Pediococcus pentosaceus, Leuconostoc mesenteroidessubsp. mesenteroides, Leuc. fallax, Leuc. lactis, Leuc. citreum and Enterococcus durans.*[84] *Ekung, eup* and *hirring* are some common indigenous fermented bamboo products of Arunacla Pradesh, Northeast India. Tamang ang Tamang[85] isolated *Lactobacillus plantarum, L. brevis, L. casei, L. fermentum, Lactococcus lactis, and Tetragenococcus halophilus* for these products and studied their functionality.

Some film yeast viz., *Saccharomyces cerevisiae* J1, *Candida krusei* J2 and *Candida krusei* J3 etc. were isolated from fermented bamboo shoots of Thailand. All the species tolerated 2.5% NaCl concentration and clove extract (3% w/v) inhibited all yeast strains within 12 h. At low concentration of 0.75% (w/v) clove extract could inhibit film yeast in fermented bamboo shoot.[86] Lactic acid bacteria strain *Enterococcus faecalis* N1-33, isolated from edible fermented bamboo shoot, displayed inhibitory activity against other lactic acid bacteria and other Gram-positive food spoilage and pathogenic bacteria. The bacteriocin-activity and ability of metabolizing low cost carbohydrates of strain N1-33 is having great potential for use in food biopreservation.[87]

Table 2.2. Fermented bamboo shoot product of India

Regions	**Local Name of Products**
Sikkim	*Mesu*
Manipur	*Soidon, Soibum* and *Soijim*
Arunachal Pradesh	*Ekung, Eup, Hikhu, Hiring* and *Hithyi*
Meghalaya	*Lung-siej or Syrwa*
Orissa	*Kardi, Handua*
Tripura	*Godhak*
Assam	*Khorisa, Miyamikhri*

Sources: Tamang & Tamang 2009;[88] Mao & Odyuo 2007;[89] Jeyaram et al. 2009;[78] Panda & Padhy 2007[90]

2.4.2 Influence of fermentation on bamboo shoots

Nirmala et al.[10] studied the changes in nutrient components in shoots (*Dendrocalamus giagnteus*) after fermentation and canning. The freshly harvested shoots were richer in nutrient components as compared to canned and fermented shoots. Fresh shoots have higher quantities of macronutrients such as amino acids, proteins, carbohydrates, fat and fibre than the fermented and canned shoots except vitamins (C and E) and mineral elements like calcium, iron, potassium and phosphorous. Nutritional profiles for fresh and fermented shoot are given in Table 2.3.

Carbohydrate, an ideal source of energy was found to be decrease by 70% after fermentation of shoot. Ash content slightly decreased during fermentation. The crude fibre content increased significantly during fermentation. However, fermented shoots contained more amount of acid detergent fibre (3.28 g/100 g fresh wt) and lignin compared to the raw shoots. The fermented shoots have higher amounts of cellulose (18.5%) than the raw shoots. Fermentation also leads to decrease in crude protein, amino acid and fat. Vitamin C and E content in fermented shoot reported as 1.090 and 0.210 mg/100g which was significantly less compared to fresh shoot. However, fermented shoots showed same amount of Cd, Co, Mn, Ni, P and Se content as in the raw shoots.

Table 2.3. Comparisons of fresh and fermented shoot of *D. giganteus* (g/100g)

Nutrients	Fresh shoots	Fermented shoots
Amino acids	3.863	2.005
Proteins	3.108	2.570
Carbohydrates	5.103	1.504
Moisture	90.70	88.83
Fat	0.387	0.315
Ash	0.890	0.780
Starch	0.506	0.455
Nutrient dietary fibre	2.645	4.180
Acid Detergent Fibre	2.150	3.280
Lignin	0.560	1.398
Hemicellulose	0.495	0.900
Vitamin C (mg/100g)	3.280	1.090
Vitamin E (mg/100g)	0.690	0.210

Source: Nirmala et al. 2008[10]

Changes in nutritional value of fermented bamboo shoot (*soibum*) of Manipur from raw shoots (*Bambusa tulda, Dendrocalamus giganteus* and *Melocanna bambusoides*) were studied by Giri & Janmejay.[91] The process caused depletion of several amino acids, formation of diacetyl, acetoin, volatile phenols and esters, liberation of free phenols, and complete disappearance of ascorbic and aspartic acids. *Soibum* is also rich in dietary fibre, low content of lipid and absence of trans fatty acid indicated its health promoting nature. Fermentation also showed to reduce the amount of reducing sugar to a great extent by converting them to acid and leads to rises in acidity. Significant amount of K, Na, Cl, Mn, Cu etc. were present in *Soibum.*[92]

Effect of pickling process on amino acid contents, texture, pectin and microstructure of bamboo shoots were studied by Zheng et al.[93] The amino acid content of fresh bamboo shoots decreased from 16.35 g/100 g of dry weight to 6.89 g/100 g and

7.91 g/100 g of dry weight with pickling of bamboo shoots with different salt concentrations (8% and 20%) respectively. The texture of bamboo shoot decreased by 60% and 47% after 90 days of pickling process for both salt concentrations and protopectin contents of such shoot decreased by 64% and 49%, respectively.

Fermented bamboo shoots are an enriched source of phytosterol. Sarangthem & Singh[94] isolated microorganisms from the fermented bamboo shoot (soibum exudates) which involved in microbial bioconversion of phytosterol during fermentation. Those microorganisms were *Bacillus subtilis, B. licheniformis, B. oagulans and Micrococcus luteus*. Concentration of phytosterol was reported to increase from 0.18 to 0.61 % dry wt during fermentation of *Bambusa balcooa* shoots. However, all the isolated microorganisms were responsible for bioconversion of metabolites from fermented bamboo shoots into phytosterols.

Fu et al.[95] analyzed volatile aroma active components in fermented bamboo shoots using gas chromatography and mass spectrometry. Static and dynamic headspace extractions of volatile compounds were conducted by solid phase microextraction (SPME) and by cryogenic focusing purge and trap extraction. The gas chromatography detected a total of 29 aroma-active peaks were in bamboo headspace. The 10 most important components were p-cresol, methional, 2-heptanol, acetic acid, (E,Z)-2,6-nonadienal, linalool, phenyl acetaldehyde, and three unknowns. Also SPME and purge and trap extraction method identified 70 various volatile compounds, which includes acetaldehyde, 4-ethylbenzaldehyde and several others.

Bamboo shoot contains cyanide in varying proportions. It contain up to 0.16% total cyanide in the tip and 0.01% in the base.[96] However, cyanide content is reported to decrease substantially following harvesting. Different modern and traditional bamboo shoot fermentation methods help to decrease the cyanide contents. Adi women of Arunachal Pradesh use banana leaves for semi-fermentation of shoots and pressed under stones near water stream for 3-4 months to reduce bitterness.[6]

Fermented bamboo shoots also use as preservative in nuggets prepared from desi spent hen for extending its shelf life. Lean meat of desi spent hen was minced and blended along with other non-meat ingredients and fermented bamboo shoot (10%). The

emulsion stability, cooking yield, moisture, crude protein, total ash and sensory score of nuggets added with fermented bamboo shoots were reported significantly high ($p<0.01$) compared to the normal (control) nuggets. However, storage studies of nuggets showed lower value of pH and thiobarbituric acid as well as total plate count, psychrophillic count and yeast and moulds counts were reported less in fermented bamboo shoots treated nuggets in comparison to control products.[97]

2.4.3 Traditional fermented bamboo shoot products of Assam

Microbial preservation of bamboo shoots refers to extend in storage life and enhanced safety of foods using the natural micro flora and their antibacterial compounds. The people of Assam have been using Lactic acid fermentation of bamboo shoots to enhance their shelflife without the aid of modern amenities like refrigeration, canning and vacuum packaging. Fermentation of bamboo shoots extends their shelflife for over a year or even sometimes more. The fermented shoots are used in local cuisines, as medicine and in pickle making. These techniques of bamboo shoot fermentation have been perfected over hundreds of years based on trial and error. The local people might not be able to explain the scientific side of these processes like the biochemistry or the microbiology, but, they know how to provide favourable conditions for the fermentation and thereby promote beneficial microbial growth for getting the desired fermented product. These products include *Khorisa, Poka Khorisa, Khorisa Pani, Kahudi* and *Miyamikhri*. Traditionally bamboo shoot fermentation is done in earthen pots as shown in Fig. 2.2. Bamboo has been a very significant resource plant in the life of the ethnic populace, its usage extending and visible in every aspect of their existence in this region. Such usage is also observed at every place wherever bamboo is available as a natural resource.

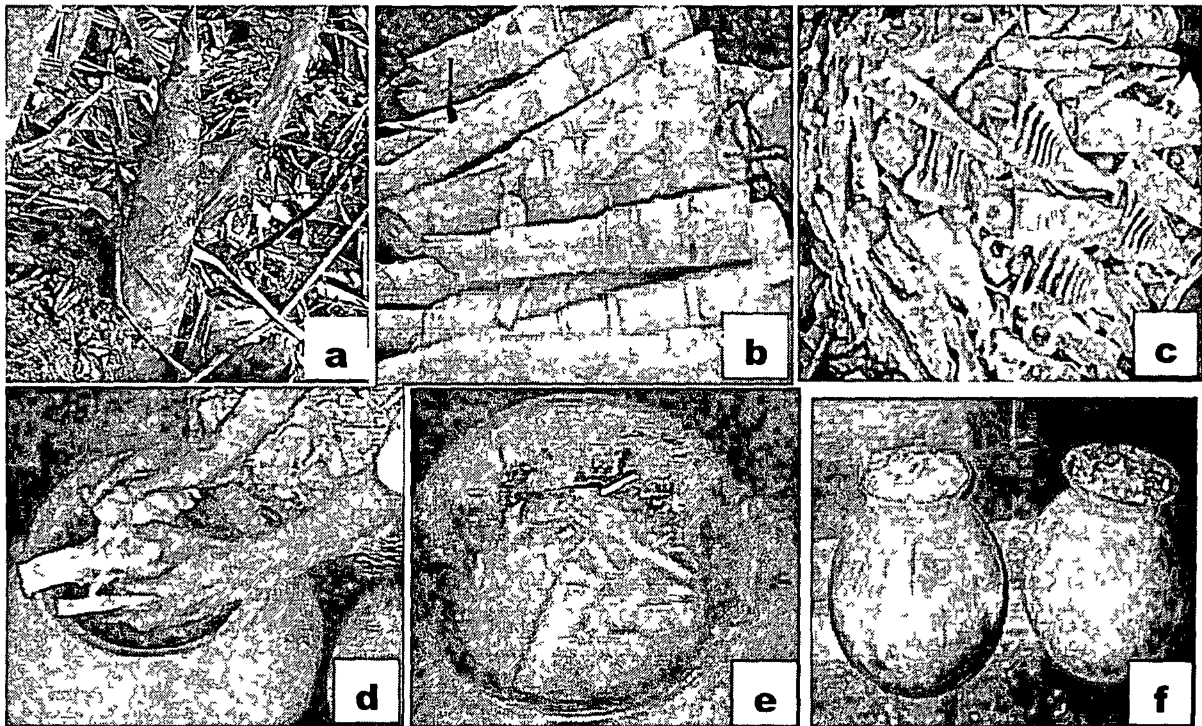

Fig. 2.2. Bamboo shoot fermentation, (a) Young bamboo shoots; (b) Peeled bamboo shoots; (c) Sliced bamboo shoots; (d) Slices filling in earthen pots; (e) & (f) Fermentation in earthen pots

2.4.3.1 ***Khorisa***

Khorisa is an ethnic fermented bamboo shoot of Assam. It is produced during the monsoons, when bamboo shoots are available. The process of making *khorisa* is quite similar to some of the other bamboo shoot fermentation techniques of the Northeastern regions. The bamboo shoots are harvested and then the outer surface is peeled off and the white inner part is used for making *khorisa.* The bamboo shoots are then washed and hammer-milled in a traditional wooden husking pedal called as *dheki.* It breaks down the bamboo shoots into mash of pulp. This is then packed inside earthen pots. The pots are smoked prior to packing In some regions small dried pieces of *Garcinia pedunculata* Roxb , locally known as *borthekera* are mixed along with the bamboo shoot pulp as an acidifier.[78] Additionally, dried chillies are also placed inside the earthen pots along with small amount of water. All the ingredients are mixed and

mildly pressed into the earthen pots and then mouth is tied with banana leaves (Fig. 2.3(a)). The entire system is made facultatively anaerobic. It is then allowed to ferment naturally for a period of 4-12 days depending upon shoot species, regions and locality. A mild acidic taste and sour smell indicate the completion of fermentation, and the entire pulp is removed from the pots. The excess water is removed by pressing and is sun dried for 2-3 days. When the moisture content reduces substantially and the product becomes crispy, it is stored in jars for further use. It is used in the traditional cuisines like fish, meat, and sweets. The cuisines cooked with *khorisa* are a good appetizer for the indigenous population (Figure 3a). The method of *khorisa* preparation is somewhat similar to *soidon,* a fermented bamboo shoot product of Manipur and group of Lactobacillus species are mainly responsible for fermentation of bamboo shoot.[88]

2.4.3.2 *Poka khorisa*

Poka khorisa (*khorisa tenga*) is also an ethnic fermented bamboo shoot of Assam. It is whitish in colour with a faint aroma and sour taste. However, it is not dry and crispy like *khorisa* and moist in nature. The smell and taste of *poka khorisa* is a real appetizer for the indigenous population of Assam. Locally grown young edible bamboo shoots of Bhaluka baah (*Bambusa balcooa*), Kako baah (*Dendrocalamus hamiltonii*) are defoliated, hammer-milled in a traditional wooden husking pedal called as *Dheki.* The bamboo shoots are then mixed with dried *Garcinia pedunculata* Roxb. *(Borthekera)* and dried chillies, and are packed inside pre-roasted earthen pots and pressed mildly. The mouth of the earthen pot is tied with banana leaves, and is left to ferment anaerobically for 4-12 days. Completion of fermentation is indicated by the typical *poka khorisa* smell. The pulp is taken out and the excess water is soaked out by pressing, and then the solid fermented product is stored in jars. It is used in cuisines, pickle making and as medicine. *Poka khorisa* is also mixed with edible oils, chillies and salt (pickled) and can be kept in closed containers for up to two years. The non-pickled fermented *poka khorisa* can also be kept in closed jars for more than a year. Like *khorisa,* group of Lactobacillus species are mainly responsible for *poka khorisa* preparation (Fig. 2.3(b)).

2.4.3.3 *Khorisa pani*

Khorisa pani is another ethnic fermentation product of Assam. However, it is not solid in nature like *khorisa* or *poka khorisa*. It is liquid in nature and has a sour acidic taste, similar to *poka khorisa* (Fig. 2.3(c)). It is produced during the fermentation of bamboo shoots. When bamboo shoots are fermented in earthen pots, a sour liquid is produced. When the fermentation process is stopped the produced liquid is collected in bottles and is used in making curry, meat, sour fish curry, etc. The liquid however, does not stay good for more than 7 days. The liquid is usually used up within a week, and any remaining liquid is discarded. The *khorisa pani* is also thought to possess medicinal properties. This liquid is fed to children who suffer from measles or chicken pox. It is believed that, the liquid helps in quick de-pigmentation of pox marks.

2.4.3.4 *Kahudi*

Kahudi is one of the traditional fermented bamboo shoot products mainly consumed by people of the river island Majuli of Assam. Mustard seeds are kept submerged in *khorisa pani*, the sour liquid that is collected after fermentation of *poka khorisa*. The seeds are kept submerged for 3-4 days in the liquid. Then it is taken out and sun dried for a day and then mixed with *khorisa pani* again and then blended into a paste (Fig. 2.3(d)). The paste is then transferred to a vessel and can be consumed up to 6 months.

2.4.3.5 *Miyamikhri*

Miyamikhri is one of the traditional fermented bamboo shoot product mainly consumed by tribes of North Cachar Hills district of Assam. The young edible bamboo shoots are defoliated and made into small pieces. These small pieces are wrapped in banana leaves and then put in earthen pot to ferment for about 4-5 days (Fig. 2.3(e)). When typical *miyamikhri* smell comes out it is shifted to a glass vessel. The local people use it for a year even as a pickle or mix with curry and serve it.[98]

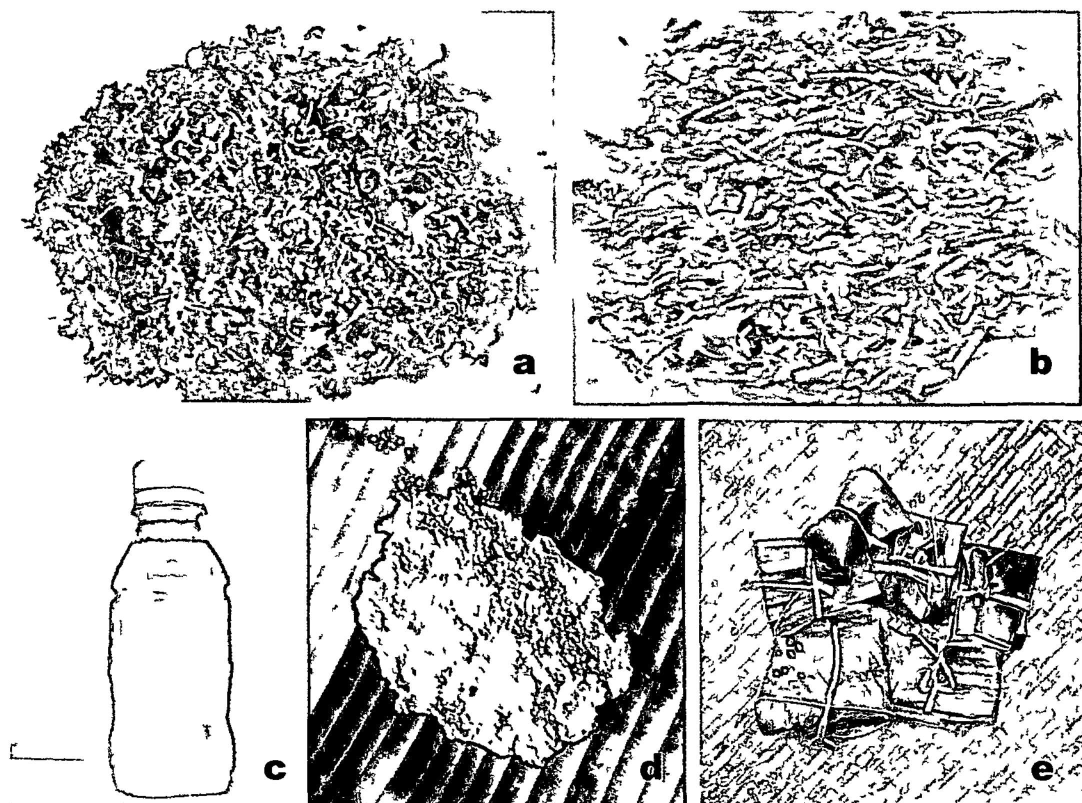

Fig. 2.3. Fermented bamboo shoot products of Assam, (a) *Khorisa*, (b) *Poka Khorisa,* (c) *Khorisa pani,* (d) *Kahudi* and (e) *Miyamikhri*

2.5 Antimicrobial metabolites

2.5.1 Bacteria as biopreservative

Recent approaches in preservation technology are directed towards alternative preservation offered by natural biochemical or biological systems or combinations of biological systems with a much reduced use of salt and chemical additives. This approach is in line with trends in consumer and industry demands for food products with less chemical preservatives.[99] However, researcher searching for bacteria with new antimicrobial properties that could be used as protective starter cultures for foods, as probiotic and/or antibiotic properties.[100] Antimicrobial metabolites of fermentation starter culture microorganisms that have the potential for use as biopreservitives in many foods that otherwise could be spoiled or involved in foodborne diseases from the growth of undesirable microorganisms. The successful fermentation of food depends on the production of antimicrobial metabolites by starter cultures. The antimicrobial activity of food-grade bacteria has long been attributed to the production of metabolites such as organic acids, hydrogen peroxide, ethanol and diacetyl. It has gradually become clear, however, that additional metabolites often contribute to the antimicrobial capacity of starter cultures.[101]

Various bacterial cultures and its metabolites are identified as antimicrobial effects against *Listeria monocytogenes* and spoilage organisms, and could be used as food preservatives.[99] Not only food but soil of Northeast India's, Himalaya and other countries also used for isolating microorganisms which will found an interesting source of antibacterial and antifungal bioactive substances.[102-104]

2.5.2 Antimicrobial potential of lactic acid bacteria

Lactic acid bacteria (LAB) are naturally found in various food items specially in fermented vegetables, milk, meat and other traditional fermented food products in order to improve the flavour and texture of the product. They have been shown to enhance the stability and nutritional value of food products by preventing the growth of pathogenic and spoilage microbes. Therefore, they have traditionally been used as natural

biopreservatives of food and feed. An important property of lactic acid bacteria has been their ability to elaborate certain antimicrobial proteins known as bacteriocins. These bacteriocins are having potential usefulness as natural substitute for chemical food preservatives in the production of foods with enhanced shelf life and safety. Biopreservation refers to extended shelf life and enhanced safety of foods obtained by using the natural or added microflora and their antimicrobial products.[105-107]

The preservative effect of lactic acid bacteria during the manufacture and subsequent storage of fermented foods is mainly due to the acidic conditions that they create in the food during their development. This souring effect is primarily due to the fermentative conversion of carbohydrates to organic acids (lactic and acetic acid) with a concomitant lowering of the pH of the food, an important characteristic that leads to an increased shelflife and safety of the final product.[108] Lactic acid bacteria are capable of producing a variety of antimicrobial substances, such as organic acids, alcohols, carbon dioxide, diacetyl, hydrogen peroxide and bacteriocins. These substances are antagonistic to a wide spectrum of microorganisms, and thus can make significant contributions to their preservative action.[109-110]

The addition of plantaricin LP84, a bacteriocin produced by *Lactobacillus plantarum* to idli batter at 1% (v/w) level was able to retard the growth of the pathogenic microorganisms viz. *Bacillus cereus, Escherichia coli and Staphylococcus aureus* etc.[111] Røssland et al.[112] produced antimicrobial metabolites by strains of *Lactobacillus* or *Lactococcus* which were effective against *Bacillus cereus* in milk. Also, *Lactobacillus plantarum* isolated from raw Tenerife goats' cheese were also screened for the production of antimicrobial substances and the bacteriocin (plantaricin TF711) was active against the Gram-positive bacteria *Bacillus cereus, Clostridium sporogenes* and *Staphylococcus aureus*; and against the *Enterobacteriaceae Shigella sonnei* and *Klebsiella pneumoniae.*[113] Zhang et al.[114] assessed the antimicrobial activity of lactobacilli strains isolated from traditional Chinese fermented foods towards *Shigella sonnei, Escherichia coli* and *Salmonella typhimurium.* Bacteriocin produced by *Pediococcus pentosaceus* K23-2 isolated from Kimchi, a traditional Korean fermented

vegetable is heat stable and shows wide antimicrobial activity against Gram-positive bacteria, especially *Listeria monocytogenes.*[110]

2.5.3 Metabolites extraction and purification

Burianek and Yousef[115] developed a solvent extraction method to concentrate bacteriocin (lacidin) from the culture of *Lactobacillus acidophilus* OSU133. The culture supernatant fluid was mixed with different organic solvents viz., hexane, ether, chloroform, isopropanol, chloroform–isopropanol, and chloroform–methanol etc. The study shows the rapid and efficient separation of bacteriocin from culture supernatant fluid by chloroform and is effective than other solvents.

2.6 Microwave assisted extraction

Microwave assisted extraction (MAE) is a relatively new extraction technique, which utilizes microwave energy to heat the solvent and the sample to increase the mass transfer rate of the solutes from the sample matrix into the solvent.[116] MAE has a number of advantages, e.g., shorter extraction time, less solvent, higher extraction rate and lower cost, over traditional method of extraction of compounds from various matrices, especially natural products. This is cost-effective extraction methods which could be combine with advance techniques like pressurized microwave assisted extraction (PMAE) and solvent free microwave assisted extraction (SFMAE).[117]

Various conventional extraction techniques viz., liquid–liquid extraction, solid–liquid, soxhlet extraction etc. are popularly used for extraction and analytical purpose.[118-120] However, these conventional extraction methods are time consuming and the efficiency of extraction is very low. However, other techniques such as supercritical fluid extraction, pressurized liquid extraction, ultrasound assisted extraction, pressurized hot water extraction and microwave assisted extraction are mostly used for extraction to enhance extraction efficiency and yield. But for all these techniques elevated temperatures and pressures are needed to improve the overall extraction efficiency.[121]

There are two microwave technologies, namely (i) closed vessels (under controlled pressures and temperature) and (ii) open vessels (under atmospheric pressure). In closed vessels, the solvent is heated above its boiling point at atmospheric pressure to enhance its extraction rate and efficiency. This system allows temperature control and also has high sample throughput as several vessels could be used in a single extraction process. They are placed on a turntable to ensure homogenous heating. The main drawback is that volatile solutes could partition into headspace. Therefore, the vessels must be cooled to room temperature before opening to avoid loss of volatile solutes. This step increases the overall extraction time. In open systems, the maximum extraction temperature is determined by the boiling point of the solvent at that pressure.[121]

MAE is a simple, cheap procedure than solvent extraction method, and also has less polarity limitations for the extractant. It offers higher degree of reproducibility, simplified manipulation, shorter extraction time, lesser use of solvent and high extraction rate compared to conventional solvent extraction methods.[122] Conductive and convective processes to heat the sample is used in conventional solvent extraction methods, whereas microwave heating occurs by direct energy transfer to the sample.[123-124] Microwave heating is volumetric in nature so microwave irradiation efficiently produces internal heating by coupling microwaves with polar components inside the solvent and the sample. According to the cell-wall broken theory,[125] there are certain solvents which are microwave transparent, while some are microwave absorbing. By using microwave transparent solvents, there is more energy for the plant material to absorb. Cellular structures contain water, which absorbs the microwave energy. This creates a sudden increase in temperature, results in the rupture of the cell wall and release of constituents into the surrounding solvent. Several studies have also used non-polar solvents which are transparent to microwave and in these cases only the sample matrix gets heated leading to release of analytes in a cold solvent.[126] This shows the higher extraction of polyphenolic compounds in acetone compared to methanol, ethanol or water.[127] Higher extraction of polyphenolic compounds was observed, when solvent polarity was modified by addition of water in the solvent.[128] Microwave extraction shows promising advantages over conventional solvent extraction system and is an

efficient method for extracting active biological compounds.[129-133] Polyphenolic compounds from waste peanut shells,[134] grape seeds,[128] citrus mandarin peels[135] and tea leaves[127] have also been successfully extracted by MAE technique.

2.7 *Garcinia pedunculata* Robx.

Garcinia pedunculata Robx. (GPR) is a globose shaped fruit with fleshy aril, found mostly in the states of Northeast India. It belongs to the genus *Garcinia* and family Clusiaceae (Guttiferae). The mature fruit is eaten cooked or raw and is locally known as Borthekera in Assam, a Northeastern State of India. The fruit of GPR is roundish red brown with a diameter ranging between 8 to 12 cm and juicy interior with edible arils.[136] The fruit usually matures during the month of April and is collected, cut into small pieces and sun dried. Dried pieces of the fruit are stored and used by the indigenous people throughout the year. The raw and dried GPR is shown in Fig. 2.4

Fig. 2.4. Raw and dried fruit of *Garcinia pedunculata* Robx.

The indigenous people of Northeast India use it for various medicinal uses. One survey showed that, among different plants GPR is popularly used in treating diabetes mellitus and related symptoms for the primary health care of the people living in rural Dhemaji district of Assam.[137] It is also used for preparing herbal recipes during the cultural festival (Rongali Bihu) of Assam.[138] In Arunachal Pradesh GPR is preserved and used for stomach disorder and for a treatment against blood dysentery;[139-140] however, in Meghalaya it is consumed as raw.[141] The traditional starter cultures used for preparing fermented bamboo shoot product of Manipur, India is made by mixing acidic juice extract of GPR fruit (1 to 1.5 kg) with rice washed water (10–15 L).[83] Soidon is fermented bamboo shoot product of Manipur and during its preparation GPR is added in the fermenting vessel to enhance its flavour.[88]

The water extract of the dried pellets of GPR are used as antidiarrhoeic and antidysentric[142] and it is rich in benzophenones, pedunculol, garcinol and cambogin.[143] High antioxidant activity has been reported in GPR by Gogoi et al.[144] and Mudoi et al.[145] GPR is a rich source of secondary metabolites including xanthones, flavonoids, benzophenones, biflavonoids, lactones and phenolic acids with wide range of biological and pharmacological activities.[146-148] The crude hexane and chloroform extracts from the fruit rinds of GPR showed antibacterial activity against food borne pathogens and spoilage bacteria such as such as *Bacillus cereus, Bacillus coagulans, Bacillus subtilis, Staphylococcus aureus* and *Escherichia coli.*[149]

2.8 Edible coatings and films

2.8.1 Functions and materials

Edible films and coatings are thin layers of edible materials applied on food products that play an important role on their conservation, distribution and marketing. Some of their functions are to protect the product from mechanical damage, physical, chemical and microbiological activities.[150] An edible coating is a thin layer of edible material formed as a coating on a food product, and this film is a preformed, thin layer,

made of edible material, which once formed can be placed on or between food components.[151]

The use of films and coatings in food applications and especially highly perishable products such as horticultural produce, is conditioned by the achievement of diverse characteristics such as cost, availability, functional attributes, mechanical properties, optical properties, the barrier effect against gases flow, structural resistance to water and microorganisms and sensory acceptability. These characteristics are influenced by parameters such as the kind of material implemented as structural matrix, the conditions under which films are preformed and the type and concentration of additives.[152-153]

Edible coatings and films are usually classified according to their structural material. Edible coatings and films are generally produced from renewable natural and abundant biodegradable polymeric materials such as polysaccharides, proteins, lipids, or the combination of these components.[154-155] These materials are generally combined with the aim of taking advantage of the properties of each compound and the synergy between them. The mechanical and barrier properties of these films not only depend on the compounds used in the polymer matrix, but also on their compatibility.[156] Various materials like carboxymethyl cellulose, casein, alginate, gum from different plant sources, pectin, starch of potato, maize, cassava, wheat gluten etc. were successfully utilized by different authors. However, $CaCl_2$ is added during coating and film preparation as a cross linking material which are affecting mechanical properties, water solubility, moisture content, film thickness etc.

Tongdeesoontorn et al.[154] studied the effect of addition of carboxymethyl cellulose (CMC) in different proportion (0, 10, 20, 30 and 40 %w/w total solid) on mechanical properties of cassava starch based film. Results concluded that the addition of CMC to the cassava starch films increased tensile strength and reduced elongation at break of the blended films. The barrier and mechanical properties of corn starch-based edible film also got improved with addition of citric acid (CA) and CMC. The water vapor barrier property and the ultimate tensile strength were improved significantly as the CA percentage increased from 0 to 10% (w/w), however, with addition of CMC at

the level of 15% (w/w) the starch films showed the lowest water vapor permeability values.[157] Fazilah, et al.[158] prepared edible films from sago starch: alginate mixtures with ratios of 1:0, 4:1, 3:1, 2:1, 1:1 and 0:1. The physical and mechanical properties of films were modified with the addition of calcium chloride. Ghanbarzadeh et al.[159] also prepared modified starch/CMC composite films and studied the effects of CMC addition on some physical properties of the resulted blend films. The addition of CMC at the level of 20% w/w starch caused an increase in the ultimate tensile strength of film without any significant decrease in the strain to break.

2.8.2 Antimicrobial and antibrowning edible films and coatings

Edible coatings might also serve as carriers of food additives such as antibrowning and antimicrobials agents, colourants, flavours, nutrients and spices. Edible films and coatings with antimicrobial properties have innovated the concept of active packaging, being developed to reduce, inhibit or stop the growth of microorganisms on food surfaces.[160] Incorporating antimicrobial compounds into coating and film have been shown to be an efficient alternative in the control of food contamination, improve its safety and shelf life.[161] Spoilage and pathogens could be reduced by incorporating antimicrobial agents into edible films and coatings.[162] Some of the more commonly used antimicrobials include benzoic acid, sorbic acid, sodium benzoate, citric acid, lysozyme, potassium sorbate, lactoferrin, bacteriocins and plant-derived secondary metabolites, such as essential oils and phytoalexins.[163-166]

Rojas-Graü et al.[163] used apple puree-alginate edible coating as carrier of antimicrobial agents to prolong shelf-life of fresh cut apples. Cellulose acetate based mono and multilayer films including potassium sorbate as an antimicrobial agent were used for its controlled release during package storage.[167] Natural plant extract of different commodity like olive, rosemary, onion, capsicum, cranberry, garlic, oreganum etc. could be use for the development of antimicrobial film and coating.[168]

The microbiological stability of food products plays an indispensable role in its quality, but also sensory aspects are essential parameter for its acceptability. The edible films and coatings become successful to control the food browning and polyphenol

oxidase activity of food product.[169] Polyphenol oxidase (PPO) is the main enzyme responsible for these changes in vegetable tissues that contain phenolic or polyphenolic molecules.[150]

Some researchers have proved the effectiveness of edible films and coatings on the control of browning processes and polyphenol oxidase activity. Alginate and gellan-based edible coatings were used as carriers of antibrowning agents for application on fresh-cut apples.[170] Significant reduction in the rates of O_2 depletion and CO_2 production was also observed in coated sample. Chitosan-coating treatments effectively retarded the enzymatic browning of minimally processed apples during storage and they effectively retarded or avoided tissue softening.[171] Hui-Min et al.[172] studied the effect of coating of carrageenan, carboxymethyl cellulose (CMC) and sodium alginate and their combinations on browning parameters of fresh cut peach fruits during storage at 5 °C.

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Chapter 3

Effect of blanching temperature and time on physicochemical properties of bamboo shoot

3.1 Introduction

India is one of the rich genetic resources of bamboo with 136 indigenous exotic species under 23 genera under cultivation. Bamboo shoots are low in fat and calorie but rich in different nutrient like protein, vitamin, mineral, fiber etc.[1] It also contains lignin and phenolic compounds which might contribute to its anti-microbial and anti-oxidant activity.[2] In Assam, India bamboo shoots generally consumed either raw or processed because of its exotic taste, flavour and medicinal value. Some of the edible species that are suitable for processing available in Assam are *Dendrocalemvs giganteus* (Worra), *D. hamiltonii* (Kako), *D. strictus* (Lathi bans), *Melocanna baccifera* (Tarai, Arten), *Bambusa balcooa* (*Bhaluka*), *B. tulda* (Jati), *B. polymorpha* (Jama betwa, Betwa), *B. nutans* (Kotoha), *B. pallida* (Bijuli, Bakhal).[3]

Heat processing is applied to various vegetables to increase its shelf life and nutritive value along with other properties while it also helps to reduce the anti-nutrient components.[4] Blanching is a cooking process to stop various enzymatic reactions, to reduce microbial load in food, to soften tissues for an easier canning step and a shorter cooking time and to eliminate intracellular air to prevent oxidation.[5] Heat treatment such as hot water blanching has been increasingly used to control pests, insects and fungi rot. It also induces resistance to chilling injury during storage at low temperature.[6] Heat treatment inhibits disease incidences, respiration, ethylene production and various enzymatic activities of bamboo shoots during storage at 20°C. Heat treatment like hot water blanching significantly delays the tissue lignificaton.[2]

Time and temperature of blanching may helps to inactivate enzymes like peroxidase which is one of the most heat stable enzymes and often used as a marker of completion of blanching.[7] Ascorbic acid, pigments like β-carotene are sensitive to heat treatment. Negi et al.[8] has reported that KMS treatment during blanching successfully reduces the loss of chlorophyll, carotene and ascorbic acid content of savory beet, amaranth and fenugreek followed by the low temperature drying which helps in the better retention of the pigments and vitamin C. Blanching of soybean degrades its chlorophyll content but the loss nutritive including sugar, amino acid, and vitamin are minimal.[9]

Blanching is a heat processing method applied to food and the changes in food during the heating process can be expected as loss in turgor in cells, loss of integrity of the cell membranes and partial degradation of cell wall components. Firmness of Brussel sprouts with increase in radical scavenging activity, total flavanoids and ascorbic acid content were observed after blanching operation at 50 and 100^{0}C for 5 and 3 min respectively. This may be attributed to the loss of integrity of cell membranes and organelles.[10] However, the loss of soluble sugar during post harvest treatment like hot water blanching is due leaching of sugar into water of soybean.[11]

In regular practices bamboo shoot are boiled for before consumption for particular time. The boiling time is depending on locality, traditional practices and use for removal of bitterness of shoot. But the effect of boiling/ blanching time on nutritional components is not considered during process. With regards to removal of anti-nutrient components some valuable nutrients may get loss. The objective of this work is to find out the nutritional potential of some edible bamboo shoots of Assam and further investigate the change in different nutritional composition like protein, carbohydrate, fat, fibre and ash of fresh bamboo shoot after hot water blanching at different time-temperature combination. Different parameters like colour, texture, ascorbic acid, total phenolic content and radical scavenging activity of fresh and blanched sample were further analyzed.

3.2 Material and methods

3.2.1 Collection of raw materials and sample preparation

Bamboo shoots of *Dendrocalamus hamiltonni* (Kako), *Bambusa balcooa* (Bhaluka), *Bambusa pallida* (Makal/ Bijuli), *Bambusa tulda* (Jati) species were collected from Tezpur, Nagaon and Karbi Anglong in Assam, India. The shoots were transported to the laboratory within 24 hours of collection, where shoots were defoliated and washed. The unwanted parts were removed and the soft edible portions were stored at 4°C for further analysis. All the shoot samples were analyzed for moisture content, protein, fat, carbohydrate, crude fiber, ash, vitamin C, total phenol, antioxidant activity etc.

3.2.2 Blanching treatment

The shoots of *Bambusa balcooa* species were taken for this study. The soft edible portions were cut into uniform size of $1cm^3$ and use for blanching treatment. For blanching treatment shoots were immersed in water bath at 75, 85 and 95 ^0C for 5, 10, 15, 20, 25 and 30 min and collected after reaching pre-established time. After blanching, the samples were cooled in cooling water bath for 2 min and excess moisture was removed from the surface of the shoot. An unheated sample was taken as a control. Fresh and blanched shoots were analyzed for moisture content, protein, fat, carbohydrate, crude fiber, ash, vitamin C, total phenol, antioxidant activity, texture and colour. During preliminary experiments the shoot was blanched at 75 $^{\circ}C$ for 5 min and checked for the presence of enzyme polyphenol oxidase and it was found to be absent. Therefore all temperature and time combinations were taken above this level.

3.2.3 Proximate analysis

Bamboo shoots were analyzed for moisture, protein, fat, carbohydrate, crude fiber, and ash according to the standard AOAC[12] methods. Fat in the samples was determined by extracting a known weight of powdered sample with petroleum ether using Socs plus (SCS6). Crude fiber and protein in the samples were determined using Fibro plus (FES06) and Kel plus apparatus (Pelican Equipment, Chennai, India) respectively. The nitrogen content was converted to protein by multiplying with a factor of 6.25.

3.2.4 Estimation of vitamin C (L-Ascorbic Acid)

Fresh and blanched bamboo shoots were weighed accurately to 5g and extracted in 4% oxalic acid solution by homogenization followed by centrifugation at 3000 rpm for 15 min. 5 ml of supernatant was collected and Vitamin C was estimated by titrating against 4% oxalic acid using 2, 6-dichlorophenolindophenols (DCPIP) as indicator.[13] Ascorbic acid standard solution was prepared for standard curve.

3.2.5 Estimation of total phenolic content

Bamboo shoot (1 g) was extracted with 10 ml of 80% methanol and centrifuged at 10000g at room temperature. Residue was reextracted (five times) with of 80% methanol and centrifuged. Supernatant was collected and used for the analysis of total phenolics and antioxidant activity. The total phenolics in the sample were estimated using Folin-Ciocalteu reagent (FCR) procedure as described by Bray and Thorpe[14] Supernatant was evaporated to dryness and residue was dissolved in 5 ml distilled water. From this mixture 0.2-2 ml of aliquots were taken in different test tubes and volume of 3 ml was made using distilled water. FCR of 0.5 ml was added in it and after 3 minutes 2 ml of 20% sodium carbonate was added to each test tube. The mixture was heated on a water bath at 100°C for 1 minute and then cooled. Absorbance was measured at 750 nm in spectrophotometer (Spectronic 20D+, Thermo Scientific, USA). The results are expressed as mg phenol/ 100 g of sample as Gallic acid equivalent.

3.2.6 Estimation of free radical scavenging activity

To check the antioxidative property of the bamboo shoot 0.1mM of 2,2 diphenyl 1-picrylhydrazyl solution (DPPH) was prepared. DPPH is a commercial oxidising radical used to be reduced by antioxidants. The disappearance of the DPPH radical absorption at a particular wavelength is monitored by the reduction in optical density. Blanched sample were extracted in methanolic (5 gm in 20 ml) solution and supernatant obtained was centrifuged at 5000 rpm for 30 min. After centrifugation 5 ml of supernatant and 5 ml of DPPH solution was kept at dark for 30 minutes for complete reaction to takes place. The anti radical activity was determined by spectrophotometer at 517 nm based on the reaction with stable radical DPPH.[15] Control for the experiment was prepared by adding 5 ml DPPH and 5ml methanol. The DPPH radical scavenging activity was calculated according to the following equation (Eq. 3.1).

$$\% \text{ Free radical scavenger activity} = [(\text{Control absorbance} - \text{Sample absorbance})/\text{Control absorbance}] \times 100 \quad (3.1)$$

3.2.7 Colour measurement

The colour of bamboo shoots samples was measured using a Hunter Colour Lab (Ultrascan VIS, Hunter Lab. Inc., USA). The results were expressed in terms of *L*, *a* and *b* values. *L*, *a* and *b* values indicate lightness, redness (+)/greenness (–) and yellowness (+)/ blueness (–), respectively.[16]

3.2.8 Texture measurement

Texture of fresh and blanched bamboo shoot were measured in Texture Analyser (TA-HDPlus, Stable Microsystems, UK) according to ASTM standard Method D882.[17] The maximum force value is related to the firmness of the bamboo shoots. The measurements were performed in a Texture Analyser at a constant test speed of 0.5 mm/sec; however, pre-test speed and the post-test speed were 1mm/sec and 5mm/sec respectively. The trigger force of 5 g, P/2 cylindrical probe and 5kg load cell was used for test purpose.

3.2.8 Statistical analysis

The effect of blanching on nutritional compoment were performed taking three replicates and data were reported as mean ± SD. Single factor ANOVA was used to determine the critical difference of means, and variance among the different samples were checked at significance level $p \leq 0.05$.

3.3 Results and discussion

3.3.1 Nutritional analysis of bamboo shoot

Four different varieties of bamboo shoots were analyzed to check their nutritional potential (Table 3.1). Moisture content for all the species was recorded above 90%, which show the high perishability of shoot. The highest protein content of 3.42 g/100g was found in *B. balcooa* followed by 3.34 g/100g for *B. pallida*, 3.32 for *B. tulda*, and 3.28 g/100g for *D. hamiltonii*. Fat content of all species of shoots were found low and the values varies from 0.31 to 0.67 g/100g. *B. tulda* shoot contains highest (4.7

g/100g) amount of carbohydrate compared to other varieties. The carbohydrate of *D. hamiltonii, B. balcooa* and *B. pallida* had estimated 4.46, 4.08 and 3.89 g/100g respectively. The ash content in the shoots ranged from 0.82-0.90 g/100g of fresh shoot. It was highest in *B. pallida* and lowest in *B. Tulda.* L-ascorbic acid of four varieties of bamboo shoots were varies from 1.39 to 2.72 mg/100g and it was highest in *B. balcooa.* All the above results are comparable with the finding of Nirmala et al.[18] on *Dendrocalamus giganteus* shoot.

Total phenolics and antioxidant activity of *B. balcooa* shoot were reported as 101.65 mg/100g and 27.12 % DPPH radical scavenging activity and it was high compared to all other species. The results of total phenolics and antioxidant activity are in line with the study of Satya et al.,[19] but the values found during this study were comparatively less than reported values. In view of nutritional status of all four species of bamboo shoot, *B. balcooa* found to be rich in protein, vitamin C, good amount of carbohydrates, crude fiber, reducing sugar etc and high amount of total phenolics and antioxidant activity. However, *B. balcooa* extensively used for preparing different dishes and fermented products. Therefore, for all further studies the shoots of *B. balcooa* were taken.

Table 3.1. Nutritional composition of different species of bamboo shoot on fresh weight basis

Parameters	*D. hamiltonii*	*B. balcooa*	*B. pallida*	*B. tulda*
Moisture (g/100g)	90.71±1.46	91.12±132	91.56±1.18	91.93±1.28
Protein (g/100g)	3.28±0.34	3.42±0.12	3.34±0.18	3.32±0.22
Fat (g/100g)	0.67±0.05	0.52±0.01	0.31±0.07	0.46±0.03
Carbohydrate (g/100g)	4.46±0.16	4.08±0.36	3.89±0.44	4.70±0.27
Ash (g/100g)	0.88±0.03	0.86±0.07	0.90±0.05	0.82±0.04
Crude fiber (g/100g)	3.88±0.41	3.51±0.32	3.16±0.54	3.92±0.25
Vitamin C (mg/100g)	1.45±0.14	2.72±0.18	2.10±0.09	1.39±0.21
Total phenols (mg/100g)	88.23±4.38	101.65±2.75	79.85±3.98	80.54±3.21
% DPPH free radical scavenging activity	23.12±1.34	27.12±1.05	19.17±0.98	21.73±1.24
Reducing sugars (g/100g)	1.3±0.07	1.33±0.11	1.25±0.12	1.26±0.05

3.3.2 Effect of blanching on nutritional component

Nutritional component of fresh bamboo shoot was evaluated on the basis of blanching time and temperature and the results are shown in Table 3.2. From the table it can be observed that blanching time and temperature have significantly influenced the nutrient like protein, carbohydrate and reducing sugar ($p \leq 0.05$). However, the influence was less on ash and crude fiber. Protein (amino acid), fat and carbohydrate (sugar) are important not only for the nutritional point of view but it also adds flavor to the food. But these components were reduced with blanching time and temperature. Blanching time and temperature affects the biological value of protein by reducing its essential amino acid content. Blanching at 95^0 C markedly reduces the protein content of bamboo shoot in comparison to 75^0C and 85^0C. At high temperature most of the labile protein gets denatured. Crude fat have been significantly affected after blanching treatment ($p \leq 0.05$). Fresh bamboo shoot contains 0.52 g/100g fat, after blanching for 30 min was reduced to 0.38, 0.28 and 0.19 g/100g at 75 ^{0}C, 85 ^{0}C and 95 ^{0}C respectively. Duration of blanching treatment had been also affected the fat content. Blanching for 5-10 min has better retention of fat than long duration blanching for 20-30 min. The reduction in fat might be due to melting and oxidation of fat at high heat treatment, which would allow it to transfer from sample to water during blanching at higher temperature and increasing time.[20]

Variation in carbohydrate and reducing sugar after hot water blanching was recorded and it can be observed that the maximum loss corresponds to the high temperature long duration blanching of 95^0C for 20-30 min. Blanching treatment has a significant reduction ($p \leq 0.05$) in carbohydrate and reducing sugar components by 4.08 to 2.25 g/100g and 1.33 to 0.87g/100g respectively. Most of the sugar like glucose, fructose and sucrose was destroyed during hot water blanching. The molecular size of sugar and duration of blanching affected the content of sugar and loss of this water soluble sugars might be correlated with the leaching into water during blanching.[9] Crude fiber and ash content were almost remaining unaffected with blanching temperatures and time.

Table 3.2. Effect of blanching on different nutritional component

Parameters	Temp. (°C)	Blanching Time (Min)								
		0	5	10	15	20	25	30	Means	CD
Protein (g/100g)	75	3.42	3.31	3.26	3.17	3.02	2.95	2.9	**3.15**	Withein Row: 0.138 Column: 0.212
	85	3.42	3.23	3.14	3.05	2.84	2.72	2.54	**2.99**	
	95	3.42	3.15	3.04	2.86	2.58	2.37	2.24	**2.81**	
	Means	**3.42**	**3.23**	**3.15**	**3.03**	**2.81**	**2.68**	**2.56**	**2.98**	
Fat (g/100g)	75	0.52	0.48	0.45	0.41	0.4	0.38	0.38	**0.43**	Withein Row: 0.036 Column: 0.055
	85	0.52	0.44	0.41	0.37	0.33	0.3	0.28	**0.38**	
	95	0.52	0.39	0.37	0.31	0.27	0.22	0.19	**0.32**	
	Means	**0.52**	**0.44**	**0.41**	**0.36**	**0.33**	**0.30**	**0.28**	**0.38**	
Carbohydrate (g/100g)	75	4.08	3.92	3.83	3.73	3.65	3.57	3.43	**3.74**	Withein Row: 0.260 Column: 0.397
	85	4.08	3.77	3.71	3.52	3.37	3.24	3.11	**3.54**	
	95	4.08	3.66	3.54	3.3	3.07	2.54	2.25	**3.21**	
	Means	**4.08**	**3.78**	**3.69**	**3.52**	**3.36**	**3.12**	**2.93**	**3.50**	
Ash (g/100g)	75	0.86	0.86	0.85	0.85	0.84	0.84	0.84	**0.85**	Withein Row: 0..010 Column: 0.016
	85	0.86	0.85	0.84	0.83	0.83	0.83	0.83	**0.84**	
	95	0.86	0.82	0.81	0.8	0.8	0.79	0.79	**0.81**	
	Means	**0.86**	**0.84**	**0.83**	**0.83**	**0.82**	**0.82**	**0.82**	**0.83**	
Crude fiber (g/100g)	75	3.51	3.51	3.51	3.51	3.51	3.51	3.51	**3.51**	Withein Row: 0..004 Column: 0.006
	85	3.51	3.51	3.51	3.51	3.51	3.5	3.5	**3.51**	
	95	3.51	3.51	3.5	3.5	3.5	3.5	3.5	**3.50**	
	Means	**3.51**	**3.51**	**3.51**	**3.51**	**3.51**	**3.50**	**3.50**	**3.51**	
Reducing sugars (g/100g)	75	1.33	1.28	1.22	1.18	1.14	1.1	1.07	**1.19**	Withein Row: 0.037 Column: 0.057
	85	1.33	1.23	1.2	1.15	1.11	1.04	0.98	**1.15**	
	95	1.33	1.18	1.14	1.09	1.06	0.94	0.87	**1.09**	
	Means	**1.33**	**1.23**	**1.19**	**1.14**	**1.10**	**1.03**	**0.97**	**1.14**	

3.3.3 Effect of blanching on L-ascorbic acid content

Ascorbic acid content in fresh bamboo shoot was found to be 2.72 mg/100g. Blanching temperature significantly reduced the ascorbic acid content of bamboo shoot ($p \leq 0.05$) (Fig. 3.1). Retention of ascorbic acid is higher in 75^{0}C which was gradually reduced at 85 and 95^{0}C. It is also affected by the duration of blanching. Blanching for short time (5- 10 min) retain more ascorbic acid while at 20-30 min the losses were high. Loss of ascorbic acid might be due to the leaching of content into water. L-ascorbic acid is very soluble in water and are not stable at high temperature.[20] Vegetable tissue suffers

various changes in cell permeability and vacuole membrane upon high temperature treatment, which leaches the nutrients. Similar results were found on by Olivera et al. (2008-[10]) on Brussels sprouts during blanching. However, disruption of cell during blanching leads to the migration of ascorbic acid into the blanching medium which may accounts for the high loss of ascorbic acid from the vegetables.[21]

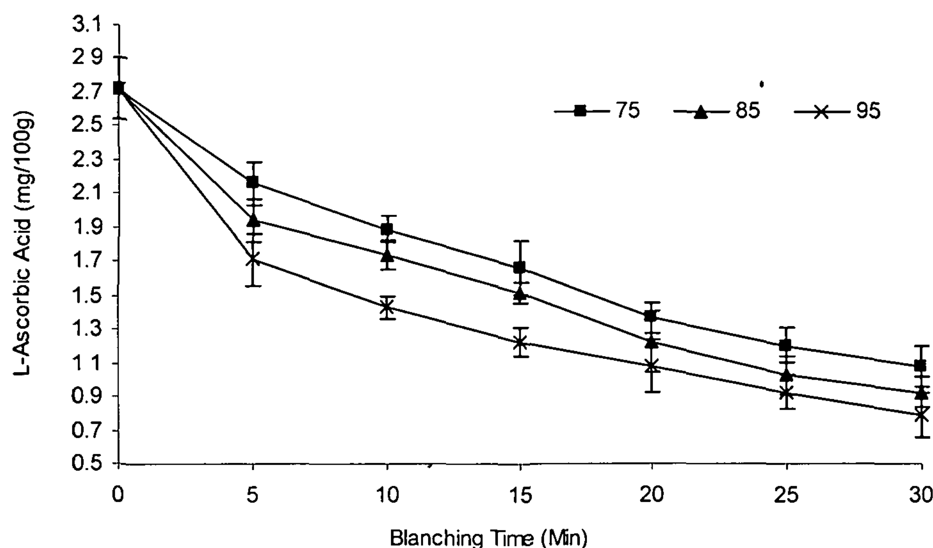

Fig. 3.1. Effect of blanching temperature on ascorbic acid content

3.3.4 Effect of blanching on total phenolic content

Fresh bamboo shoot showed the total phenolic content of 101.65 mg/100g which was get degraded during blanching operation. The total phenolic value for the samples blanched at 75, 85 and 95^{0}C for 10, 20 and 30 min are indicated in the Fig. 3.2. The results clearly indicate that there is a significant loss of phenolic content with increase in temperature and duration of blanching ($p \leq 0.05$). Maximum loss can be observed at 95^{0}C for 30 min of blanching and highest retention of phenolic reported at 75^{0}C for 5 min. At the end of blanching, the retention of phenolic at 75, 85 and 95^{0}C were 59.57, 45.26 and 39.34 mg/100g respectively. High intensity heat treatment leads to the maximum loss of phenolic content which may be due to several reasons like thermal degradation, leaching or diffusion of component into water etc.[22] Similar results were reported by Jaiswal et al.[23] during blanching of cabbage. Enzyme like PAL

(phenylalanine ammonia-lyase), PPO (polyphenol oxidase) plays an important role during phenol synthesis in plant. PAL is the first key enzyme in the biosynthesis of the phenolic component. Increase activity of PAL leads to the increase in synthesis of phenols.[2] Thus it can be attributed that during heat treatment these enzymes gets inactivated which leads to the reduction of phenolic components.

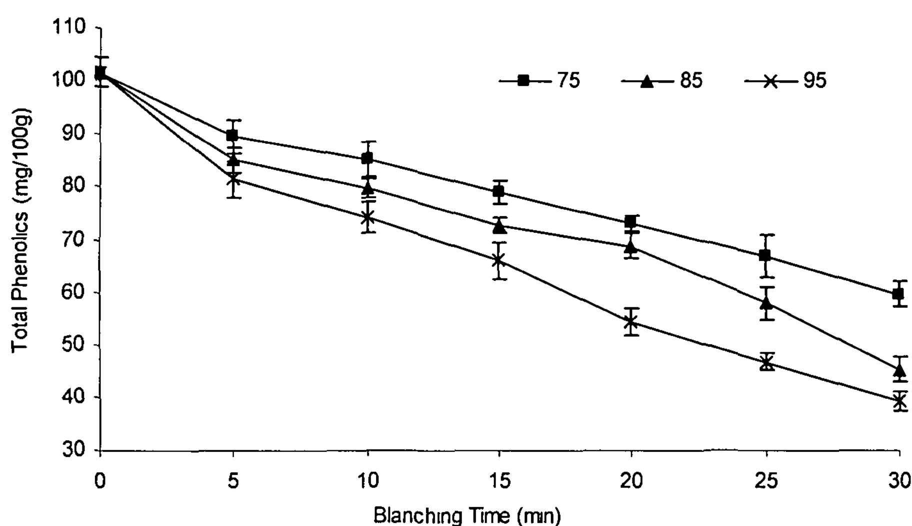

Fig. 3.2. Effect of blanching temperature on total phenolic content

3.3.5 Effect of blanching on free radical scavenging activity

DPPH is a stable free radical which is used to interpret the antioxidative property of sample. Antioxidant property of fresh and blanched sample were assayed and shown in Fig. 3.3. The profile obtained after blanching of bamboo shoot, showing a decreasing antioxidative activity after blanching operation. Graph depicts the significant loss of antioxidative property as the temperature and time of the blanching increases ($p \leq 0.05$). Antioxidative property of bamboo shoot was closely related to the presence of different phytochemicals, phenols, tocopherol, ascorbic acid and their synergistic effects. Antioxidative capacity cannot be correlated with a single compound, but attributed to synergistic and additive effects between different inherent phytochemicals.[24] Different studies have suggested that not only the amount of the phenolic content but molecular structure also affects the antioxidative property.[25]

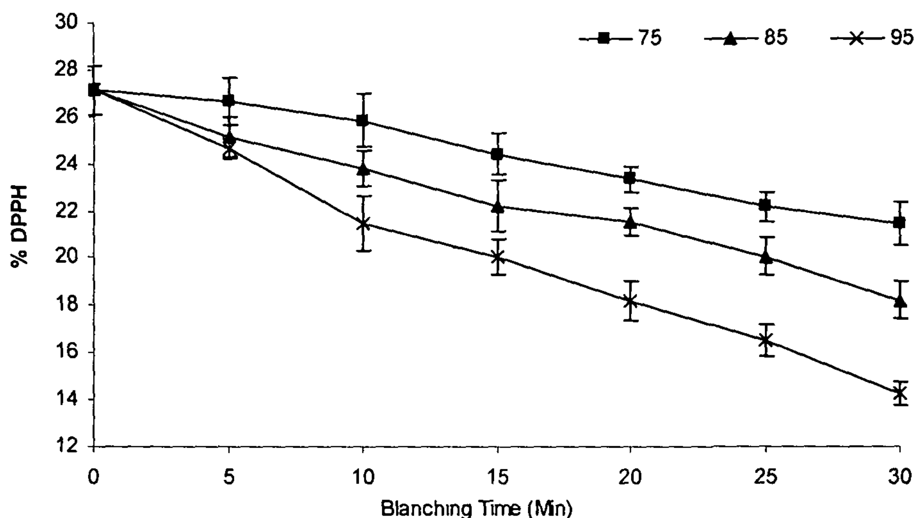

Fig. 3.3. Effect of blanching temperature on free radical scavenging activity

3.3.6 Effect of blanching on colour of the bamboo shoot

Colour is one of the most important parameter which indicates the quality and freshness of any food. Colour parameter of fresh and blanched bamboo shoot at different time and temperature are shown in Fig. 3.4. The L value depicts the lightness of the sample. Fresh sample has higher L value (71.26) comparative to blanched sample which were decreased further as the blanching time and temperature increased. Bamboo shoots blanched at 75^{0}C and 85 ^{0}C have shown a little colour change with increase in blanching time. While there were more changes in colour values were observed in the sample blanched at 95 ^{0}C. At higher blanching temperature (85-95^{0}C), excessive loss in the natural colour pigments and decreased in lightness of the shoot were observed. This might be due to the non enzymatic browning of the bamboo shoot due to high temperature treatment. Gonçalves et al.[22] follows the same trends of decreased in L and a value with increasing blanching time for carrot, but b value were not in line with this study. In this case b value get increased with temperature and time this might be due to more yellowness form on blanched shoot at high temperature. Blanching alters the chloroplast integrity where the chlorophyll pigments are embedded and results in the formation of pheophytin as the time and temperature of blanching progresses.[26]

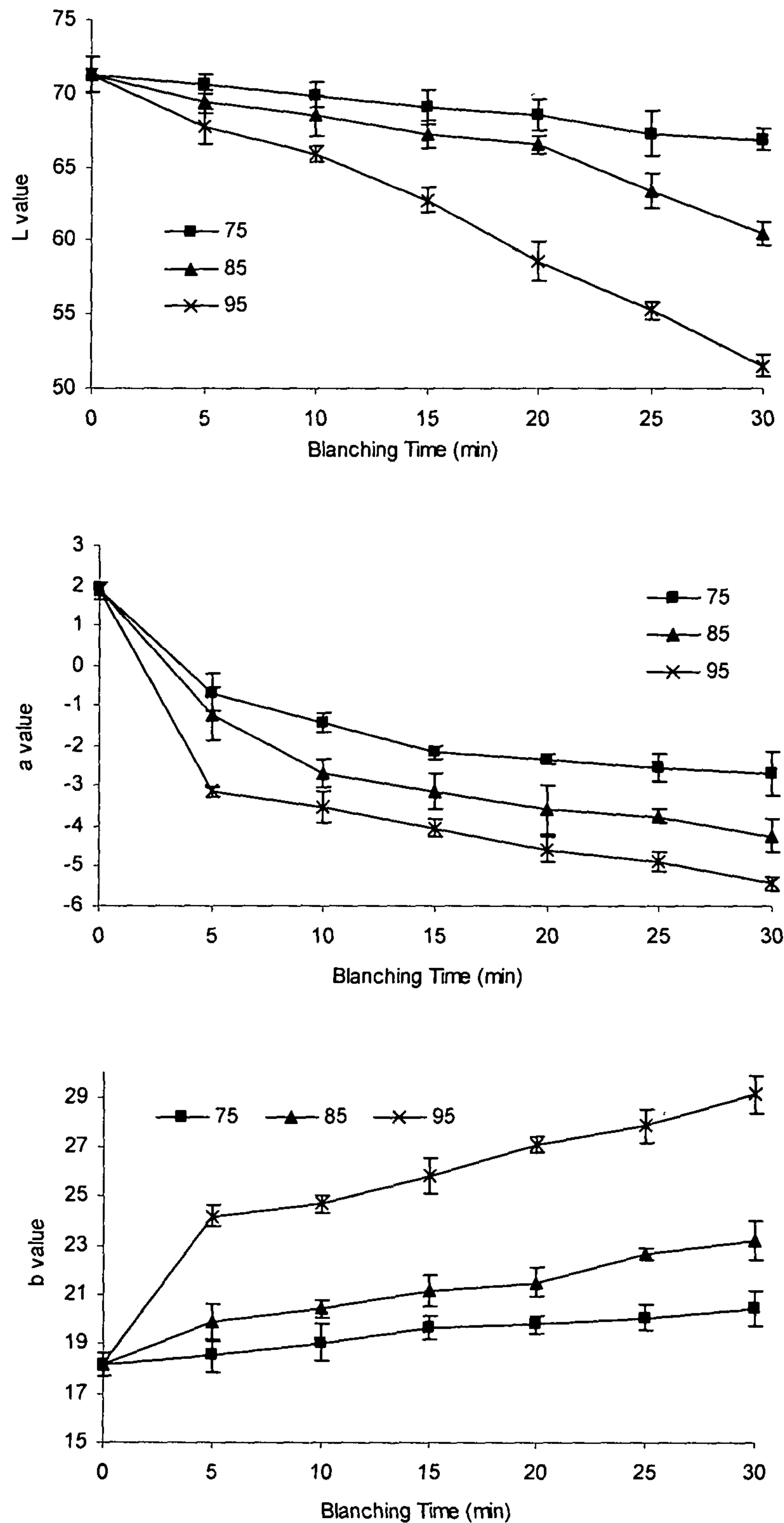

Fig. 3.4. Effect of blanching temperature on colour of bamboo shoot

3.3.7 Effect of blanching on texture

Bamboo shoot firmness continuously decreases with the increasing time and temperature of blanching. Fresh bamboo shoot exhibited firmness of 547.83 g. The texture degradation curve of blanched bamboo shoot at various temperatures (75-95^{0}C) and time (5-30 min) are shown in Fig. 3.5. Textural loss was found to be more in first 5 minutes of blanching which consistently increased with increase in temperature and time. Most of the degradation of texture was occurred during 30 minutes of blanching at 95^{0}C.[22] Firmness of the fresh and blanched bamboo shoot can be attributed to the loss of lignin and cellulosic components of cell wall. There is decrease in lignin and cellulose with increase in time and temperature of blanching.[27]

Softening of tissue after hot water blanching is due to the decomposition of pectin content with some other biochemical changes. Significant changes in the textural properties of blanched bamboo shoot can be observed at different temperature and time ($p \leq 0.05$). Bamboo shoots blanched for 5 to 10 min have shown better retention of textural properties than 20 to 30 min of blanching. At the end of 30 min of blanching, the retention of texture at 75, 85 and 95 ^{0}C were 70.74, 59.84 and 46.86% respectively. The textural behavior is consistent with the result found by Zheng et al.[1]

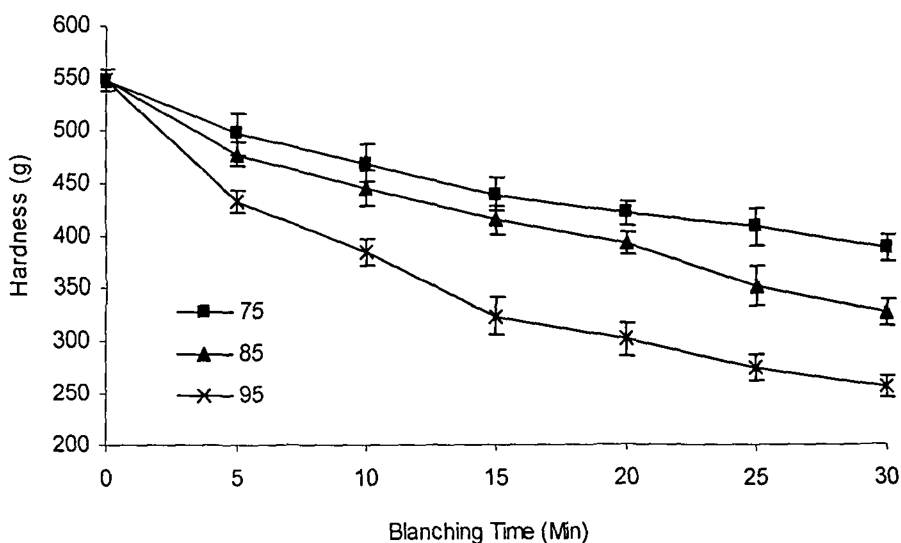

Fig. 3.5. Effect of blanching temperature on texture of bamboo shoot

3.4 Conclusion

Blanching in hot water was carried out in order to inactivate various enzymes and to improve the quality and to increase shelf life of vegetable. Effect of hot water blanching on different physical and nutritional quality like total soluble sugar, protein, dietary fiber, fat vitamin, ascorbic acid, phenolic content and radical scavenging activity were evaluated. Blanching of bamboo shoot leads to degradation of various nutrients. Loss was maximum at 95^0C for 20-30 min and minimum at 75^0C for 5-10 min of blanching. Texture was also affected by the blanching operation. Blanching results in soft texture, while there was decreased in lightness and increase in greenness and yellowness observed. Loss of antioxidative property, total phenolic content and ascorbic acid might be related to the migration or leaching of component into the water. Proper combination of time and temperature of blanching is very important to retain the nutrients and quality of bamboo shoot. Low temperature and short time blanching have better retention of the entire nutritional component along with colour and textural properties. Therefore low temperature treatment with short duration was most suitable method of blanching.

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Optimization of osmotic dehydration process, its mass transfer kinetics and effect of centrifugal force and pulsed vacuum on osmotic dehydration

4.1 Introduction

The young shoots of bamboo grown mainly in rainy season of the year are used as vegetable in the South-East Asian countries. Bamboo shoots are highly nutritious and potentially rich sources of dietary fibers, antioxidants[1], amino acids, minerals, vitamins and low in calories. The protein content of the shoots is also high, and contains fewer amounts of fats; however, it is rich in essential fatty acids.[2-4] It is also known to contain phytosterols which are known for their cholesterol-reducing activity.[5] Bamboo shoots also have high cellulosic content for which it could be used as appetizer.[6] Potential use of bamboo shoot as nutrition rich food has created the importance of preserving it to overcome its seasonality. Various drying processes have adverse effect on the nutritional and physical quality of bamboo shoot; however, osmotic dehydration could be the best options for preserving its nutrients.

Osmotic dehydration is a semi-drying process applied to high moisture fruits and vegetables to reduce the moisture content to an optimum level so that the shelf-life of the product is increased without much deterioration in sensorial and nutritional status. In this method partial removal of the moisture is achieved using the process of osmosis, where the food entity to be dehydrated is kept in hypertonic solution so that exosmosis of water from the food takes place through membrane in virtue to maintain equilibrium. It is a useful treatment of dehydration compared to the other dehydration processes, as it does not bring adverse and irreversible changes in physical and nutritional status of food material like other dehydration processes.[7] Osmotic dehydration is also quite energy and cost effective process. Osmotic agents generally used are common salt (NaCl), other salts (KCl, $CaCl_2$), sugar (sucrose), reducing sugars (glucose, fructose, arabinose etc.) or combination of salt and sugar.[8] Though combination of two osmotic agents gives higher water loss, but for vegetables and meat, salt is singly used as it gives the salting effect which is acceptable[9], and for fruits, sugar is used for its candying effect.

The osmotic dehydration process and its mass transfer kinetics has been studied for many food entities such as apple[10], banana[11], pineapple[12], mushroom[13], kiwifruit[14] etc. However, studies on osmotic dehydration of bamboo shoots and its mass transfer

kinetics are seldom found in literature. Basically osmotic dehydration consists of moisture migration, and further studies revealed that along with moisture flux, an opposite direction flux, i.e. solid migration from the osmotic solution to the sample occurs. A third type of flux i.e. leaching of soluble solids from the sample to solution, which is often negligible, but harmful towards sensorial and nutritional status of the food is also observed[15]. The rate of mass transfer during osmotic dehydration depends upon various factors viz., temperature and concentration of solution, size and geometry of the solid, solution to solid mass ratio and degree of agitation.[16]

Osmotic dehydration is recently being studied in diverse fields. Application of new technologies on osmotic dehydration to enhance its effect is becoming frequent field of study. Osmotic dehydration with application of centrifugal force[17-18] and vacuum pressure[19-21] was studied in various fruits and vegetables. Centrifugal force enhanced water loss and limited solid gain during osmotic dehydration, while vacuum pressure helps to enhance mass transfer.[22] It is seen that both centrifugal force and vacuum pressure have positive effect towards enhancing the water loss, though effect of both the techniques is not yet compared.

The present study was undertaken to study the effect of the process time, solution temperature and salt concentration on osmotic dehydration of bamboo shoot and these variables were optimized using response surface methodology (RSM). The mass transfer kinetics was also studied to obtain water and solute diffusivity during osmotic dehydration of bamboo shoot using salt as osmotic agent. Effective diffusivities of water and osmotic solute were experimentally determined and correlated as a function of temperature by Arrhenius equation. In addition of centrifugal the effects of centrifugal force and pulsed vacuum on the osmotic dehydration at the optimized level were examined and compared.

4.2 Materials and methods

4.2.1 Materials

Fresh bamboo shoots (*Bambusa balcooa*) were collected from Tezpur, Assam (India) and immediately kept in refrigerated condition. For experimentations, bamboo shoots were brought to room temperature and then they were defoliated, removed unwanted parts, washed and cut into the pieces of approximately 1cm^3 sizes. The average moisture content of the bamboo shoot was found to be 93.30 % on a wet basis. The used osmotic agent (salt) was food grade and purchased from a local market. The osmotic solution was prepared by stirring the salt with distilled water.

4.2.2 Optimization of process variables for osmotic dehydration

4.2.2.1 Experimental design

A face centered central composite design of response surface methodology (RSM) was used to optimize the parameters affecting osmotic dehydration of the sample. The ranges of the input variables such as temperature, time, and salt concentration were taken as 30-50 °C, 120-240 minutes and 5-25 % respectively.[23] The statistical software Design-Expert version 8.0.7 was used for design of experiments. Total numbers of experimental run were 20, including 6 centre points. The levels of the input variables in coded (x_i) and uncoded (X_i) form are given in Table 1. Optimum combination of parameters was obtained numerically by considering maximum water loss; and minimum solid gain, hardness and colour change. A second degree polynomial equation given below (Eq. 4.1) was fitted in each response to study the effect of variables and to describe the process mathematically.[24]

$$y_k = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta_{ii} x_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{ij} x_i x_j \quad (4.1)$$

Where, β_o, β_i ,β_{ii} , β_{ij} are coefficients and x_i, x_j are coded independent variables, n=3.

Table 4.1. Values of independent variables at three levels of CCRD design

	Levels in coded form		
	-1	0	1
Temperature (°C) (X_1)	30	40	50
Time (min) (X_2)	120	180	240
Salt conc. (%) (X_3)	5	15	25

4.2.2.2 Experimental setup

The experiments were conducted with designed experimental runs with three replicates of preweighed rectangular pieces of bamboo shoot keeping them in the shaker incubator (Sartorius Stedim Biotech CERTOMAT IS) with 150 rpm. After each experiment, the samples were taken out of the salt solution, rapidly and mildly washed with distilled water, blotted with absorbent paper and weighed. The osmotically dried samples were checked for solid gain (SG), water loss (WL), hardness and colour change (dE) as discussed in section 4.2.5.

4.2.3 Mass transfer kinetics during osmotic dehydration

4.2.3.1 Osmotic dehydration

Bamboo shoots of approximately 1 cm^3 sizes were taken for study. Osmotic dehydration study was carried out in a water bath incubator shaker (Jain scientific glass works, Ambala, India) which was preset at the required temperature. The optimized process parameters were used for this study. The optimized values of process time and salt concentration obtained were 129 minutes and 12.10% respectively at 10:1 solution to sample ratio. The osmotic solution containing 12.10% sodium chloride was taken in 250 ml beaker and placed in a hot water bath. When the osmotic solution attained the required temperature the weighed bamboo shoot samples were dipped in the osmotic solution and then kept for the required time period. Proportions of osmotic solution and shoots pieces (10:1) were kept constant for all experiments.

4.2.3.2 Determination of water and solute diffusivities

The experiments were done in all the five levels of temperature viz., 25, 30, 35, 40, and 45°C. At each temperature, samples were taken out of the shaking incubator at particular time interval of 20, 40, 60, 80, 100 and 120 minutes. Maximum time limit of 120 minutes was selected vis-à-vis optimized process time of 129 minutes as discussed in section 4.3.1.3. Each sample were washed with water and spread onto absorbent paper to remove free water present on the surface. Osmotically dried samples were kept in hot air oven for 24 h at 105±1°C and then reweighed to calculate solid gain (SG), weight reduction (WR), water loss (WL), and moisture content (MC) as discussed in calculation section.

The diffusivity of the water and solute was determined using Fick's second law of diffusion for rectangular parallelepiped having sides 2a, 2b, and 2c (Eq. 4.2 & 4.3).[16, 25-26]

$$M_r=[(m_t-m_\infty)/(m_o-m_\infty)]=\sum_{n=1}^{\infty} C_n^3 \exp\{-D_{ew}tq_n^2[(1/a^2)+(1/b^2)+(1/c^2)]\} \quad (4.2)$$

$$S_r=[(s_t-s_\infty)/(s_o-s_\infty)]=\sum_{n=1}^{\infty} C_n^3 \exp\{-D_{es}tq_n^2[(1/a^2)+(1/b^2)+(1/c^2)]\} \quad (4.3)$$

Where, M_r and S_r are the moisture and solid ratio; the subscripts o, ∞ and t represent relevant initial concentrations, at equilibrium, and at any time; D_{ew} and D_{es} are the effective diffusivity of water and solute, respectively, and C_n is equal to $2\alpha(1+\alpha)/(1+\alpha+\alpha^2q_n^2)$ where, q_n's are the nonzero positive roots of the equation $\tan q_n = -\alpha q_n$. Here α is the ratio of volume of solution to that of each piece. A set of experimentations were performed for the set of equilibrium osmotic dehydration at all five temperatures by keeping fresh preweighed bamboo shoot in 12.10% salt concentration solution at 10:1 solution to sample ratio for 8 h. When samples are expected to reach an equilibrium position with no change in WL and SG, moisture contents of those shoots were determined.

Considering only the first term of the above equations to be significant and other terms to be negligible (which can be done when the Fourier number=D_et/A^2 value is

more than 0.1, and A being defined below), the equations reduces to the following equations (Eq. 4.4 & 4.5).[16,27]

$$-\ln (M_r/C_1^3) = q_1^2[(D_{ew}t)/A^2] \quad (4.4)$$

$$-\ln (S_r/C_1^3) = q_1^2[(D_{es}t)/A^2] \quad (4.5)$$

Where, $1/A^2 = [(1/a^2) + (1/b^2) + (1/c^2)]$. In this case a=b=c, hence $1/A^2 = (3/a^2)$. The values of D_{ew} and D_{es} can be calculated from the slopes of the regression lines obtained by plotting $-\ln (M_r/C_1^3)$ and $-\ln (S_r/C_1^3)$ against t.

Calculated D_{ew} and D_{es} values were fitted to Arrhenius type of equation to calculate activation energy (Eq. 4.6 & 4.7).

$$D_{ew} = D_{ow} \exp(-E_{aw}/RT) \quad (4.6)$$

$$D_{es} = D_{os} \exp(-E_{as}/RT) \quad (4.7)$$

Where, D_{ow} and D_{os} are the reference diffusivities of water and solute at infinitely high temperature, R is the ideal gas constant (J/mol K), T is temperature (K), E_{aw} and E_{as} are the respective activation energy (J/mol).

Above equations can be represented in a linear form and activation energy could be obtained from the slope of the resulting straight line.[28]

$$\ln D_{ew} = \ln D_{ow} + (-E_{aw}/RT) \quad (4.8)$$

$$\ln D_{es} = \ln D_{os} + (-E_{as}/RT) \quad (4.9)$$

Further, the texture, colour and microscopic observation of the fresh and osmotically treated bamboo shoots at all the five levels of temperature use for mass transfer kinetics study viz. 25, 30, 35, 40, and 45°C for 120 min was carried out to examine the effect of temperature on these parameters. Other osmotic drying conditions (12.10% salt concentration and 10:1 solution to sample ratio.) were kept constant.

4.2.4 Effect of centrifugal force and vacuum on osmotic dehydration

Effects of centrifugal force and vacuum pressure on osmotic dehydration were checked to get the best level of these two factors taking the optimized level of the input parameters (i.e. at 37 °C for 129 min and 12.10 % salt concentration). The five levels of

centrifugal force (1600, 2000, 2400, 2800, and 3200 rpm) were used during osmotic dehydration and these samples were referred as CFOD. With another set of experiments, the levels of vacuum pressure of 200, 300, 400, 500, and 600 mm Hg were applied during osmotic dehydration and these samples were referred as VOD. The vacuum was appied for 10 minutes followed by normal osmotic dehydration for 10 min and this process was continued till the end of total osmotic dehydration duration. The results of CFOD and VOD were compared with the results of the control sample which was treated under same optimized conditions in shaking incubator at 150 rpm. For each set of experiment solid gain, water loss, hardness, rehydration ratio, colour change, and microscopic observation were calculated as discussed in calculation section.

4.2.5 Calculation of responses

4.2.5.1 Water loss and solid gain

Osmotically dried samples were kept in hot air oven for 24 hours at 105±1 °C and then reweighed to calculate solid gain (SG), weight reduction (WR), water loss (WL), and moisture content (MC) using the following equations.[12,29]

$$SG\ (\%) = [(S-S_o)/M_o] \times 100 \quad (4.10)$$

$$WR\ (\%) = (M_o-M)/M_o \times 100 \quad (4.11)$$

$$WL\ (\%) = WR+SG = [(M_o-M)+(S-S_o)]/M_o \times 100 \quad (4.12)$$

Where, M_o- Initial weight of sample (g); M - Weight of sample after osmotic dehydration (g); S_o- Initial dry matter in sample (g); S –Dry matter in sample after dehydration (g)

4.2.5.2 Hardness

The texture for the shoot was evaluated by measuring the penetration force using a puncture test. The maximum force value is related to the firmness of the bamboo shoots. Hardness of sample was measured by using texture analyzer (TA-HDi, Stable Microsystems, UK) with a 2 mm diameter probe (pretest speed: 2 mm/s, test speed: 1 mm/s, posttest speed: 10 mm/s, trigger force: 5.0 g and distance: 2 mm).

4.2.5.3 Colour

The colour of the bamboo shoot samples were measured using a Hunter Colour Lab (Ultrascan VIS, Hunter Lab., Inc.). The results were expressed in terms of L, a and b values. The L value represents lightness component on surface that the value ranges from 0 to 100 while a and b values are chromatic components of redness to greenness and blueness to yellowness.[30] In addition, the total colour change (dE) values were calculated (Eq. 4.13) from the Hunter L, a and b scale and used to describe the colour change during osmotic dehydration of bamboo shoot. For each type of sample five colour measurements were taken and the average values were used.

$$dE = \sqrt{(L_o-L_t)^2 + (a_o-a_t)^2 + (b_o-b_t)^2} \quad (4.13)$$

Where, L_o, a_o, b_o are the initial colour measurements of fresh bamboo shoot cubes and L_t, a_t, b_t are the colour measurements after osmotic dehydration for 120 min.

4.2.5.4 Rehydration ratio

The rehydration of dehydrated bamboo shoots were determined by soaking a known weight of each sample in a sufficient volume of water (approximately 30 times the weight of dehydrated bamboo shoot) at room temperature and kept for 12 hours. Later the change in weight was calculated after removing the excess water with an absorbent paper.

Rehydration ratio= weight of rehydrated sample (g)/ weight of dehydrated sample (g)

(4.14)

4.2.5.5 Microscopic observation

Microscopic observation was carried on the thin slices of the dehydrated sample using a light microscope (DM 3000, Leica, Germany) equipped with a CCD camera (Leica DFC 440C) and Leica Application Suite (LAS) software. The slices were stained with 0.1 % methylene blue solution and covered with cover slip. Stained slice was checked under microscope at different magnifications.[31]

4.2.5.6 Statistical analysis

All the analyses of centrifugal force and vacuum pressure effects on osmotic dehydration were performed taking three replicates and data were reported as mean ± SD. Single factor ANOVA was used to determine the critical difference of means, and variance among the different samples were checked at significance level $P \leq 0.05$.[32]

4.3 Results and discussion

4.3.1 Optimization of osmotic dehydration process

4.3.1.1 Fitting models

The effect of various independent variables on the responses i.e. SG, WL, hardness and colour change were analysed and represented in Table 4.2. The independent and dependent variables were fitted to the second order model and tested for adequacy and fitness by analysis of variance. The effects of linear, quadratic and interaction terms on each of the responses were observed (Table 4.3). All the quadratic models showed high significant effects ($p < 0.001$) on response variables. The goodness of the fitting of model is generally shown by the correlation coefficients being significantly high ($R^2 > 0.9$). In these experiments, R^2 for SG, WL, hardness and colour change were reported as 0.95, 0.99, 0.90, and 0.93 respectively, which showed the model to be significantly fitted for the experiment.

4.3.1.2 Influence of input variables on responses

The results in the Table 4.3 clearly define the effect of linear, quadratic and interaction effects of the independent variables on solid gain, water loss, hardness and colour change. Among the linear terms, temperature evinced significant effect on all the response parameters ($p<0.001$), whereas concentration shown significant effect on all the parameters except colour change, which was found to be nonsignificant.[23-33] Quadratic effect of temperature, concentration and its interaction term was found nonsignificant, but they were positively correlated with solid gain (Fig. 4.1 (b)). Effect of time was not significant and its interaction with temperature and concentration was less effective (Fig. 4.1 (a),(c)). Water loss was significantly affected by linear

coefficients of temperature, concentration, and their interaction term ($p < 0.001$). Therefore, with increasing temperature and concentration, water loss was found to be increased (Fig. 4.1 (b)). Increase in solution concentration resulted in an increase in the osmotic pressure gradients and hence, higher water loss was obtained. The results are in agreement with Azoubel et al.[34] during osmotic dehydration of cherry tomato in NaCl solution (with and without sucrose). Quadratic effect of temperature showed significant effect ($p < 0.01$) on water loss, but it was correlated negatively (Table 4.3). Time also shows positive significant effect on water loss ($p < 0.01$), but its quadratic and interaction terms with other parameters revealed nonsignificant, however, they were correlated positively (Fig. 4.2 (a),(c)). Hardness was significantly affected by linear coefficients of temperature ($p < 0.001$) followed by linear term of concentration ($p < 0.01$). All other linear, quadratic and interaction terms were nonsignificant. Effect of all interaction term on hardness can be clearly seen from Fig. 4.3(a),(b),(c). The linear term of temperature had significant effect on colour of shoot ($p < 0.001$). With increasing in temperature, colour change values (dE) were found high, it might be due to exposure of bamboo shoot at high temperature. However, all other terms were nonsignificant and its effect on colour change could be seen from Fig. 4.4(a),(b),(c).

4.3.1.3 Optimization of the process variables

A numerical optimization technique based on the desirability approach was used to determine the workable optimum conditions for osmotic dehydration of bamboo shoots having higher response values. The main criteria for constraints optimization were to maximize water loss, and minimize solid gain, hardness and colour change of the dehydrated product. The independent variables were kept in the experimental range for the optimization process. The optimized values of temperature, time and salt concentration obtained were 37 °C, 129 minutes and 12.10 % respectively. The predicted solid gain, water loss, hardness and colour change values evaluated under the optimum conditions are 4.58 %, 19 %, 495 g and 14.38 respectively. The experiments were carried out in triplicate at the obtained optimum combination and the results demonstrated a good relationship between the predicted and experimental values. The experimental values at optimized conditions for solid gain, water loss, hardness and

colour change were obtained as 4.40±0.23 %, 19.50±1.30 %, 501.12±7.85 g and 13.92±0.21 respectively.

Table 4.2. Coded and real values of independent variables and experimental responses of osmotic dehydration process

Run No.	Coded and real values of the Independent Variables			Experimental Responses			
	Temp. (°C) x_1 (X_1)	Time (min) x_2 (X_2)	Conc. (%) x_3 (X_3)	SG (%)	WL (%)	Hardness (g)	dE
1	-1 (30)	-1 (120)	-1 (5)	2.12±0.03	12.76±0.67	410.6±10.33	12.51±0.34
2	-1 (30)	-1 (120)	1 (25)	6.25±0.08	14.76±0.71	475±7.97	12.56±0.56
3	-1 (30)	0 (180)	0 (15)	4.52±0.07	13.58±0.75	490.6±3.98	11.23±0.39
4	-1 (30)	1 (240)	-1 (5)	3.28±0.05	12.92±0.91	475±5.28	13.15±0.44
5	-1 (30)	1 (240)	1 (25)	6.5±0.11	15.14±0.62	509.8±6.90	13.25±0.21
6	0 (40)	-1 (120)	0 (15)	6.74±0.09	20.31±0.77	541±8.14	15.58±0.12
7	0 (40)	0 (180)	-1 (5)	5.49±0.15	18.57±0.38	450.26±7.57	14.91±0.28
8	0 (40)	0 (180)	0 (15)	5.59±0.22	22.58±0.77	528.23±11.59	14.37±0.47
9	0 (40)	0 (180)	0 (15)	6.92±0.28	21.18±0.85	550.6±8.92	16.68±0.40
10	0 (40)	0 (180)	0 (15)	5.37±0.13	23.12±0.98	607.6±5.10	17.37±0.35
11	0 (40)	0 (180)	0 (15)	5.56±0.09	22.46±0.49	598.65±6.70	15.4±0.58
12	0 (40)	0 (180)	0 (15)	6.27±0.16	21.88±0.54	580.8±5. 82	17.13±0.25
13	0 (40)	0 (180)	0 (15)	5.83±0.13	22.05±0.81	543.23±5.92	14.04±0.56
14	0 (40)	0 (180)	1 (25)	10.68±0.16	24.36±0.83	614.8±10.39	14.58±0.25
15	0 (40)	1 (240)	0 (15)	7.35±0.09	23.85±0.51	519.7±7.28	15.89±0.18
16	1 (50)	-1 (120)	-1 (5)	8.74±0.13	21.31±0.54	641±4.95	21.52±0.42
17	1 (50)	-1 (120)	1 (25)	12.68±0.16	28.31±0.59	688.5±8.54	23.43±0.59
18	1 (50)	0 (180)	0 (15)	11.45±0.09	26.14±0.82	682.4±5.48	19.3±0.66
19	1 (50)	1 (240)	-1 (5)	9.27±0.16	23.77±0.41	660.2±7.90	24.27±0.28
20	1 (50)	1 (240)	1 (25)	13.74±0.13	30.55±0.54	741±6.98	24.8±0.34

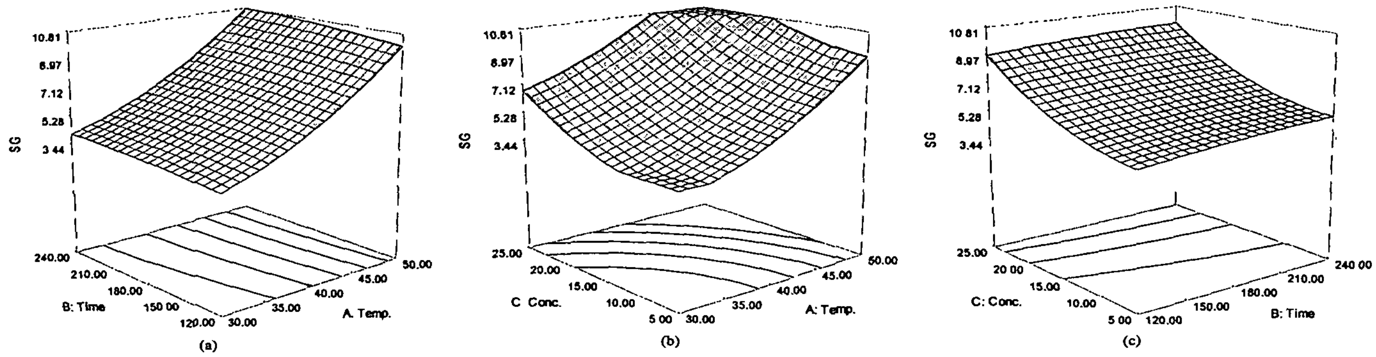


Fig. 4.1. SG as a function of (a) time and temp, (b) conc. and temp, (c) conc. and time

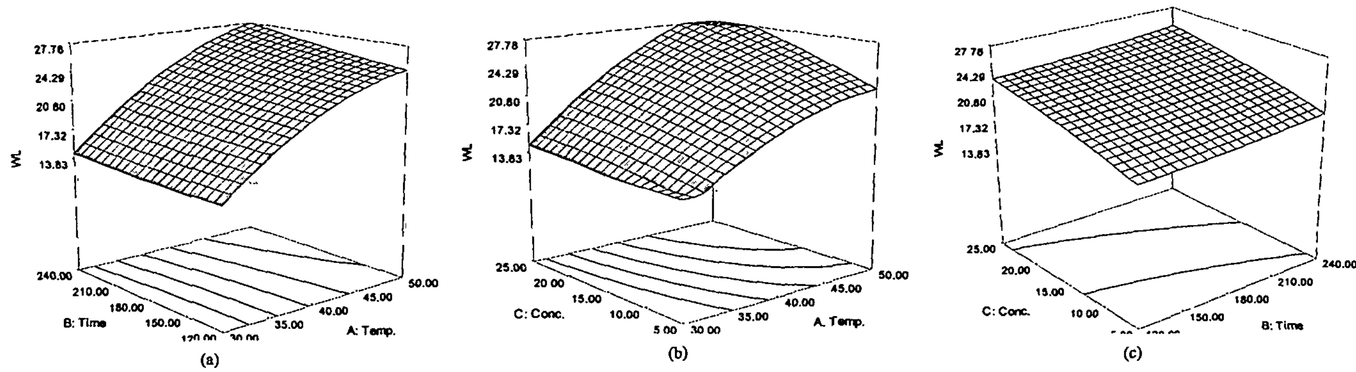


Fig. 4.2. WL as a function of (a) time and temp, (b) conc. and temp, (c) conc. and time

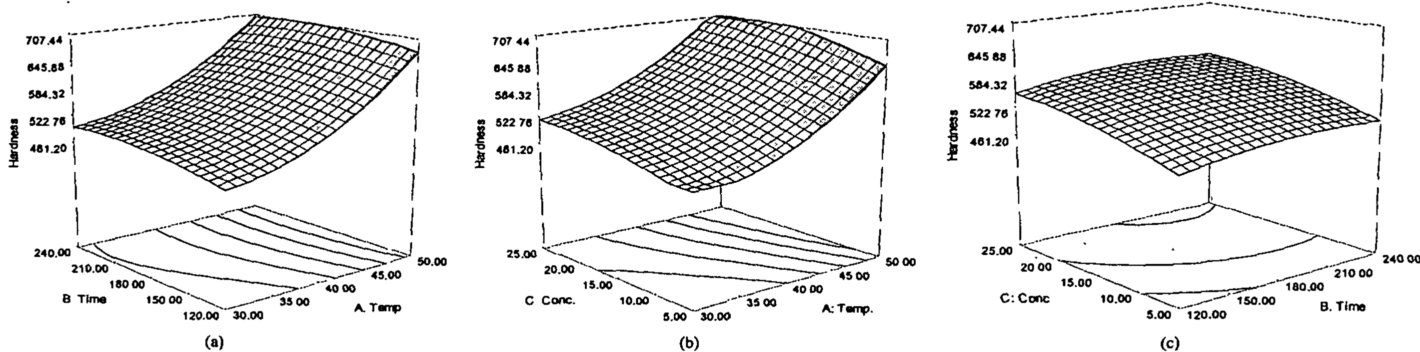


Fig. 4.3. Hardness as a function of (a) time and temp, (b) conc. and temp, (c) conc. and time

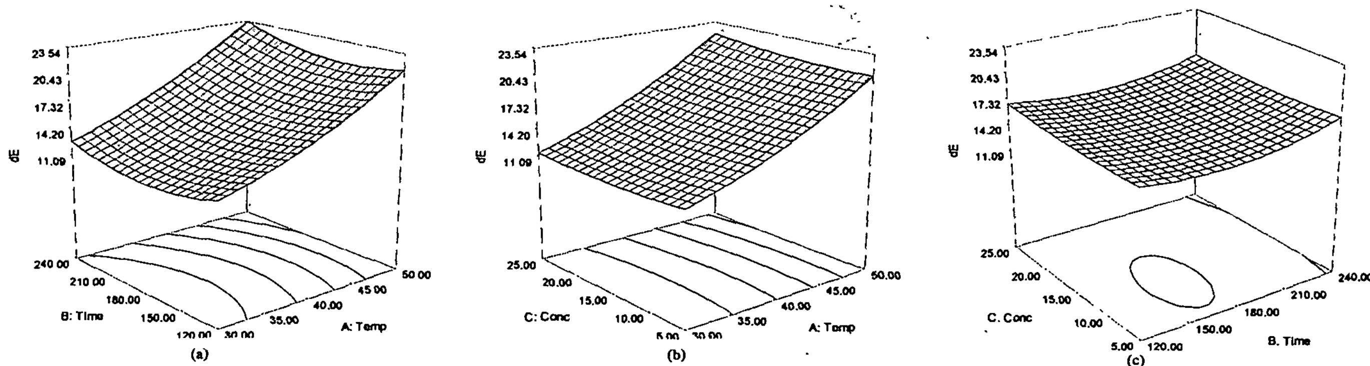


Fig. 4.4. Colour change as a function of (a) time and temp, (b) conc. and temp, (c) conc. and time

Table 4.3. Regression coefficients of responses calculated on coded levels

Parameters	Solids gain (%)	Water loss (%)	Hardness (g)	Colour change (dE)
Intercept (β_o)	6.42	22.07	559.15	15.23
Temp. (β_1)	3.32***	6.09***	105.21***	5.06***
Time (β_2)	0.36	0.88**	14.96	0.58
Conc. (β_3)	2.09***	2.38***	39.20**	0.23
Temp. × Time (β_{12})	0.022	0.52	-3.44	0.35
Temp. × Conc. (β_{13})	0.13	1.20***	3.64	0.29
Time × Conc. (β_{23})	-0.047	0.000	0.46	-0.17
(Temp.)^2 (β_{11})	0.81	-2.00**	40.91	0.93
(Time)^2 (β_{22})	-0.13	0.22	-15.24	1.40
(Conc.)^2 (β_{33})	0.91	-0.40	-13.06	0.41
R^2	0.9552	0.9887	0.9058	0.9314
F value	23.68	97.23	10.69	15.08
P value for model	< 0.0001	*< 0.0001*	0.0005	0.0001
P value for model lack of fit	*0.0902*	*0.3534*	*0.2920*	*0.4696*

* Significant at $p < 0\ 05$; ** Significant at $p < 0.01$; *** Significant at $p < 0.001$

4.3.2 Mass transfer kinetics during osmotic dehydration

4.3.2.1 Determination of water and solute diffusivities

The moisture content and solid gain data of each temperature taken at a particular interval of time (20 min) and at equilibrium condition, the M_r and S_r values corresponding to each time interval and at each temperature were calculated. The α value was calculated to be 10 and hence q_1 value against level 0.10 (=1/α) is 1.6320. The graphs were plotted taking -ln (M_r/C_1^3) against t (Fig. 4.5(a)) and -ln(S_r/C_1^3) against t (Fig. 4.5(b)) at each temperature. From the slopes of the graph, effective water diffusivity (D_{ew}) and effective solute diffusivity (D_{es}) were calculated (Table 4.4). The effective diffusivity values were calculated and it was observed that D_{ew} values were in

the range of 3.36×10^{-9} to 10.20×10^{-9} m^2/s and D_{es} values were in the range of 3.20×10^{-9} to 25.31×10^{-9} m^2/s in the temperature range of 25-40 °C. Similar trend of D_{ew} values were found between 5.86×10^{-9} and 2.505×10^{-8} m^2/s for tomato slices[35]; however, Rastogi and Niranjan[16] reported D_{ew} and D_{es} values as 0.538×10^{-9} m^2/s and 0.713×10^{-9} m^2/s respectively for pineapple.

Both D_{ew} and D_{es} values showed increasing trend with temperature. The effect of temperature and the values of effective diffusivity coefficients are similar to those reported in the literature for fruits and vegetables products.[36-40] The D_{ew} and D_{es} values for some fruits and vegetables were reported in the range of 5.084×10^{-11} to 21.335×10^{-11} m^2/s and 3.854×10^{-11} to 10.822×10^{-11} m^2/s for apple slice[41]; 1.48×10^{-9} to 3.24×10^{-9} m^2/s and 0.53×10^{-9} to 1.54×10^{-9} m^2/s for cubical shapes pineapple[42]; 1.030×10^{-8} to 3.549×10^{-9} m^2/s and 1.117×10^{-8} to 8.540×10^{-9} m^2/s for rectangular slabs of watermelon.[28]

In reference to Arrhenius equation (Eq. 4.6 and 4.7), the values of D_{ow}, D_{os}, E_{aw}, and E_{as} were obtained by plotting $\ln D_{ew}$ and $\ln D_{es}$ against 1/T (K) (Fig. 4.6(a) and (b)); Table 4.5). By putting the values in Arrhenius equation (Eq. 4.15 and 4.16), equations were developed which gives D_{ew} and D_{es} for bamboo shoot at any temperature (K). The equations derived were given below:

$$D_{ew} = 0.22425 \exp(44668.65/RT) \quad (4.15)$$

$$D_{es} = 3630496.595 \exp(85939.0877/RT) \quad (4.16)$$

Where; D_{ew} and D_{es} are expressed in terms of m^2/s, T is temperature (K), R is gas constant (J/mol K).

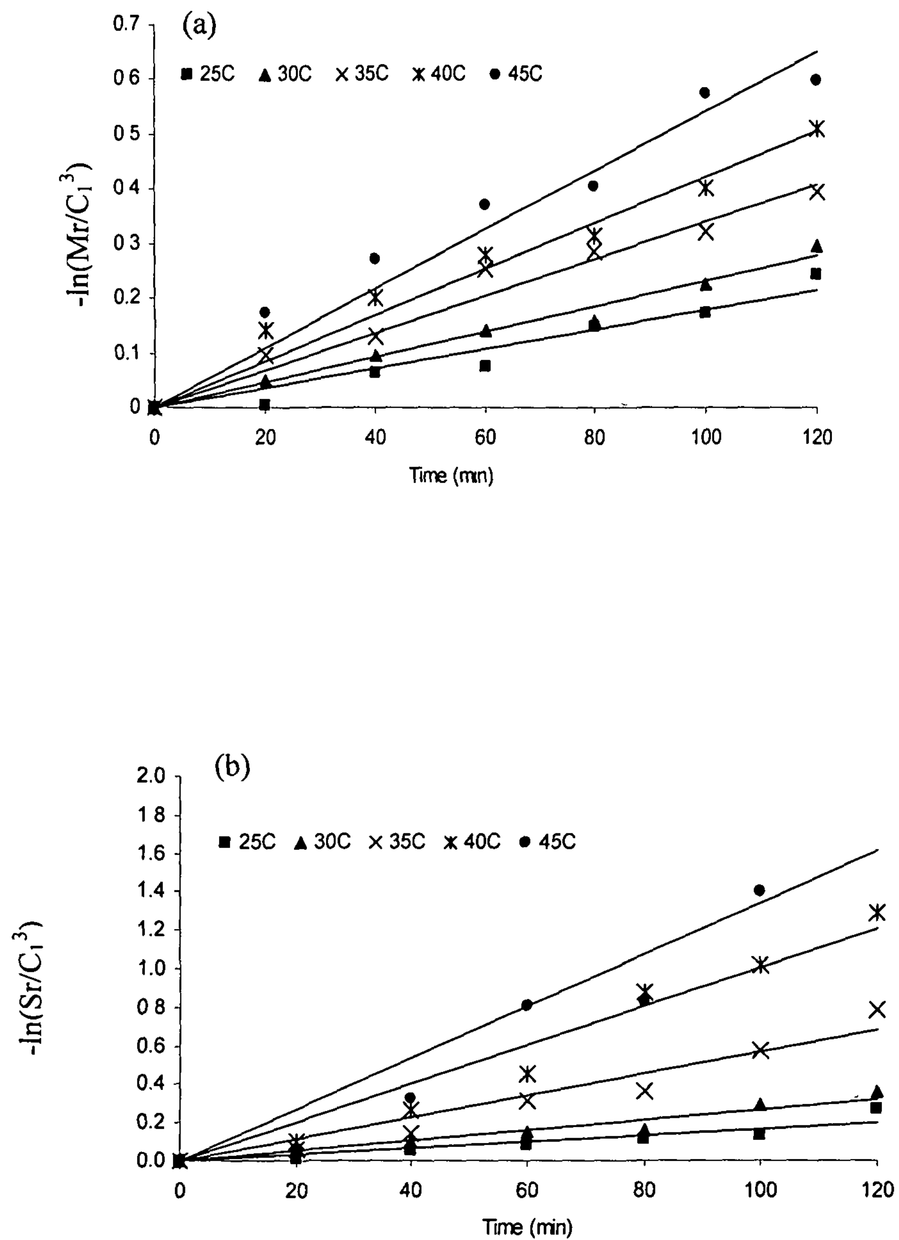
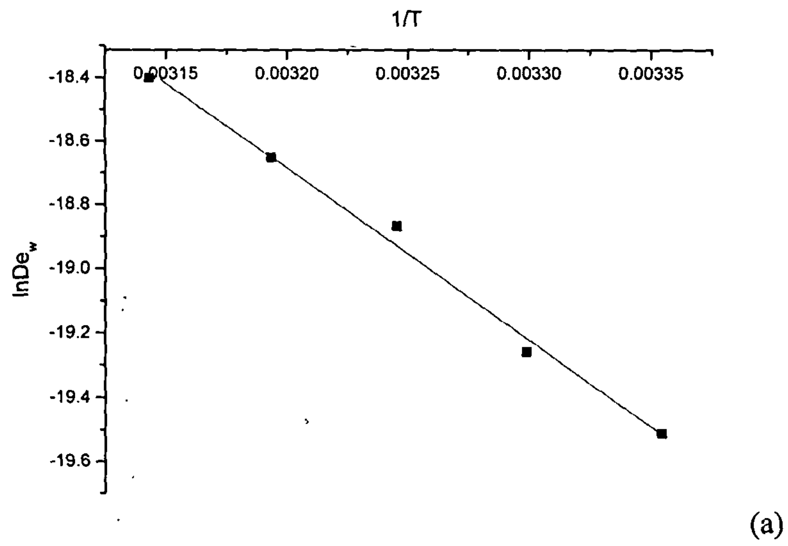
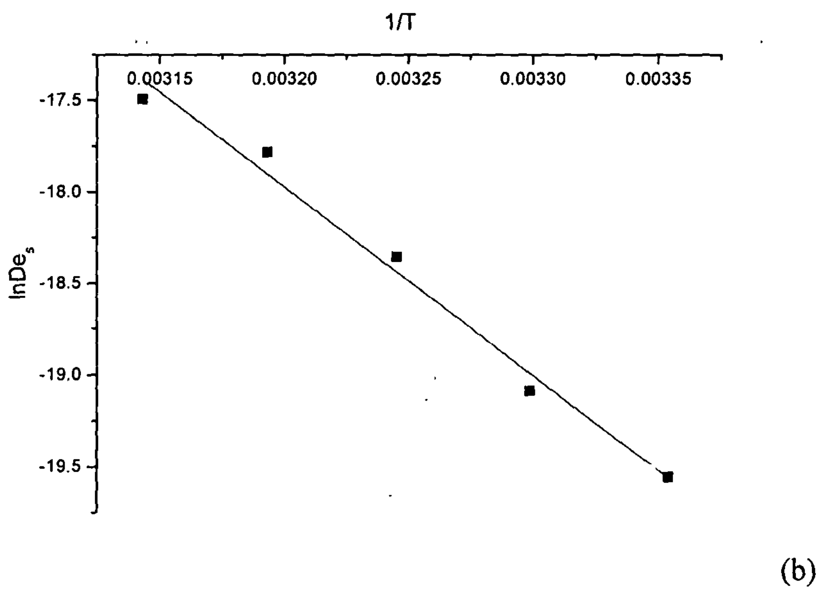


Fig. 4.5. Plots of (a) -ln (M_r/C_1^3) against t and (b) -ln (S_r/C_1^3) against t combined for all the temperatures



(a)



(b)

Fig. 4.6. Plots of (a) $lnDe_w$ against 1/T (K) and (b) $lnDe_s$ against 1/T (K)

Table 4.4. Effective water diffusivity and effective solute diffusivity at different temperatures

Temperature	Effective Diffusivity (m^2/s)	
(°C)	D_{ew}	D_{es}
25	3.36×10^{-9}	3.20×10^{-9}
30	4.32×10^{-9}	5.13×10^{-9}
35	6.39×10^{-9}	10.68×10^{-9}
40	7.94×10^{-9}	18.95×10^{-9}
45	10.20×10^{-9}	25.31×10^{-9}

Table 4.5. Coefficients of Arrhenius equation for effective water and solute diffusivity

Parameters	**D_o (m^2/s)**	**E_a (kJ/mol)**	**R^2**
Effective water diffusivity (D_{ew})	0.2243	44.67	0.9902
Effective solute diffusivity (D_{es})	3630496.5953	85.94	0.9810

4.3.3 Effect of temperature on texture, colour and microscopic observation of bamboo shoots.

The texture, colour and microscopic observation of the fresh and osmotically treated bamboo shoots at all the five levels of temperature viz., 25, 30, 35, 40, and 45°C for 120 min was carried out to examine the effect of temperature during osmotic dehydration.

4.3.3.1 Texture analysis

Hardness of shoot depends on water loss and solid gain of the sample. The hardness of fresh shoot was observed as 550.85 g. The osmotic drying temperature showed significant effect on hardness of bamboo shoot at $p \leq 0.05$. A gradual decrease in the hardness value reported with an increase in temperature of osmotic dehydration from 25 °C to 45 °C (Table 4.6). This could be explained by the fact that higher loss of water makes the tissue of the sample softer and reduction of firmness due to reduction of the turgor pressure and cell integrity.[43] Hardness value of bamboo shoot treated at 25 °C was 535.41 g which reduced to 464.23 g at treatment of 45 °C. Abraão et al.[44] reported that the osmodehydrated fruit became more brittle and less tough as the water content reduced and the solid content increased, and this might be related to the reinforcement of the cell walls due to the increase in sucrose concentration in the osmodehydrated fruit tissues.

4.3.3.2 Colour analysis

The osmotically dried shoots at various temperatures showed lower *L* values than the fresh shoot and effect of temperature was found significant at $p \leq 0.05$; however, above 35 °C the effect was not much significant. The *L* value decreased gradually along with increase of osmotic dehydration temperature from 25 °C to 45 °C (Table 4.6). This could be correlated with more water loss with increasing temperature, as it supports the increasing shrinkage and darkness of the samples. The temperature of the osmotic dehydration process had a more pronounced effect on the final colour of the osmodehydrated sample than the final solid content and with increase in temperature there was a decrease in the lightness of the samples.[44] The effect of temperature on *a* and *b* values was found less significant. However, both the values decreased with increase in temperature of osmotic dehydration. The dE value which gives change of colour with the fresh sample recorded the highest (10.18) at temperature of 45 °C and lowest (5.32) at 25 °C. The effect of temperature on colour change value was found significant at $p \leq 0.05$.

4.3.3.3 Microscopic observation

Microscopic observation of fresh and osmotically dehydrated bamboo shoots at all the five levels of temperature for 120 min are given in Fig. 4.7. Fresh sample contained spherical intact cell and it appeared turgid with an apparent consistent cell wall structure. However, gradual deformations in tissues were observed with increase in osmotic dehydration temperature, which might lead to more water loss during osmotic dehydration of bamboo shoot. At a temperature of 25°C and 30°C very slight deformation in cell structure was noted but further increasing in temperature from 35 to 45°C, collapse of tissues and folding of cells walls occurred. This could be correlated with more water loss and higher water diffusivity. The similar deteriorating effect on fruit cell structure were studied by various authors and their study revealed that with respect to temperature, time of osmotic dehydration, rehydration temperature, ultrasound pre-treatment and high pressure treatment were responsible for deterioration of cell structure.[16, 45-49]

Table 4.6. Texture and colour value of fresh and osmotically dehydrated bamboo shoots at different temperature

	Hardness (g)	L-value	a-value	b-value	dE
Fresh Shoot	550.85±7.72^{a}	69.13±4.68^{a}	2.27±0.59^{a}	25.66±1.78^{a}	---
25 °C	535.41±5.45^{b}	66.76±2.24^{a,b}	1.68±0.19^{b}	20.93±0.67^{b}	5.32±1.01^{a}
30 °C	521.84±3.12^{c}	63.92±1.73^{b,c}	1.55±0.25^{c}	18.96±0.49^{b}	7.07±0.33^{b}
35 °C	507.28±8.29^{d}	61.74±0.8^{c}	1.38±0.42^{d}	18.88±1.51^{b}	8.82±1.53^{c}
40 °C	484.93±9.82^{e}	61.52±2.44^{c}	1.35±0.09^{d}	18.39±0.66^{b}	9.01±1.71^{d}
45 °C	464.23±7.43^{f}	60.17±3.85^{c}	1.30±0.12^{d}	17.07±0.58^{b}	10.18±1.29^{e}

All data are the mean ± SD of three replicates Mean followed by different letters in the same column differs significantly ($P \leq 0.05$)

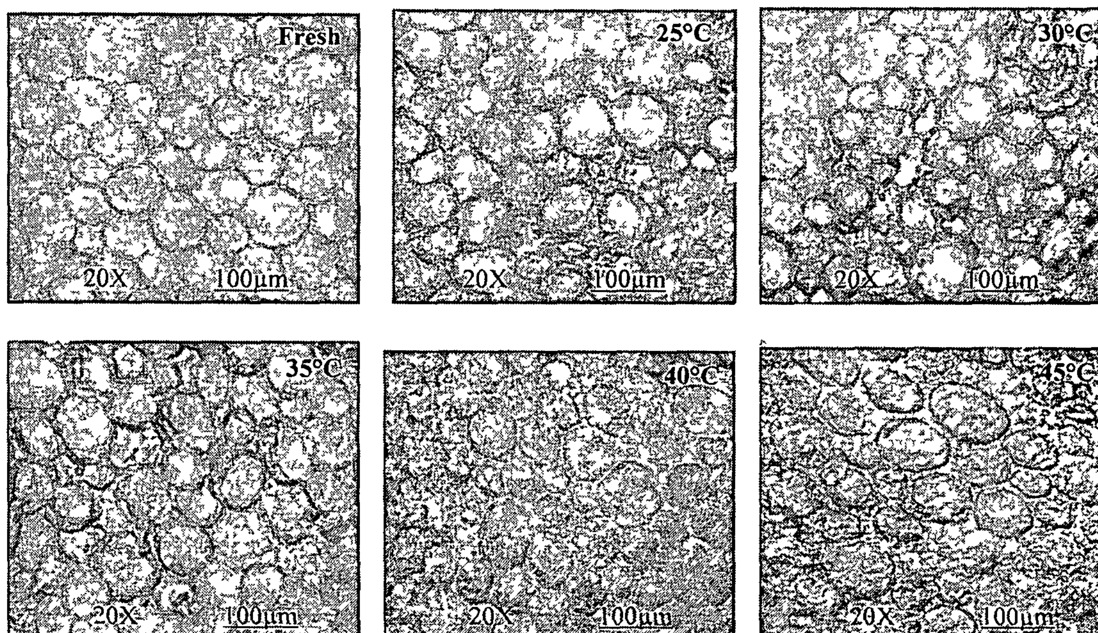


Fig. 4.7. Micrographs of the fresh and osmotically dehydrated bamboo shoot at all temeparture levels (under 20X magnification). Osmotic dehydration was carried out at all the five levels of temperature viz., 25, 30, 35, 40 and 45°c for 120 min.

4.3.4 Effect of centrifugal force and vacuum on osmotic dehydration

The bamboo shoots were osmotically dried using optimized process conditions under various centrifugal forces and vacuum pressure. Effects of centrifugal force and vacuum on various parameters viz. water loss, solid gain, hardness, colour change, rehydration ratio and microscopic observations are discussed below.

4.3.4.1 Water loss

The control sample has a water loss of 19.50 g/100g. The samples under centrifugal force initially showed a gradual increase in water loss pattern along with the increase of centrifugation force (Table 4.7). The results of the present study are inline with Amami et al.[18] in case of carrot. However, after centrifugation level of 2800 rpm, the water loss became almost constant. It could be explained by the fact that

centrifugation permits more accessibility of the hypotonic intracellular water to come out of the sample due to osmosis, and made an equilibrium with the outside hypertonic salt solution. There was a highest increase of around 7% water loss of the centrifuged sample compared to control. The samples treated under pulsed vacuum initially showed a gradual increase in water loss until application of vacuum pulse up to 400 mm Hg, and then it decreased continuously with further increase of vacuum pressure to 600 mm Hg (Table 4.8). Mujica-Paz et al.[50] also observed similar results by applying vacuum. This could be explained by the fact that initially due to application of the pulse vacuum, hydrodynamic mechanism occurs[51] which might have increased the interfacial area to increase the capillary action.[31] A highest increase of 12% water loss of the vacuum treated sample was obtained as compared to the control.

4.3.4.2 Solid gain

Under controlled conditions, 4.40% solid gain was reported. Although, a gradual decrease in solid gain ranging from 4.35 to 3.27% with increase in level of centrifugation was observed (Table 4.7). The same results were obtained by Azuara et al.[17] and Amami et al.[18] This pattern could be explained by the fact that centrifugal force exerts a centrifugal pressure on the sample which retards the movement of solutes to and from the solution. The highest decrease of around 1.13% solid gain was observed compared to the control with application of centrifugal force. In case of vacuum treated samples, a pattern of gradual increase in solid gain was observed (Table 4.8), which is similar to the work of Mujica-Paz et al.[50], Deng and Zhao[43] and Maneepan and Yuenyongputtakal.[31] It could also be explained by hydrodynamic mechanism which is responsible for increasing interfacial area thus increasing the pore size which facilitates the easy transfer of solutes to the sample from the solution. The level of entrapment of solutes inside the samples increased gradually with an increase of level of pulsed vacuum pressure. It might be attributed to vacuum which promotes outflow of the gas from inside the sample and compression of the residual gas takes place while restoring the atmospheric pressure with an uptake of osmotic solution containing solute.[31] The

highest increase of around 1.30 % solid gain was observed compared to the control due to application of vacuum.

4.3.4.3 Hardness

Hardness of shoot depends on water loss and solid gain of the sample. The hardness of the control was noted 501.12 g. The centrifuged samples showed a gradual decrease in the hardness level with an increase level of centrifugation (Table 4.7). This could be explained by the fact that higher loss of water makes the tissue of the sample softer and reduction of firmness due to reduction of the turgor pressure and cell integrity.[43] Present results can be corroborated with the findings of Maneepan and Yuenyongputtakal.[31] Again, in case of vacuum treated samples, hardness firstly decreased from 440.53 g to 359.57 g until pressure level of 400 mm Hg, and afterwards it was again increased (Table 4.8). The trend is exactly opposite to the trend of water loss, justifying the reduction of firmness with increased water loss. But the hardness levels of the vacuum treated samples are higher than that of centrifuged samples, though the water loss levels are higher in the former case. The results could be explained by the increased solid gain level in the vacuum treated samples, which might have imparted the hardness. However, control samples evinced the highest hardness proving the highest firmness level, and it was evident that firmness decreased with both treatments on osmotic dehydration.

4.3.4.4 Rehydration ratio

The rehydration ratio under controlled conditions was recorded 0.97 and ratio increased from 0.96 to 1.06 for the sample treated under centrifugation level of 1600 to 2800 rpm. However, at 3200 rpm, it slightly decreased to 1.03 (Table 4.7) and indicated that up to certain level of centrifugal force (2800 rpm) cellular disintegration is less; hence during rehydration it takes up water easily. Above this level, the cellular disintegration starts to occur, which is not desirable for osmotic dehydrated samples. Again in case of vacuum treated sample, rehydration ratio increased gradually from

0.94 to 0.96 up to 400 mm Hg and then it becomes constant, showing that increased level of vacuum do not affects the rehydration ratio (Table 4.8). It is evident that vacuum at any level disintegrates cells, which is proved by the lower values of rehydration ratio of the vacuum treated samples as compared to all other samples.

4.3.4.5 Colour change

The values of L, a, b and their overall difference from fresh sample shown as dE were obtained from the Hunter colour lab. The trend of L was studied basically, as it is related to shrinkage. L value which gives whiteness index, should be decrease according to the increasing level of shrinkage, which is again linearly related to the water loss.[52] In case of samples under centrifugal force, the L value decreased gradually along with increase of centrifugal force (70.38 to 63.54). Further increase in water loss corroborates the increasing shrinkage and darkness of the sample (Table 4.7). The dE value which gives change of colour with the fresh sample, was recorded highest (13.62) at centrifugal force of 3200 rpm and it was less than that of the control. The samples treated under pulsed vacuum showed decrease in L value from 69.25 to 52.85 for vacuum level of 200 to 400 mm Hg, and then it increased to 64.78 at 600 mm Hg. The trend is exactly fitted with the water loss pattern, and justifying the pattern of shrinkage (Table 4.8). The dE value was recorded highest (13.35) in sample treated under 200mm Hg vacuum pressure but recorded less than that of control (13.92).

4.3.4.6 Microscopic observation

Microscopic observation of fresh, conrol, CFOD and VOD treated shoots are presented in Fig 8. Fresh sample contained oval and spherical intact cell, while control evinced slight change in shapes of cell (Fig. 4.8 (a) and (b)). Gradual deformations in tissues were observed with increase in centrifugal force, which might lead to more water loss during osmotic dehydration (Fig. 4.8 (c) to (g)). Cell structure is also highly affected with increase in vacuum pressure (Fig. 4.8 (h) to (l)).

Table 4.7. Effect of centrifugal force on osmotic dehydration of bamboo shoot

Sample	Water loss (g/ 100g)	Solid gain (g/100g)	Hardness (g)	Rehydration ratio	L	dE
Control	19.50±1.30[a]	4.40±0.23[a]	501.12±7.85[a]	0.97±0.004[a]	71.25±0.35[a]	13.92±0.21[a]
CFOD 1600	21.95±0.48[b]	4.35±0.08[a]	452.32±5.14[b]	0.96±0.002[a]	70.38±0.75[a]	13.12±0.14[b]
CFOD 2000	23.05±0.19[c]	3.89±0.03[b]	408.36±4.56[c]	0.98±0.001[a]	66.15±0.62[b]	10.27±0.15[c]
CFOD 2400	24.10±0.23[c]	3.90±0.04[b]	373.46±6.80[d]	0.98±0.003[a]	65.46±0.83[b]	8.31±0.09[d]
CFOD 2800	27.24±0.31[d]	3.48±0.06[c]	302.53±7.54[e]	1.06±0.015[b]	64.18±0.46[c]	10.94±0.17[e]
CFOD 3200	27.14±0.41[d]	3.27±0.07[d]	277.57±6.80[f]	1.03±0.011[c]	63.54±0.36[c]	13.62±0.19[a]

All data are the mean ± SD of three replicates. Mean followed by different letters in the same column differs significantly ($P \leq 0.05$).

Table 4.8 Effect of pulsed vacuum on osmotic dehydration of bamboo shoot

Sample	Water loss (g/ 100g)	Solid gain (g/100g)	Hardness (g)	Rehydration ratio	L	dE
Control	19.50±1.30[a]	4.40±0.23[a]	501.12±7.85[a]	0.97±0.004[a]	71.25±0.35[a]	13.92±0.21[a]
VOD 200	22.04±0.23[b]	4.85±0.05[b]	440.53±3.75[b]	0.94±0.004[b]	69.25±0.87[b]	13.35±0.16[b]
VOD 300	26.35±0.45[c]	5.08±0.06[c]	433.27±3.59[b]	0.95±0.002[bc]	64.36±0.65[c]	7.09±0.19[c]
VOD 400	31.28±0.38[d]	5.13±0.04[c]	359.57±4.65[c]	0.96±0.002[c]	52.85±1.15[d]	13.12±0.28[b]
VOD 500	28.46±0.28[e]	5.44±0.06[d]	390.31±5.58[d]	0.96±0.001[c]	60.48±1.21[e]	4.86±0.24[d]
VOD 600	24.81±0.43[f]	5.70±0.04[e]	476.23±6.32[e]	0.96±0.003[c]	64.78±0.84[f]	8.56±0.23[e]

All data are the mean ± SD of three replicates. Mean followed by different letters in the same column differs significantly ($P \leq 0.05$).

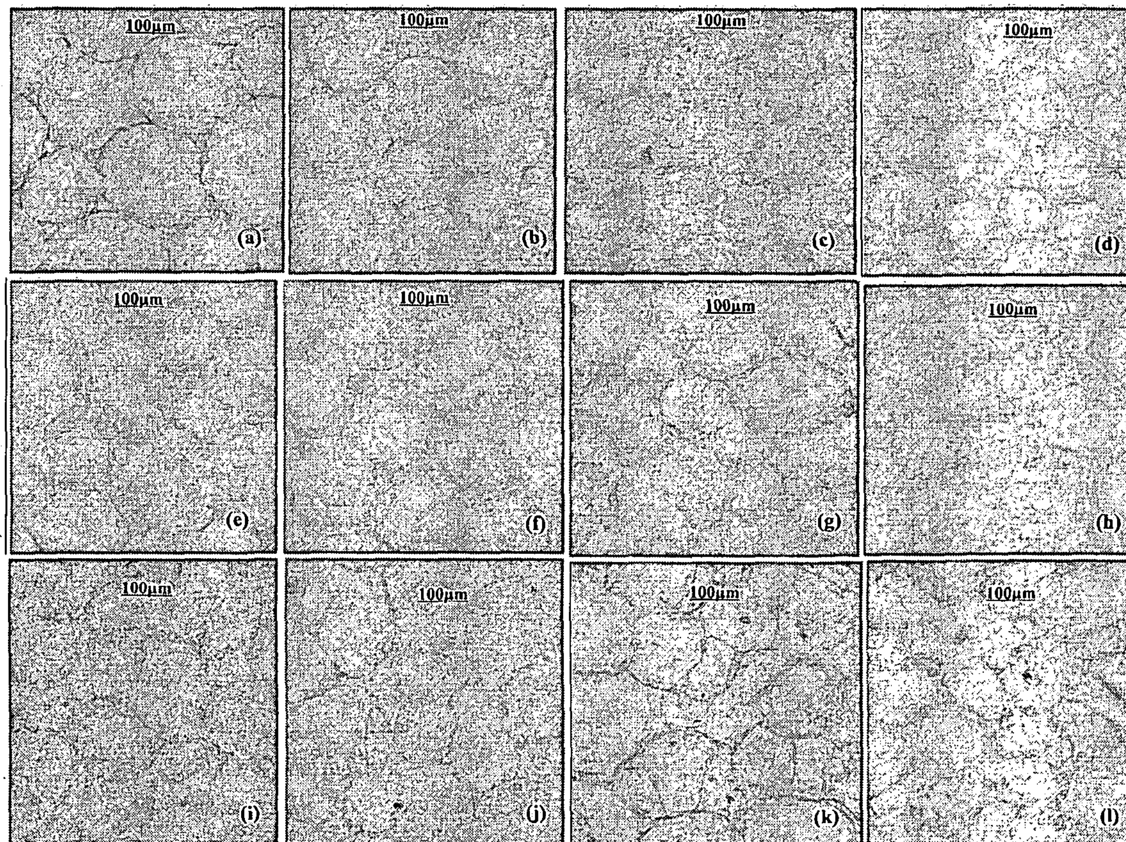


Fig. 4.8. Micrographs of (a) fresh, (b) control, (c) CFOD 1600, (d) CFOD 2000, (e) CFOD 2400, (f) CFOD 2800, (g) CFOD 3200, (h) VOD 200, (i) VOD 300, (j) VOD 400, (k) VOD 500, (l) VOD 600

4.4 Conclusion

Bamboo shoots were osmotically dried by varying different process variables and it was optimized using RSM. The optimum values for temperature, time, and salt concentration were found as 37 °C, 129 min, and 12.10 % respectively. The optimized temperature of osmotic dehydration (37 °C) is very desirable in terms of economy and nutritional requirement.

Mass transfer kinetics during osmotic dehydration of bamboo shoot reveled that the effective diffusivity of water as well as solute was dependent on osmotic solution temperature. Higher values of temperature showed more water loss through the shoot and more solid gain by shoot. Fick's diffusion model for unsteady state mass transfer was implemented to calculate the effective diffusion coefficients during osmotic dehydration of bamboo shoot. The effective water and solute diffusivity values were in the range of 3.36×10^{-9} to 10.20×10^{-9} m^2/s and 3.20×10^{-9} to 25.31×10^{-9} m^2/s for the temperature range of 25-40°C. Textures of bamboo shoot were affected with an increase in temperature of osmotic dehydration. The hardness values showed decreasing trend with increase in temperature and hence firmness gets reduced. At higher osmotic dehydration temperature, reduction in brightness of shoot, change in colour (dE) value and also damage to the cell structure were observed.

Vacuum pressure up to a certain level (VOD 400) brought more water loss compared to that of the centrifugal force. But, at the same time, solid gain is also quite more. As high solid gain is not desirable during osmotic dehydration, therefore, the use of centrifugal force for more water loss and lesser solid gain is suggested. The best levels of centrifugal force and vacuum pressure were recorded were 2800 rpm and 400 mm Hg respectively.

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Chapter 5

Influence of fermentation on bamboo shoot quality with and without addition of *Garcinia pedunculata* Roxb. fruit

5.1 Introduction

Bamboo shoots are young edible sprouts that come out from the ground. Tender young shoots are edible and are very popular in the traditional cuisines of various tribes and communities of South, South-East and East Asia. They are mainly used fresh, dried, shredded, canned, pickled or fermented.[1] A total of 17 amino acids are present in bamboo shoots, out of which eight are essential for the human body.[2] Minerals mainly consisting of chromium, copper, iron, potassium, calcium, manganese, zinc, less amounts of phosphorus, and selenium are found in bamboo shoots.[3] The lipid content of shoot is very less and the main fatty acids present in lipid are palmitic, linoleic and linolenic acids.[4] Both fresh and fermented bamboo shoots are rich in vitamins and phytosterols. Phytosterols act as neutraceuticals[5] and are precursors of many pharmaceutically active steroids found in plants.[6]

Fermented bamboo shoot is an important part of the traditional foods in the Northeastern state of India. Fermentation of bamboo shoots not only helps to extend storage life but also enhanced safety of foods using the natural microflora and their antibacterial compounds. Such traditional fermented food will be a potential source of lactic acid bacteria.[7-8] It also adds specific flavour, aroma and taste to the fermented product. Khorisa is a traditional fermented bamboo shoot product of Assam, India and it is important part of diet of both rural and urban people and is extensively used as a main ingredient in different food items like meat, fish preparations, preparing pickles etc. In the process of khorisa fermentation, small quantities of dried fruit of *Garcinia pedunculata* Roxb. (Local name: Borthekera) are added along with the shoot for fermentation, as a possible acidifier. The fruit of the garcinia tree is globose in shape and is 8-12 cm in diameter with fleshy aril. The fruit is used by the indigenous people as an antiscorbutic, astringent, cooling, cardiotonic and emollient.[9] The fruit is rich in antioxidants, but has low phenolic compounds.[10]

Effects of bamboo shoot fermentation and aging on nutritional and sensory qualities of Soibum, a traditional fermented bamboo shoot product of Manipur (India) were studied by Giri and Janmejay.[11] The changes in nutrient contents and texture of bamboo shoots during pickling process were studied by Zheng et al.[12] However, the studies on fermentation and microbiology of *khorisa* is yet be made. Therefore the present investigation was undertaken to examine the physicochemical

and microbial changes during the fermentation of young bamboo shoots in the process of making *khorisa*. The study also makes a comparison between the two types of *khorisa* viz. fermented with and without the use of *Garcinia pedunculata* Roxb., with reference to its nutritional and safety point of view.

5.2 Materials and methods

5.2.1 Materials

Bamboo shoots (*Bambusa balcooa*) were collected from Nagaon, Assam, India. The shoots were transported to the laboratory within 24 h of collection, and then shoots were defoliated and washed. The unwanted parts were removed and the soft edible portions were stored at 4°C for further analysis. The fresh shoot was subjected to chemical analysis, and then they were used to prepare khorisa. Mature *Garcinia pedunculata* Roxb. was harvested from local horticultural orchard of Nagaon, Assam (India), and transported to the laboratory within 24 h. They were washed, shredded uniformly and dried in a cross airflow tray drier (IKON, India) at 40 °C for 24 h (moisture content 12%) and kept in sterile containers and stored at 4 °C till further use.

All microbiological growth media, supplements, anaerobic system (Mark II), anaerobic gas packs (3.5 litre) and anaerobic indicator tablets were obtained from HiMedia Laboratories (India). Acetonitrile (HPLC grade) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Merck (Darmstadt, Germany). Water used in all the experiments was double distilled and deionised (Elix Millipore Water Purification System, USA). The carbohydrate standards (raffinose, trehalose, maltose, sucrose, melibiose, lactose, glucose, xylose, galactose, rhamnose, inositol, arabinose and fructose) and organic acid standards (oxalic acid, tartaric acid, formic acid, pyruvic acid, lactic acid, acetic acid, citric acid, succinic acid, propionic acid) were of HPLC grade and obtained from Sigma (Sigma Aldrich, USA). All other chemicals used in the study were obtained from Merck (India).

5.2.2 Bamboo shoot fermentation and its kinetics

The fermentation of bamboo shoot was carried out in two batches in two replicates. In first batch bamboo shoots were grated and packed tightly in pre-sterile 500g capacity glass jars (Batch-1) and in the second batch pieces of *Garcinia*

pedunculata Roxb. were mixed (1%) along with grated bamboo shoot and packed in pre-sterile 500g capacity glass jars (Batch-2). The glass jars were incubated at 32°C in an incubator (New Brunswick Scientific, USA) for a period of 12 days for natural anaerobic fermentation. Fermentation kinetics was studied for a period of 12 days at regular intervals of 48 hours (2 days) for both the batches. Each batch consists of 7 jars to avoid cross contamination. One jar was taken out at an interval of 2 days from each batch and analyzed variation in pH, acidity, total phenol, antioxidant activity, reducing sugar and microbial count. The methods are discussed below.

5.2.3 Proximate analysis

Fresh and fermented bamboo shoots were analyzed for moisture, ash, protein, carbohydrate and fat, according to the standard AOAC[13] methods. The nitrogen content was converted to protein by multiplying with a factor of 6.25. Vitamin C was estimated on fresh shoot by titrating against 4% oxalic acid using 2, 6-dichlorophenolindophenols (DCPIP) as indicator.[14]

5.2.4 Estimation of total phenolics and antioxidant activity

Sample (1 g) was extracted with 10 ml of 80% methanol and centrifuged at 10000g at room temperature. Residue was reextracted (five times) with of 80% methanol and centrifuged. Supernatant was collected and used for the analysis of total phenolics and antioxidant activity. The total phenolics in the sample were estimated using Folin-Ciocalteu reagent (FCR) as described by Bray and Thorpe.[15] Supernatant was evaporated to dryness and residue was dissolved in 5 ml distilled water and aliquots (0.2-2 ml) were taken in different test tubes and final volume of 3 ml was made using distilled water. FCR (0.5 ml) was added and after 3 minutes 2 ml of 20% sodium carbonate was added to each test tube. The mixture was heated on a water bath at 100°C for 1 minute and then cooled. Absorbance was measured at 650 nm in spectrophotometer (Spectronic 20D+, Thermo Scientific, USA). The results were expressed as mg phenol/ 100 g of sample as catechol equivalent.

Free radical scavenging activity was used to measure the total antioxidant activity by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay method. DPPH is a commercial oxidising radical used to be reduced by antioxidants. The disappearance of the DPPH radical absorption at a particular wavelength is monitored by the

reduction in optical density. Methanolic extract of bamboo shoot (20 μL) was mixed with 1.5 ml of DPPH solution (0.025 g DPPH in 1000 ml of methanol) and the tubes were vortexed (Vortex Shaker, LaboTech, India) immediately and allowed to react for 45 minutes in a dark environment at room temperature. The control was prepared without the addition of any sample for baseline correction. Absorbance was measured at 517 nm in a spectrophotometer (Spectronic 20D+, Thermo Scientific, USA). The free radical scavenging activity was expressed as inhibition percentage and calculated by using the following equation.[16]

% Free radical scavenger activity = [(Control absorbance – Sample absorbance)/ Control absorbance] × 100

5.2.5 Estimation of pH and acidity

Sample (10 g) was blended for 15 min with 100 ml double distilled water in a homogenizer (Remi, Mumbai, India). The pH of the slurry was determined using a pH meter (PH 510 Eutech Instruments, Cyberscan, Malayasia) calibrated with standard buffer solution (Merck, Mumbai, India). Titratable acidity was estimated by titrating the filtrate (10 ml) of the slurry with 0.1 N NaOH solution using phenolphthalein as indicator (0.1% w/v in 95% ethanol). Titratable acidity was expressed in mg lactic acid equivalent per gram of sample.[13]

5.2.6 HPLC analysis

Organic acids and carbohydrate profile of fresh and fermented bamboo shoot of both the batches were determined using HPLC system (Dionex Ultimate 3000, Germany). Organic acids were quantified using UV detection of 210 nm, on Hamilton OA C18-column and mobile phase was 0.2M sodium sulphate, adjusted with methanesulphonic acid to pH of 2.68. Standard organic acids used for analysis were oxalic acid, tartaric acid, formic acid, pyruvic acid, lactic acid, acetic acid, citric acid, succinic acid and propionic acid.[17] Carbohydrates were analysed using RI detection on Hamilton Ca-column using water as a mobile phase. Flow rate and column temperature was 0.6 ml/min and 30°C respectively for both analyses. Standard sugars used for analysis were raffinose, trehalose, maltose, sucrose, melibiose, lactose, glucose, xylose, galactose, rhamnose, inositol, arabinose and fructose.[18-19] Stock and standard solutions of organic acids were prepared in

acetonitrile:water (80:20), and those of carbohydrates were prepared in water. Calibration curve were prepared using two different concentrations of each standard. Thus, a calibration curve was prepared for each organic acid and carbohydrate.

5.2.7 FTIR spectroscopy

The infra-red spectra for all the samples were obtained with a FTIR spectrometer (PerkinElmer, USA). The equipment was operated with scanning range of 4000 –450 cm^{-1} and spectrum of 100. Sample (clear glassy disk) for FTIR analysis were prepared by mixing powdered sample with IR grade KBr using suitable press at around 12,000 psi pressure.

5.2.8 Microbial analysis

Fermented sample weighing 11 g was blended in 99 ml double distilled sterile 0.89% (w/v) sodium chloride diluents by use of a Stomacher lab-blender 400 (Seward Medical, London, UK) for 3 minutes. Appropriate serial dilutions were made and plated on plate count agar (PCA) for total plate count, the plates were incubated at 36°C for 36-48 h. Potato dextrose agar (PDA) supplemented with 10% sterile tartaric acid solution was used for yeast and mould count, the plates were incubated at 27°C in a dark environment for 36-48 h. Lactobacillus MRS agar supplemented with 3% $CaCO_3$ and 0.1% bromocresol purple indicator solution was used for estimation and enumeration of lactic acid producing bacteria. The plates were incubated at 36°C for 48-72 hours inside an Anaerobic System (Mark II) with anaerobic gas packs and anaerobic indicator tablets. The colonies that appeared were counted as colony forming units (CFU) per gram wet weight of sample.

5.2.9 Statistical analysis

All analyses were performed in triplicate and data were reported as mean ± SD. The data was assessed by analysis of variance (ANOVA) and Duncan's multiple range test. Statistical significance was defined at $p \leq 0.05$.

5.3 Results and discussion

5.3.1 Influence of fermentation on nutrients

Fresh and fermented shoot were analysed for various biochemical parameters, and the results are shown in Table 5.1. Fat content of fresh shoot was very low (0.67%), thus very less changes were observed in fermented shoot. The protein content decreased from 3.78% to 2.40% after fermentation, this may be due to denaturation of protein.[3] Carbohydrate which is an ideal source of energy content decreased in shoots after fermentation; it may be due to use of available carbohydrate by the microorganisms. The content of carbohydrate in fresh shoot was 4.50% which decreased to 1.45% and 1.39% in batch-1 and batch-2 respectively. Ash content was 0.86% and no significant change was observed during fermentation.

Vitamin C content was found less in the fermented shoots than the fresh shoots, but the reduction is more in batch-1 than batch-2. Total phenolics content were found to be increased 2-3 fold after fermentation. Fresh shoot having phenolics content of 97.5 mg increased to 255 mg and 239 mg/ 100g in batch-1 and batch-2 respectively. However, antioxidant activity was recorded to be higher in fermented shoots (49.20 and 55.35 % DPPH RSA for batch-1 and batch-2), as antioxidant capacity of bamboo shoot is closely related to L-ascorbic acid and total phenolic compounds.[20-21] The drop in pH and increase in acidity were also recorded in both the batches.

5.3.2 Fermentation kinetics of Khorisa

Fermentation kinetics for both the batches was studied for a period of 12 days at regular intervals of 48 hours (2 days). Each sample was analyzed for variation in pH, acidity, total phenol, antioxidant activity, reducing sugar and microbial count as discussed below.

5.3.2.1 Change in pH

The decrease in pH was directly proportional to the increase in fermentation time (Fig.5.1). The pH of khorisa decreased during fermentation, from initial values of 6.40 to 4.52 and 4.09 for batch-1 and batch-2 respectively. Decrease in pH is mainly because of lactic acid fermentation of bamboo shoot. Drop in pH was more

Table 5.1. Chemical composition of fresh and fermented shoot

	Fat (%)	Protein (%)	Carbohydrate (%)	Ash (%)	Vitamin C (mg/100g)	Total phenolics (mg/100g)	Antioxidant activity (%DPPH)	pH	Acidity (% LA)	Reducing sugars (g/100g)
Fresh Shoot	0.67±0.8[a]	3.78±1.4[a]	4.50±1.0[a]	0.86±0.05[a]	2.45±0.5[a]	97.5±4.2[a]	26.67±1.6[a]	6.40±0.15[a]	0.89±0.25[a]	1.37±0.05[a]
Khorisa (Batch-1)	0.44±0.1[b]	2.56±0.8[b]	1.45±1.2[b]	0.83±0.01[a]	1.09±0.2[b]	255±4.4[b]	49.20±1.5[b]	4.52±0.19[b]	2.82±0.16[b]	0.32±0.06[a]
Khorisa (Batch-2)	0.41±0.2[b]	2.40±0.5[b]	1.39±0.9[b]	0.81±0.02[a]	1.37±0.3[b]	239±5.2[b]	55.35±1.2[c]	4.09±0.23[c]	3.75±0.19[c]	0.26±0.09[a]

All data are the mean ± SD of three replicates. Mean followed by different letters in the same column differs significantly ($P \leq 0.05$). n=3

in batch-2 compared to batch-1, which might be due to addition of *Garcinia pedunculata* Roxb. It acts as an acidulant and enhances the fermentation. The end product khorisa was sour in taste with a typical fermented flavour. Above results are comparable with pH of some fermented bamboo shoot products of North East India like mesu, soidon, soibum, soijim, ekung, eup and hirring having average pH of 3.9, 4.2, 4.2, 4.1, 3.9, 4.1 and 4.0 respectively.[22-23] uring the manufacturing of jiang-sun (fermented bamboo shoots product of Taiwan), a pH of 4.2 was also observed in the day-1 fermented sample, and a pH of 3.5 was observed in the 30-day sample.[24] Medoua et al.[25] reported that, during first two days of natural fermentation of yam (*Dioscorea dumetorum*) hardened tubers, pH decreased from 5.5 to 4.8 and then to 3.9 after 14 days of fermentation.

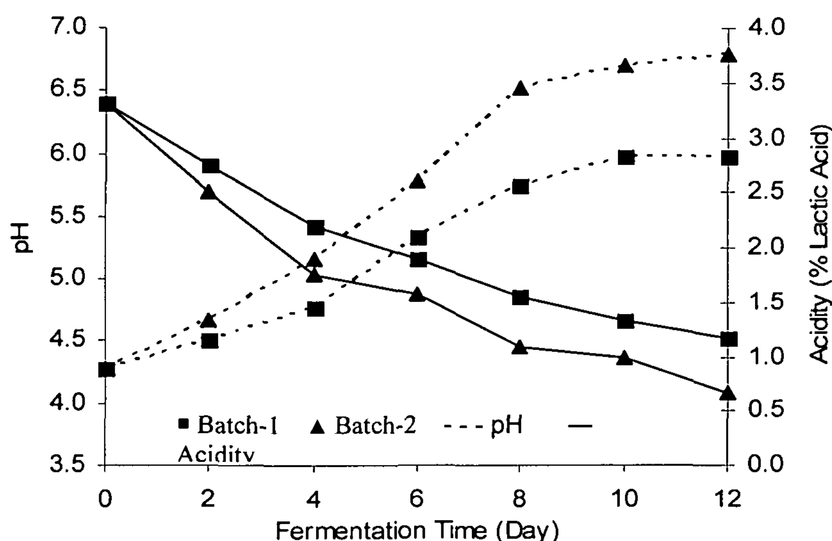

Fig.5.1. Change in pH and acidity of bamboo shoot during fermentation.

5.3.2.2 Change in acidity

Acidity was measured on the basis of % lactic acid. Increase in the acidity was found in both batches. However, distinct differences were observed in their changes over time. In batch-1 acidity changed from 0.89% to 2.82% whereas, it was slightly higher for batch-2, and values changed from 0.89% to 3.75% (Fig.5.1). The relative increase in high acidity could be due to the lactic acid production during fermentation of

bamboo shoot. Analysis of variance showed a significant effect ($p \leq 0.05$) of fermentation time on titratable acidity. Similar trend was reported for *Dioscorea dumetorum* hardened tubers.[25]

5.3.2.3 Change in total phenol content

Change in total phenol content was observed from 97.5 to 255.0 and 239.0 mg catechol equivalent/100g for batch-1 and batch-2 respectively. Analysis of variance showed a significant effect of the fermentation time. There was an exponential increase in total phenols level with fermentation time (Fig.5.2). Phenolics are usually found in conjugated forms through hydroxyl groups with sugar and glycosides in plant materials and these may catalyze during fermentation and thus lead to an increase in the content of total phenolics.[26] However, Luo et al.[27] reported that, total phenolic content of control and salicylic acid treated bamboo shoot increased progressively during storage at 1°C.

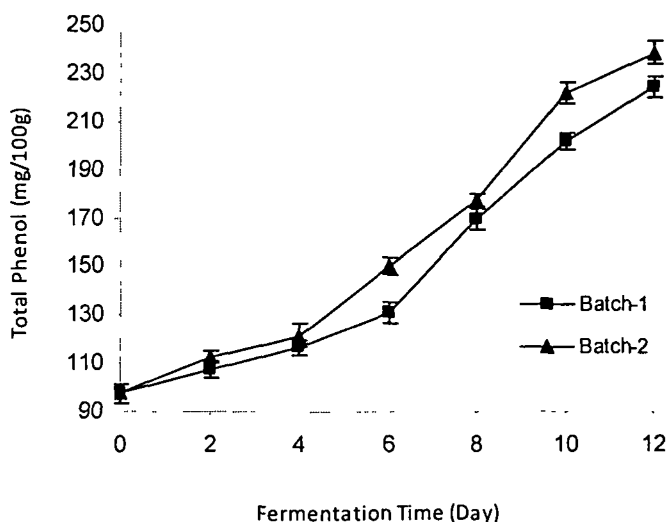

Fig. 5.2. Change in total phenol of bamboo shoot during fermentation. Vertical bars represent standard errors of means, n = 3.

5.3.2.4 Change in antioxidant activity (% DPPH RSA)

Antioxidant activity increased with time of fermentation. Values were observed to increase from 26.67 to 49.20 % for batch-1and 55.30 % batch-2. However, after 8 days there was very less change observed in both the batches but DPPH radical scavenging activity increased more for batch-2 (Fig.5.3). The results were inclining

with the antioxidant activity of lactic-fermented *Anoectochilus formosanus* (traditional Asian herb) ranged from 61% to 78% .[28]

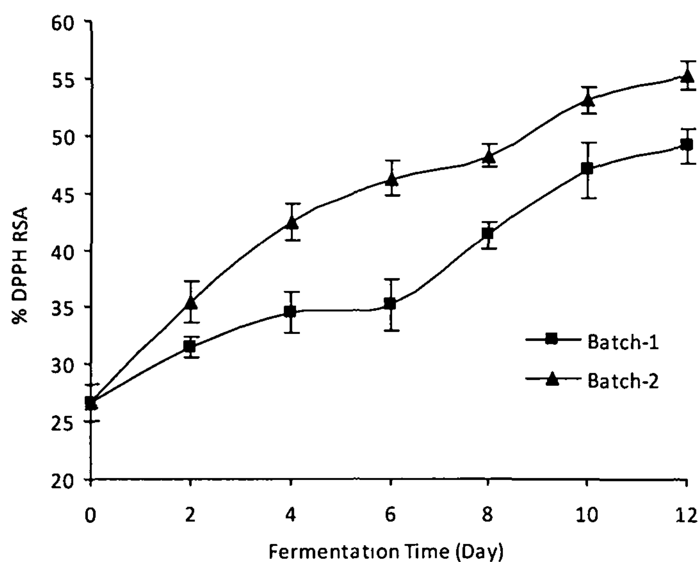

Figure 5.3. Change in antioxidant activity of bamboo shoot during fermentation. Vertical bars represent standard errors of means, n = 3.

5.3.2.5 Change in reducing sugars content

Reducing sugars like glucose is the most important substrate for microbes to undergo fermentation. Understanding the dynamics of reducing sugars will also enable to understand the fermentation mechanism. There was a sharp decrease in the reducing sugars content recorded during initial 8 days fermentation and after that it became slow. Reduction was noted from 1.37 to 0.32g/100g for batch-1 and 1.37 to 0.26 g/100g for batch-2 (Fig. 5.4). Pérez-Gregorio et al.[29] also reported similar observation in reducing sugar, during mulberries fermentation.

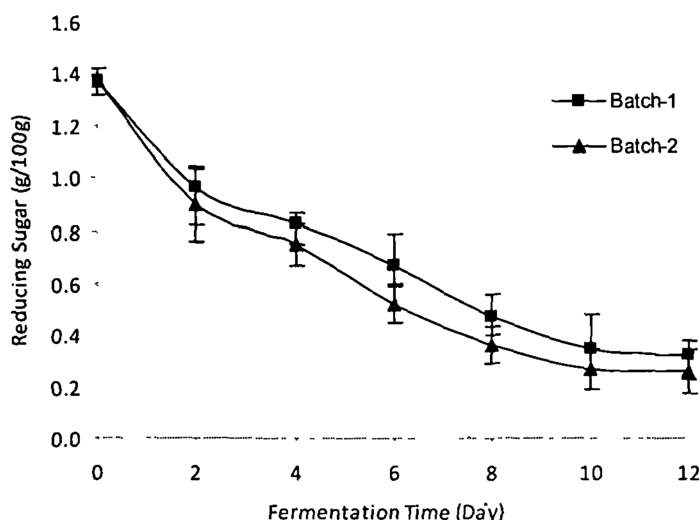

Fig. 5.4. Change in reducing sugars of bamboo shoot during fermentation. Vertical bars represent standard errors of means, n = 3.

5.3.2.6 Microbial analysis

Microbial kinetics of both the batches for total plate count (TPC), yeast and mould and lactic acid bacteria (LAB) are given in Fig. 5.5. At the onset of fermentation TPC increased manifolds and it was maximum at 6th day (6.95 log cfu/g) for batch-1 and at 4th day (7.20 log cfu/g) for batch-2, but afterward TPC decreased or almost stable in both the cases. Yeast and mould count increased from 5.18 to 6.00 log cfu/g for batch-1 and 5.18 to 6.18 log cfu/g for batch-2 upto 6th day, and afterwards it declined. At the end of 12th day, count was 4.46 and 4.51 log cfu/g for batch-1 and batch-2. LAB is mainly responsible for fermentation and imparts sour test development because of lactic acid production. LAB count was observed high during onset of fermentation and it increased till 6th day of fermentation. The values changed from 5.24 to 6.93 and 7.04 log cfu/g for batch-1 and batch-2 respectively. Growth of LAB decreased from 6th day onwards for both the batches. Similar LAB growth was also observed in other studies on lactic acid fermentation on different products. [24, 30-31] The decreased in microbial count attributed to both drop in pH of the fermented product and production of antimicrobial biometabolites by the dominant lactic acid bacteria.

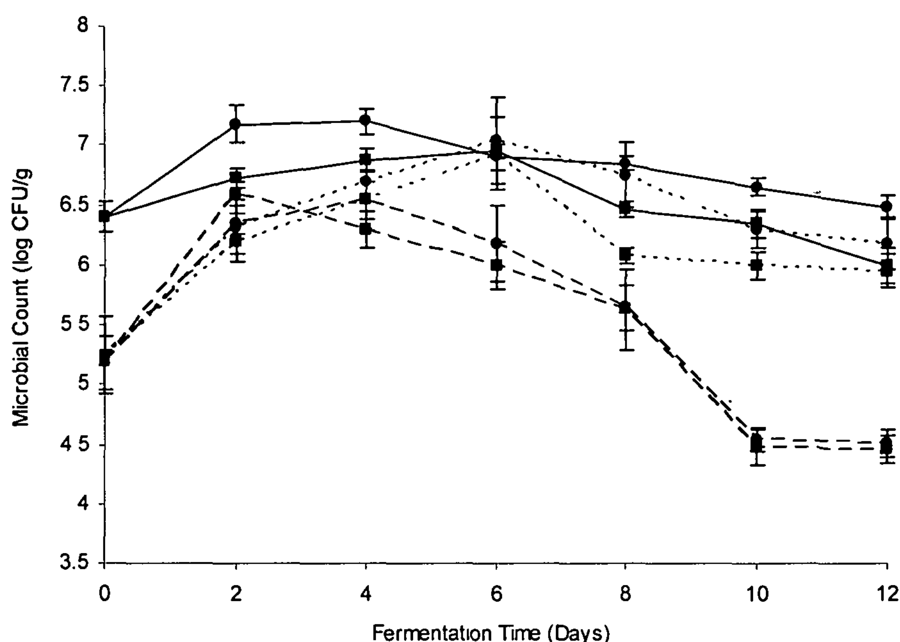

Fig. 5.5. Effect of fermentation on total plate count (—), yeast and mould count (– –), lactic acid bacteria count (····). Vertical bars represent standard errors of means, n = 3.

5.3.3 HPLC analysis of organic acids and carbohydrates

HPLC results evinced the presence of oxalic acid, tartaric acid, formic acid, pyruvic acid and lactic acid in fresh bamboo shoot. However, in fermented shoots, oxalic acid and pyruvic acid were found absent in batch-1 as well as oxalic acid and formic acid was found absent in batch-2 (Table 5.2). But the concentration of acids present in fermented shoots was found to increase. Lactic acid concentration in fresh shoot is 2.824 mg/g, which increased to 37.030 mg/g for batch-1 whereas it increased to 39.492 mg/g for batch-2. This is mainly because of lactic acid fermentation of shoot by lactic acid bacteria. Concentration of tartaric acid also found to increase from 1.733 mg/g to 32.041 mg/g and 51.021 mg/g for batch-1 and batch-2 respectively. However, other acids like acetic acid, citric acid, succinic acid and propionic acid were found absent in all the samples.

HPLC analysis of carbohydrates showed the presence of raffinose, sucrose, glucose, galactose and inositol in fresh bamboo shoot. Although raffinose, sucrose and glucose were not found in fermented shoot (batch-1), however, trehalose was recorded

during fermentation (Table 5.2). Concentration of galactose was recorded high in fermented shoot. Fresh shoot showed galactose concentration of 2.296 mg/g which increased to 31.277 mg/g and 23.480 mg/g for batch-1 and batch-2 respectively. Raffinose and glucose were retained in batch-2. Concentration of raffinose was quite high in batch-2 compared to fresh shoot, but glucose concentration gets diminished. The change in the concentration of different carbohydrates might be due to their utilization by the group of lactic acid bacteria during their cellular metabolism. However, other sugars like maltose, melibiose, lactose, xylose, rhamnose, arabinose and fructose were found absent in all the samples. Kozukue et. al.[32] reported the presence of major organic acids viz., oxalic, citric and malic acid as well as sugars viz., fructose, glucose and sucrose in raw bamboo shoots (*Phyllostachys pubescens*). However, during this study citric acid, malic acid and fructose were not detected.

Table 5.2 Characteristics of organic acids and carbohydrates present in fresh and fermented bamboo shoots.

Peak No.	Ret. time (Min)	Peak name	Amount (mg/g) Fresh shoot	Khorisa (Batch-1)	Khorisa (Batch-2)
Organic Acids					
1	3.89	Oxalic Acid	0.001	ND	ND
2	4.29, 4.23	Tartaric Acid	1.733	32.041	51.021
3	4.53, 4.57	Formic Acid	0.012	2.965	ND
4	4.63	Pyruvic Acid	0.037	ND	0.452
5	4.93, 4.89	Lactic Acid	2.824	37.030	39.492
Carbohydrates					
1	8.42, 8.70	Raffinose	0.092	ND	16.741
2	8.80	Trehalose	ND	11.022	ND
3	9.16	Sucrose	0.514	ND	ND
4	10.35, 10.37	Glucose	23.801	ND	13.119
5	11.80, 11.82, 11.73	Galactose	2.296	31.277	23.480
6	14.22, 14.12, 14.02	Inositol	0.131	0.201	0.223

ND – Not Detected

5.3.4 FTIR analysis

The FTIR spectrum pattern of shoot revealed six different peaks between 4000 and 800 cm^{-1} (Fig. 5.6). All characteristic peaks were observed in fresh and fermented samples. However, slight changes in peak intensities were observed in the fermented shoot. Gradual changes in the positions of the peak from 1650.63 (fresh shoot) to 1627.75 (batch-1) and 1626.72 (batch-2) was observed, corresponding to the stretching vibration of C=C bond. The changes were affected significantly by the fermentation of shoot. The peak assignment for identified peak and their related compounds present in fresh and fermented bamboo shoots are shown in Table 5.3.

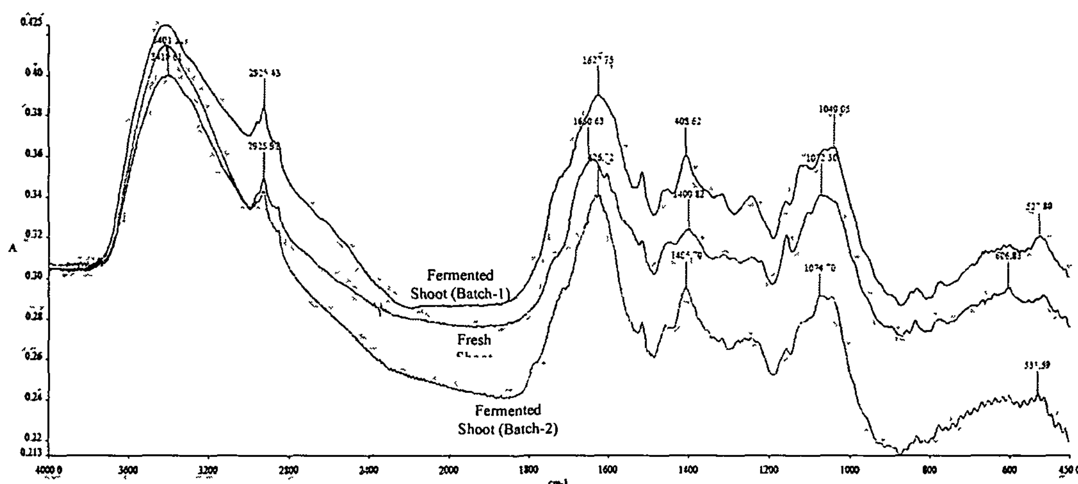

Fig. 5.6. FTIR spectrum pattern for fresh and fermented shoots of Batch-1 and Batch-2.

Table 5.3. Peak assignment and their related compounds present in fresh and fermented bamboo shoots.

Peak value			Peak assignments	Compounds
Fresh	**Fermented (Batch-1)**	**Fermented (Batch-2)**		
3410.61	3401	3401	O-H Stretching	Alcohols/ Phenols
2925.91	2925.43	2925	C-H Stretching	Alkanes (methylene)
1650.63	1627.75	1626.72	C=C Stretching	Aromatic
1400.82	1408.62	1405.70	C-H bending	Alkanes
1072.30	1040.05	1074.70	C-O Stretching	Carboxylic acid

5.4 Conclusion

Fermentation of bamboo shoots evinced various changes in physicochemical compositions. Minor changes in fat, protein, crude fibre, ash and vitamin C were recorded but carbohydrates concentration dropped markedly during fermentation. Increase in acidity and drop in pH up to 4.09 reveal the stability of fermented bamboo shoot products against contaminating microorganisms. Significant increase in total phenolics and antioxidant activity during fermentation highlighted its nutritional status and importance. Addition of *Garcinia pedunculata* Roxb. in bamboo shoot not only enhances the fermentation process but also imparts significant desirable changes in the product.

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Chapter 6

Extractions of antimicrobial biometabolites and antioxidant extract from different biological sources

6.1 Introduction

Lactic acid bacteria (LAB) are widespread and found commonly in nature, therefore there is an increased interest in this bacterium, for its ability to biopreserve. Several studies have been directed at identifying LAB that produces these bioactive antimicrobial metabolites, which may be used to control the growth of pathogenic and food spoilage microbes in food products.[1-3] LAB isolated from different sources have shown probiotic potential[4-5] and inhibitory effects on various pathogenic bacteria in the genera *Enterococcus*, *Salmonella*, and *Staphylococcus*.[6-8] Production of organic acids, undissociated organic acid molecules, bacteriocins, the competition for adhesion sites and coaggregate with pathogens are some of the several mechanisms, which have been suggested for the antimicrobial activity of lactic acid bacteria towards pathogens.[7,9] It has also been suggested that the antimicrobial activity, may also be due to other inhibitory substances.[10-11] There are also some reports where several authors have suggested that production of organic acids are the sole reason for the antimicrobial activity of lactic acid bacterial strains.[12]

Khorisa, a traditional fermented bamboo shoot product made by the indigenous people of Assam in the Northeastern regions of India. Khorisa is made by the lactic acid fermentation of fresh bamboo shoots during the monsoons. The fermented bamboo shoot product has good antibacterial and antifungal properties,[13] and the product can be stored and used up to six months to a year, without the addition of food preservatives. LAB isolated from Jiang-sun, a fermented bamboo shoot product from Taiwan, has shown bacteriocin producing capabilities.[14] The dominant lactic acid microflora in *khorisa* might also be responsible for the antimicrobial property.

Antioxidants are molecules that inhibit the oxidation of other molecules. Antioxidants are primarily composed of thiols, ascorbic acid or polyphenols.[15] They tend to minimize DNA damage, protein oxidation and lipid peroxidation in living cells caused by free radicals and various redox reactions.[16] There is an increased awareness and interest in the use of natural antioxidants. Synthetic antioxidants and preservatives are used in food industries to prolong shelf life of foods. These synthetic additives are found to cause nutrition losses, and produce toxic effects.[17] Worldwide there have been

numerous steps to substitute all synthetic antioxidants with natural ones. Many antioxidants are being identified as anticarcinogens and cardio-protective.[18-20] Neutraceutical properties of antioxidants have sparked immense interest among researchers to search plant sources for identifying antioxidants.[21-22]

Garcinia pedunculata Roxb. (GPR) is a globose shaped fruit with fleshy aril, found mostly in the states of Northeast India. It belongs to the genus *Garcinia* and family Clusiaceae (or Guttiferae). The mature fruit is eaten cooked or raw[23] and is locally known as "Borthekera" in Assam, a Northeastern State of India. The fruit usually matures during the month of April and is collected, cut into small pieces and sun dried. Dried pieces of the fruit are stored and used by the indigenous people throughout the year. The indigenous people of Northeast India use it for various medicinal uses. The water extract of the dried pellets of GPR are used as antidiarrhoeic and antidysentric[24] and it is rich in benzophenones, pedunculol, garcinol and cambogin.[25] High antioxidant activity has been reported in GPR by Gogoi et al.[26] and Mudoi et al.[27] GPR is a rich source of secondary metabolites including xanthones, flavonoids, benzophenones, lactones and phenolic acids with wide range of biological and pharmacological activities.[28-29]

Microwave assisted extraction (MAE) is a new green extraction technique that combines microwave radiation and traditional solvent extraction methods. It is a simple, cheap procedure than solvent extraction method, and also has less polarity limitations for the extractant. It offers higher degree of reproducibility, simplified manipulation, shorter extraction time, lesser use of solvent, and high extraction rate compared to conventional solvent extraction methods.[30] Conductive and convective processes to heat the sample is used in conventional solvent extraction methods, whereas microwave heating occurs by direct energy transfer to the sample.[31-32] Microwave heating is volumetric in nature so microwave irradiation efficiently produces internal heating by coupling microwaves with polar components inside the solvent and the sample. According to the cell-wall broken theory,[33] there are certain solvents which are microwave transparent, while some are microwave absorbing. By using microwave transparent solvents, there is more energy for the plant material to absorb. Cellular

structures contain water, which absorbs the microwave energy. This creates a sudden increase in temperature, and results in the rupture of the cell wall, and release of constituents into the surrounding solvent. Several studies have also used non-polar solvents which are transparent to microwave and in these cases only the sample matrix gets heated leading to release of analytes in a cold solvent.[34] This shows the higher extraction of polyphenolic compounds in acetone, compared to methanol, ethanol or water.[35] Higher extraction of polyphenolic compounds was observed, when solvent polarity was modified by addition of water in the solvent.[36] Microwave extraction shows promising advantages over conventional solvent extraction system and is an efficient method for extracting active biological compounds.[37-41] Polyphenolic compounds from waste peanut shells,[42] grape seeds,[36] citrus mandarin peels[43] and tea leaves[35] have also been successfully extracted by MAE technique.

The objective of the present work is two folds. First the extractions of secondary metabolites from *L. plantarum* isolated from *khorisa* having antimicrobial properties. Various solvents viz., ethyl acetate, petroleum ether, ethanol, chloroform etc. have been used for the purification of antimicrobial biometabolites.[44-45] Secondly to study the feasibility of microwave-assisted extraction (MAE) for the extraction of antioxidants extract from *Garcinia pedunculata* Roxb. (GPR). Box–Behnken design (BBD) combined with response surface methodology (RSM) was employed to analyze the interaction among the MAE operating factors. The effect of time of microwave bombardment on micro structural changes in plant material was also observed.

6.2 Materials and methods

6.2.1 Materials

The biometabolites were extracted from previously isolated *Lactobacillus plantarum* (identified by sugar fermentation and biochemical characterization) form fermented bamboo shoot product of Assam (*khorisa*).[46] The *L. plantarum* was cultured in MRS broth (HiMedia Labs., India) under anaerobic condition at 37°C. Indicator bacteria used for antimicrobial assays viz., *Escherichia coli* MTCC 443, *Streptococcus aureus* MTCC 740 and *Bacillus cereus* MTCC 430 were collected from the Dept. of

Food Engineering & Technology and Dept. of Molecular Biology and Biotechnology, Tezpur University (India). They were appropriately sub-cultured and used throughout the study.

For the extraction of antioxidant extract, fresh mature fruits of GPR were collected from Nagaon district of Assam, a Northeastern State of India. The exocarp and the mesocarp of the fruit were used for the purpose of study. They were cut into small pieces, washed and dried in a cross airflow tray drier (IKON, India) at 40 °C for 24 hours. 1, 1-diphenyl-2-picryl hydrazyl (DPPH) and 2, 2'-azino-bis, 3-ethylbenzo thiazoline-6-sulphonic acid (ABTS) were obtained from Merck, India. HPLC grade 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) standard was obtained from Sigma Aldrich, USA. Absolute ethanol used for microwave extraction and all other chemical reagents and solvents used in experiments were of analytical grade purchased from Merck (India), and double distilled water was used throughout the experiment.

6.2.2 Antimicrobial activity and growth kinetics of *L. plantarum*

The *L. plantarum* was tested for antibacterial activity against *Staphylococcus aureus* by agar overlay method in nutrient agar. Nutrient agar was supplemented with dextrose (20g/lt.), polysorbate 80 (1g/lt.), ammomium citrate (2g/lt.), sodium acetate (5g/lt.), magnesium sulphate (0.10g/lt.), manganese sulphate (0.05g/lt.) and dipotassium phosphate (2g/lt.) for proper growth of *L. plantarum*. Final pH of the medium was adjusted to 7±0.5 at 25°C (De Man et al. 1960) and zone of inhibition was noted.

MRS broth (50 ml) was taken in 11 different Erlynmeyer flasks to study the Growth kinetics of *L. plantarum*. Ten flasks were inoculated with 1 ml of 24 h old culture of *L. plantarum* at concentration of 7.68 McFarland,[47] and 1 flask was kept as blank. The flasks were incubated at 37°C at 100 rpm in a shaking incubator (New Brunswick Scientific, USA), till stationary phase was achieved. Each flask was taken out at intervals of 6 h and absorbance was measured in a spectrophotometer (Spectronic 20D+, ThermoFisher Scientific, USA) against blank medium at 600 nm and the growth curve was plotted between absorbance and time.[48]

6.2.3 Production and purification of biometabolites

Submerged aerobic fermentation technique was used for the production of biometabolites using *L. plantarum*. Two sets of 50 ml MRS broth in Erlenmeyer flasks were taken and each was inoculated with 24 hour old culture of *L. plantarum* (1 ml) at concentration of 7.68 McFarland. The flasks were kept for fermentation at 37°C at 100 rpm till the pre-stationary period in a shaking incubator. The pre-stationary period was decided on the basis of growth curve of *L. plantarum*. The flasks were removed from the incubator and stored at 4°C for further processing.[49]

Cell-free crude supernatants (CFCS) were obtained by centrifugation (4000×g, 4°C, and 20 min) of fermented broth. The supernatant was filtered through a 0.22μm cellulose acetate filter to remove residual cells. As the nature of the metabolite was unknown, three organic solvents viz., chloroform, hexane and petroleum ether were used for the extraction of the unknown biometabolites from the CFCS, on the basis of maximum solubility in solvent phase and maximum antimicrobial activity. CFCS (5ml) was taken in screw cap non-reactive teflon tubes, and mixed with equal quantities 1:1 (v/v) each with chloroform, hexane and petroleum ether. The tubes were kept horizontally for 4 h in a shaker (180 rpm at 25 °C). After this incubation, the solvent phase was separated and evaporated at 40°C in a vacuum oven kept at 650mm Hg for 24 h.[49] The weight of dried metabolites was noted and it was re-dissolved with 1ml of sterile double distilled water, so that the final concentration of the metabolite was known in g/ml equivalent. This was considered as cell-free purified supernatants (CFPS).

6.2.4 Antimicrobial activity of biometabolites

To neutralize the organic acid function on the antimicrobial activity, 1 ml of CFCS was adjusted to pH 6.5±0.2, using 1M NaOH solution. In order to test the heat sensitivity, 1 ml CFCS of the *L. plantarum* was heated to 100°C in a water bath for 15 min. Agar well diffusion assay[50] with slight modification was used for analyzing antibacterial activity of CFCS, CFPS, organic acid neutralized CFCS and heat treated

CFCS. Nutrient agar plates were prepared by inoculating the molten agar (at ~50°C) with 1:100(v/v) 24 h cultures of the indicator food contaminating strains *E. coli, S. aureus* and *B. cereius* at concentration of 6.50 McFarland. Sterile cork borer was used to dig wells of 8 mm diameter in the agar plates. CFCS, CFPS, organic acid neutralized CFCS and heat treated CFPS (100μl each) were loaded on to the wells. Also, 100μl of standard antibiotic tetracycline (25 μg/ml) was used as the positive control, and sterile water was used as the negative control. After loading the plates were kept at 4 °C for 4 hours, for the preparations to diffuse from the wells into the agar. The plates were then placed in an incubator for 24 h at 37 °C. The ability to inhibit growth (-, +, ++, +++) of the indicator strains was observed.

6.2.5 Analysis of organic acid composition in CFCS by HPLC

CFCS was taken and an equal volume of 20% (v/v) trichloroacetic acid was added to remove proteins. After centrifugation (10,000×g, 15 min), 1 ml supernatant was mixed with 5 ml 5 mM H_2SO_4. Finally, the mixture was filtered through 0.22 μm cellulose acetate membrane filter. Organic acid concentration in the crude extract was measured by high performance liquid chromatography (HPLC, Dionex Ultimate 3000, Germany). Acid separation from CFCS was performed by purifying the CFCS via means of solid phase extraction using Sep-Pak C 18 Plus cartridges (Waters, Milford, MA, USA). Organic acids were quantified using UV detection of 210 nm, on Hamilton Organic Acid C18-column and mobile phase was 0.2M sodium sulphate solution, adjusted with methane sulphonic acid to pH 2.68. Standard acids were used for analysis are oxalic acid, tartaric acid, formic acid, pyruvic acid, lactic acid, acetic acid, citric acid, succinic acid and propionic acid (Sigma, USA). Acid identification was performed by comparing the retention times of the samples with that of the standards of organic acid.[51]

6.2.6 Effect of biometabolites on minimum lethal dose concentration (LD_{min}) and death rate kinetics of test bacterial strains

Two-fold micro broth dilution technique was used to determine the minimum lethal dose concentration using the standard procedure[52] with slight modification. Nutrient broth (5 ml) was added to each of the tubes. The final concentration of the CFPS chloroform fraction was adjusted to 54, 27, 13.5, 6.75, 3.37, 1.68, 0.84, 0.42, 0.21, 0.10 mg/ml concentration in the tubes accordingly. An inoculums suspension of 20µl for 24 hour old culture of the indicator strains concentration of 6.50 McFarland was added to each of the tubes. Tetracycline (25 µg/ml) was used as positive control, and water was used as negative control. The tubes were incubated at 37 °C for 24 h, and then visually checked for turbidity (visible bacterial growth). The lowest concentration at which turbidity was inhibited was recorded as the LD_{min} for respective indicator strain.

Death rates kinetics of test bacterial strains cultured in nutrient broth supplemented with CFPS chloroform fraction equivalent to LD_{min} of respective pathogen was studied. Nutrient broth (5 ml) was inoculated with 100µl of the test strain concentration of 6.50 McFarland. The tubes were incubated till 24 hours at 37°C in an incubator (New Brunswick Scientific, USA). The inoculated broth (100µl) was plated on nutrient agar plates at intervals of 0, 2, 4, 8, 10, 12, 16, 20 and 24 h respectively, and incubated at 37 °C for 36-48 h. The cell count was taken as log cfu/ml.

6.2.7 Optimization of microwave assisted extraction process of antioxidant extract

The extractions were carried out in a domestic microwave oven (Samsung, India). The dried pieces of GPR were crushed and finely ground. The fine grinding was carried out in order to increase the surface area of contact with solvent. Powdered GPR (5 g) was weighed into a flat-bottomed flask. The flask was maintained with varying solvent (absolute ethanol) concentration (50-100%) and solvent to sample ratio (10:1-20:1). It was kept at room temperature for 90 minutes for leaching of the solvent into the sample,[36] and then microwave-irradiated at 180 Watt on a carousel for defined time

(4–10 min). The suspensions were irradiated with microwaves for 1 min (heating to the desired temperature about 65-70 °C) and cooling to room temperature (30±1 °C) in water bath and this cycle is continued to the pre-set extraction time. The lower microwave power (180 W) was selected for treatment to avoid boiling of solvent; as at higher power excessive boiling and evaporation of solvent was observed. However the flask was covered loosely with polyethylene to avoid evaporative loss. After microwave irradiation, the residues were then filtered and stored at 4 °C.

6.2.8 Experimental design for microwave assisted extraction

A three-variable, three-level Box–Behnken design (BBD) was applied to determine the best combination of extraction process variables for achieving higher antioxidant activity of extract obtained from GPR.[53-54] The three independent variables set were solvent concentration (%, X_1), solvent to sample ratio (v/w, X_2) and irradiation time (min, X_3), and each variable set at three levels. The independent variables were coded at three levels and their actual values selected on the basis of preliminary experimental results. Antioxidant activity of extract in terms of DPPH radical scavenging activity (DPPH RSA) and ABTS radical scavenging activity (ABTS RSA) were taken as responses. The coded and uncoded (actual) levels of the independent variables are given in Table 6.3. Experiments were augmented with 5 replications at the center point to evaluate the pure error. RSM was applied to the experimental data using a commercial statistical package, Design-Expert version 6.0.11 (Stat-Ease, Inc., Minneapolis, MN, USA). The experiments were conducted randomly to minimize the effects of unexplained variability in the observed responses as a result of external factors. Regression analysis for the experiment data was performed, and was fitted into the empirical second order polynomial model (Eq. 6.1).

$$Y = a_o + \sum_{i=1}^{n} a_i x + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} a_{ij} x_i x_j + \sum_{i=1}^{n} a_{ii} x_i^2 \qquad (6.1)$$

Where, a_o, a_i, a_{ii} and a_{ij} are the regression coefficients and x_i, x_j are the coded levels

of independent variables i and j . Model adequacy was evaluated using F ratio and coefficient of correlation (R^2) represented at 1, 5 and 10 % level of significance accordingly.

6.2.9 DPPH radical scavenging activity of antioxidant extract

Free radical scavenging activity was used to measure the total antioxidant activity by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay method. DPPH is a commercial oxidising radical used to be reduced by anti-oxidants. The disappearance of the DPPH radical absorption at a particular wavelength was monitored by the reduction in optical density. To 20µL methanolic extract of GPR, 1.5 ml of DPPH solution (0.025g DPPH in 1000 ml of methanol) was added. The tubes were vortexed (Vortex Shaker, Labtech, Korea) for proper mixture and allowed to react for 45 minutes in a dark environment at room temperature. The control was prepared by adding distilled water instead of GPR extract for baseline correction. Absorption was measured at 517nm in a spectrophotometer (Spectronic 20D+, Thermo Scientific, USA). The Free radical scavenging activity was expressed as inhibition percentage and calculated by using the following equation (Eq. 6.2).[55]

$$\% \text{ Free radical scavenger activity} = [(\text{Control absorbance} - \text{Sample absorbance}) / \text{Control absorbance}] \times 100 \quad (6.2)$$

6.2.10 ABTS radical scavenging activity of antioxidant extract

The method is based on the ability of antioxidant molecules to quench 2, 2'-azino-bis, 3-ethylbenzo thiazoline-6-sulphonic acid (ABTS) radical, a blue-green chromophore with characteristic absorption at 734 nm, compared with that of trolox, a water-soluble vitamin E analog. The addition of antioxidants to the preformed radical cation, reduces it to ABTS radical resulting in decolorization. A stable stock solution of ABTS was prepared by the reaction of a 7 mmol/l aqueous solution of ABTS with 2.45 mmol/l potassium persulfate (final concentration) and allowing the mixture to stand in

the dark at room temperature for 16 h before use.[56] The ABTS working solution was obtained by the dilution of the stock solution in ethanol to an absorbance of 0.70±0.02 AU at 734 nm. 2 ml diluted ABTS solution was added to the appropriately diluted fruit residue extracts. The contents were mixed well and absorbance was read after 2 min after mixing. The percentage inhibition was calculated against trolox standard curve prepared using 0-2.5 mM trolox and the results were expressed as mg-trolox equivalent /g dry weight.

6.2.11 FTIR spectroscopy analysis

The infrared spectra of untreated GPR powder and MAE treated residue were obtained with a FTIR spectrophotometer (Perkin Elmer, USA). MAE was carried out at optimized values of solvent concentration and solvent to sample ratio; however, irradiation time were varied at three different levels. The equipment was operated with scanning range of 4000–450 cm^{-1} and spectrum of 100. Sample (clear glassy disk) for FTIR analysis was prepared by mixing powder sample with IR grade KBr using a suitable pressure of 12,000 psi.

6.2.12 Microscopic observation

The cellular microstructures were observed for untreated GPR and MAE treated GPR slices, in order to check for damage in the cellular structure (mesocarp and exocarp) and parenchyma, as proposed by the Broken cell wall theory,[39] which would in turn facilitate the release of chemical substances inside the cells, into the surrounding solvent. Microscopic observations were carried out using a trinocular microscope (DM 3000, Leica Microsystems, Wetzlar, Germany) equipped with a CCD camera (Leica DFC 440C) and Leica Application Suite (LAS) software on thin slices of untreated GPR and MAE treated GPR. The slices were stained with 0.1% methylene blue solution for a short time and then washed to remove the excess stain. Stained slice was covered with cover slip and checked under the microscope at a magnification of 40X.[57]

6.2.13 Statistical analysis

All the experiments used for biometabolites extraction were performed three times independently and each assay was performed in duplicate. Results were expressed as means ± standard deviation. The level of significance was analyzed by ANOVA ($P < 0.05$). RSM was applied to the experimental data of MAE using a commercial statistical package, Design-Expert version 6.0.11 (Stat-Ease, Inc., Minneapolis, MN, USA).

6.3 Results and discussion

6.3.1 Antimicrobial activity and growth kinetics of *L. plantarum*

The *L. plantarum* were tested for antibacterial activity against *Staphylococcus aureus* by agar overlay method in nutrient agar. A large zone of inhibition was recorded, which show the strong antimicrobial activity of *L. plantarum* against *S. aureus*. The fermentation growth curve of the strain was studied in order to have an idea of the period when it reaches its stationary phase, as this phase is considered to give maximum yield of secondary biometabolites.[58-59] The growth curve of *L. plantarum* was plotted between absorbance of MRS broth inoculated and time (Fig. 6.1). Stationary phase was achieved at 24th h of incubation.

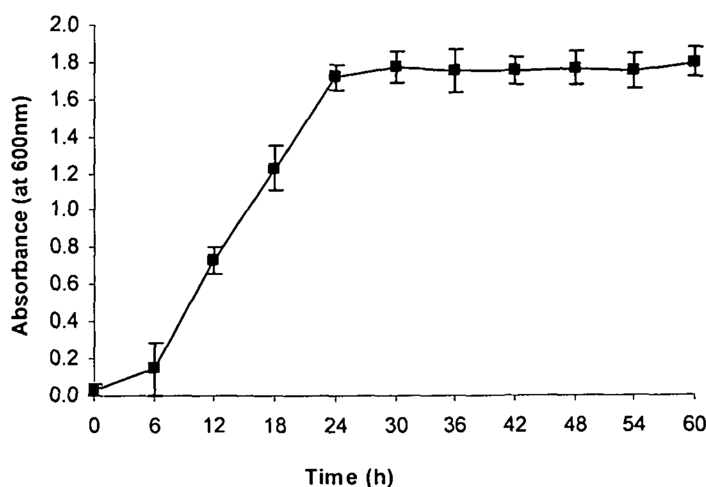
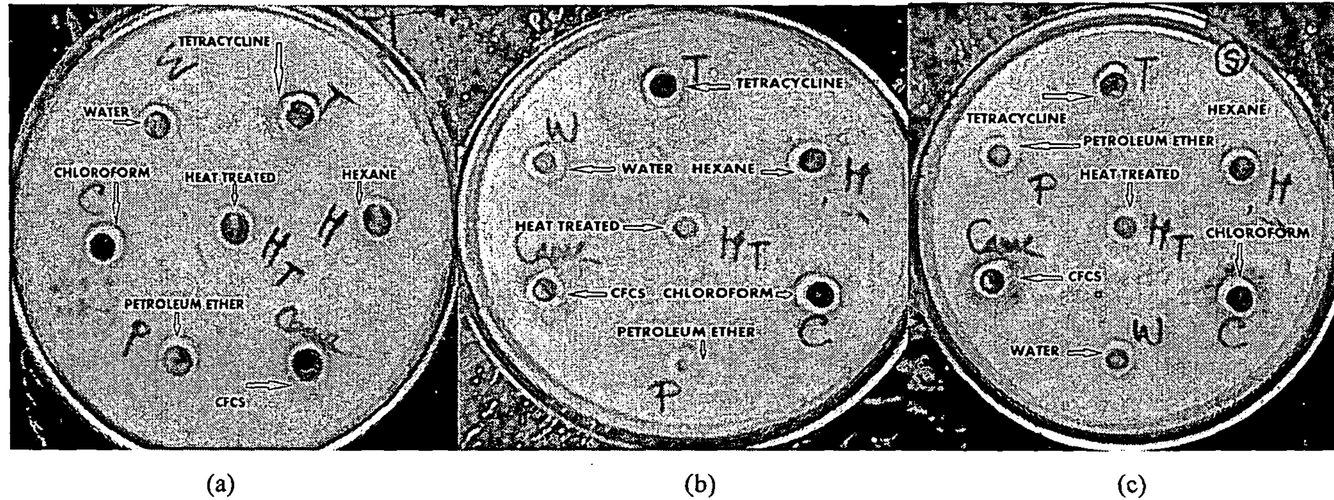


Fig. 6.1 Growth curve of *L. plantarum* isolated form fermented bamboo shoot. Vertical bars represent standard errors of means, n = 3.

6.3.2 Purification of biometabolite and its antimicrobial activity

Bacterial metabolite was extracted in organic solvents, as it gives higher yields compared to other techniques.[60] Three organic solvents viz., chloroform, hexane and petroleum ether were used for the extraction and concentration of biometabolites from the CFCS, on the basis of maximum solubility in the solvent and antibacterial activity. The yield of biometabolite was found to be highest in the chloroform extract (0.054g/ml) followed by hexane (0.052g/ml). However, petroleum ether fraction shows the lowest yield (0.026g/ml).

CFCS showed antimicrobial activity against all the three test pathogens by the agar well assay (Fig. 6.2). The pH of the CFCS was found to be 4.2 at the pre-stationary phase. When the CFCS was adjusted to pH 6.5, the antimicrobial activity slightly diminished. This might be an indicator of organic acids presence in CFCS. The diminishing of antimicrobial activity upon adjustment of pH might be due to the negation of the inhibition effects of acids upon the test pathogens. The same cause can also be attributed to the slight loss of antimicrobial activity in the CFPS fractions of chloroform and hexane. The purified extracts, most noticeably the chloroform extract showed good inhibition against the test pathogens. The hexane fraction showed positive inhibition against *S. aureus* and *B. cereus,* but did not show any inhibition against *E. coli.* The petroleum ether fraction did not result in positive inhibition of any of the test pathogens. However, the antimicrobial activity totally diminished after heat treatment of the CFPS (Table 6.1).



(a) (b) (c)

Fig. 6.2. Inhibition zones of different extracts against (a) *E. coli,* (b) *S. aureus* and (c) *B. cereus*

Table 6.1. Antimicrobial activity of different extracts against test pathogens*

Indicator Strain	Inhibition by CFCS	Inhibition by CFCS (pH 6.5±2)	Inhibition by CFPS			Inhibition by CFPS Heat Treated (100°C for 15 min)			Inhibition by Tetracycline (Positive Control)	Inhibition by Water (Negative Control)
			Hexane	Chloroform	Petroleum ether	Hexane	Chloroform	Petroleum ether		
E. coli	+	+	–	+	–	–	–	–	+++	–
S.aureus	++	+	+	+	–	–	–	–	+++	–
B. cereus	++	+	+	+	–	–	–	–	+++	–

*Symbols inside the table refer to the size of the inhibition zone diameter observed with growing cells: +, 1 mm; ++, 2 mm; +++, >2–5 mm; –, absence of an inhibitory zone. CFCS, Cell-free crude supernatants; CFPS, Cell-free purified supernatants

6.3.3 HPLC analysis of organic acids

HPLC analysis confirmed that the main organic acid present in the CFCS was lactic acid with an amount of 2850.50 ppm. Moreover, tartaric acid and formic acid were also found and the content was found to be 625.67 ppm and 245.39 ppm respectively (Fig. 6.3). Relatively low amounts of formic acid were produced by the strain. No oxalic acid, pyruvic acid, acetic acid, citric acid, succinic acid and propionic acid were detected in the CFCS. It is well established that antibacterial activity is shown by organic acids.[61] Makras et al.[7] reported that an antimicrobial activity of *Lactobacillus rhamnosus* GG and *L. casei* Shirota was entirely owed to the production of lactic acid. Organic acids, especially succinic, fulfill a barrier effect on pathogenic bacteria.[62] In addition, organic acids in the undissociated form enters the pathogenic cell and dissociates it inside cytoplasm, due to decrease of intracellular pH and eventually the accumulation of the ionized organic acid causes the death of the pathogenic bacteria.[63]

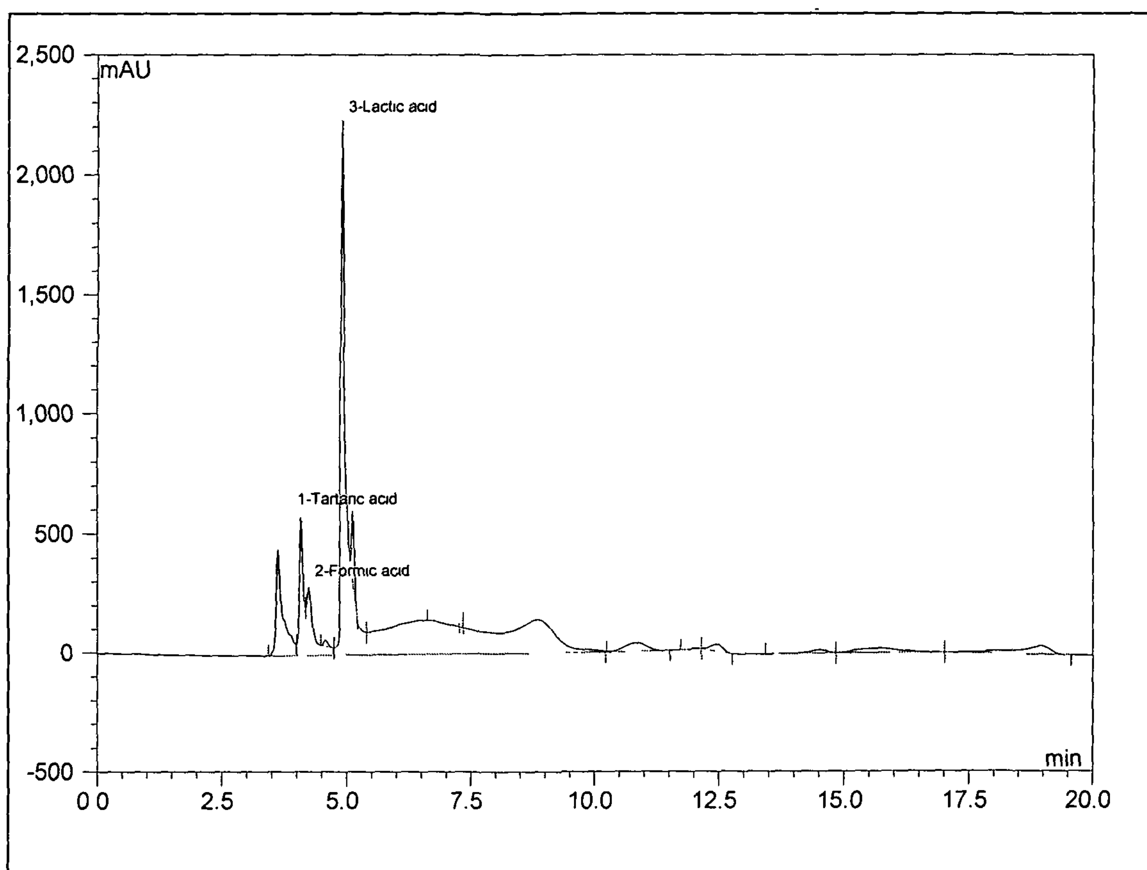


Fig. 6.3() HPLC spectrum of organic acids available in CFCS

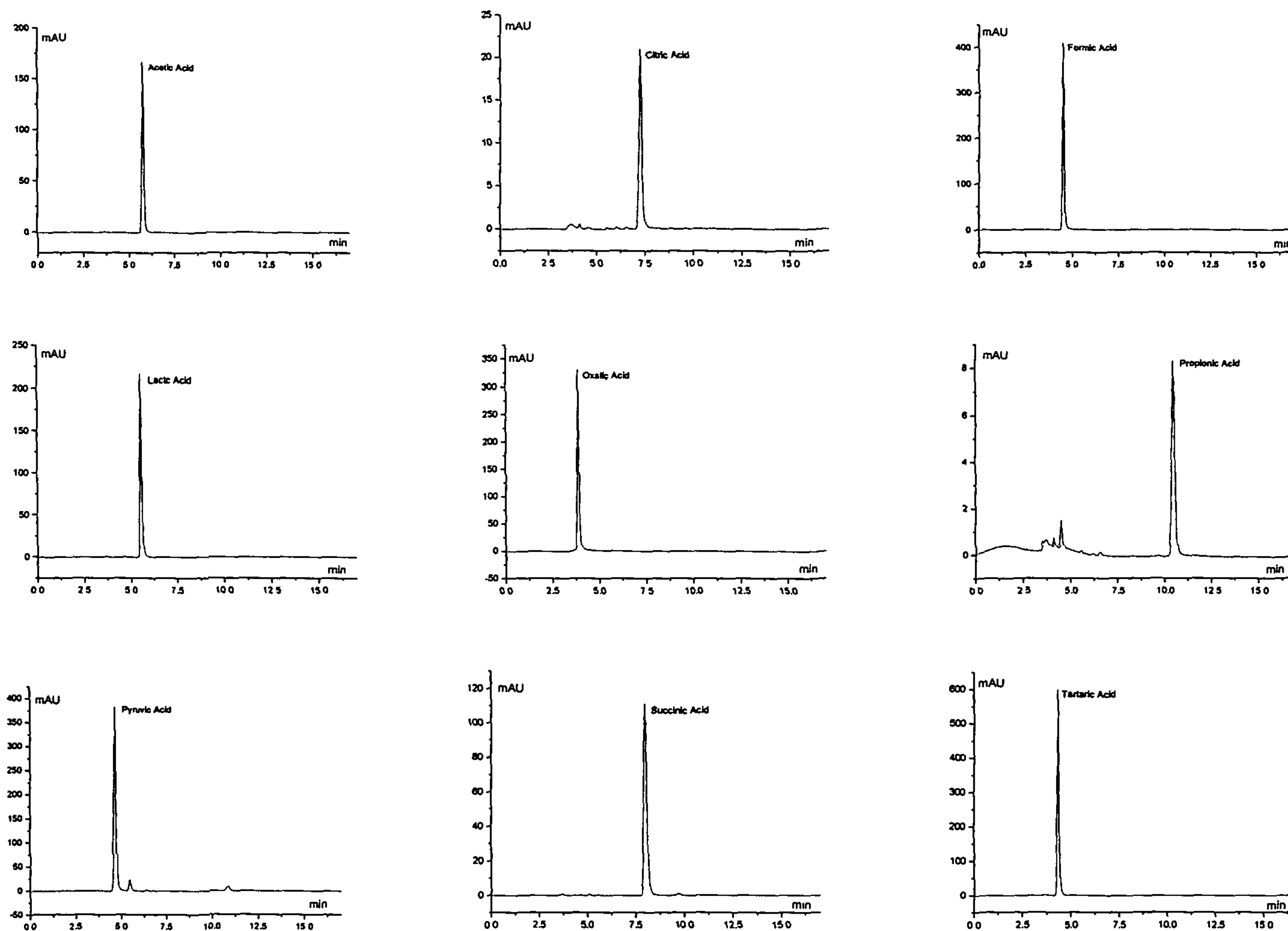


Fig. 6.3(b). HPLC chromatograms of standard acids

6.3.4 Minimum lethal dose concentration and death rate kinetics of the CFPS chloroform fraction

The minimum lethal dose concentration of CFPS chloroform fraction against different bacterial strains were reported as 27 mg/ml for *E. coli*, 1.68mg/ml for *S. aureus* and 1.68mg/ml for *B. cereus* (Table 6.2). The CFPS showed strong antimicrobial activity against two indicator strains viz., *S. aureus* and *B. cereus*. The antimicrobial activity against *E. coli* was fairly lower, as higher concentrations of CFPS were required for the inhibition of the test strain. Based on death rate kinetics of enteropathogens used in this study, bacterial viable count after inoculating with the CFPS was less than 1 cfu/ml before 12 h for *E. coli*, 12 h for *B. cereus* and less than 16 h for *S. aureus*. Differences in LD_{min} values of bacteria might be related to differential susceptibility of bacterial cell wall, which is the functional barrier and minor differences present in outer membrane in the cell wall composition.[64]

Table 6.2. Death rates kinetics of test bacterial strains cultured in nutrient broth supplemented with biometabolite equivalent to LD_{min} of respective pathogen

Incubation Time (h)	0	2	4	8	10	12	16	20	24	LD_{min} Values (mg /ml)
E. coli (Log cfu/ml)	3.48	3.08	2.89	2.21	1.51	0	0	0	0	27
S. aureus (Log cfu/ml)	4.29	3.87	3.70	3.60	3.00	2	0	0	0	1.68
B. cereus (Log cfu/ml)	6.30	3.00	3.34	2.78	3.00	0	0	0	0	1.68

6.3.5 Model fitting for microwave assisted extraction process

During microwave assisted extraction process the effect of solvent concentration (X_1), solvent to sample ratio (X_2) and irradiation time (X_3) on DPPH RSA and ABTS RSA of GPR were studied and results are shown in Table 6.3. The coefficient of regression of the intercept, linear, quadratic and interaction terms of the model were calculated using the least square technique. Regression analysis and ANOVA were used for fitting the model and to examine the statistical significance of the terms and the results of ANOVA were given in Table 6.4. The correlation coefficients (R^2) for the responses DPPH RSA and ABTS RSA were 0.9297 and 0.9256 respectively. Based on

t-statistics, the regression coefficient significant at 95% probability levels were selected for developing the model given below [Eq. 6.3 & 6.4].

DPPH RSA= $6.46349 + 0.62942\ X_1 + 6.77674\ X_2 + 2.01375X_3 - 3.46616E\text{-}003\ X_1^2 - 0.12634\ X_2^2 - 0.70412\ X_3^2 - 0.029778\ X_1X_2 + 0.056457\ X_1X_3 - 0.033600\ X_2X_3$

(6.3)

ABTS RSA= $-39.78199 + 1.14460\ X_1 - 0.11886\ X_2 + 6.32008\ X_3 - 5.17547E\text{-}003\ X_1^2 + 0.027017\ X_2^2 - 0.36926\ X_3^2 - 0.010300\ X_1X_2 - 0.031777\ X_1X_3 - 9.70667E\text{-}003\ X_2X_3$

(6.4)

Where, X_1, X_2 and X_3 are independent variables viz., solvent concentration (%), solvent to sample ratio (v/w) and irradiation time (min), and each variable set in the range of 50-100%, 10:1-20:1 and 4–10 min respectively.

F-value for the lack of fit was insignificant ($P > 0.05$) thereby confirming the validity of the model. The model F value of 10.29 and 9.68 implies that the model is significant. There is only a 0.28% and 0.34% chance that a model F value this large could occur due to noise. The value of coefficient of variation (C.V.) was 5.73% and 11.42%, suggested that the model was reliable and reproducible. The results indicated that the model could work well for the prediction of antioxidant activity of extract from GPR.

6.3.6 Effect of process variables on DPPH radical scavenging activity

The results given in Table 6.4 show the linear, quadratic and interaction effects of the three factors on the responses. Linear and quadratic term of irradiation time showed the significant effect on DPPH RSA at the 99% and 95% level respectively. However, other terms did not affect significantly on DPPH RSA. The effect of solvent to sample ratio and time on DPPH RSA is shown in Fig. 6.4(a). DPPH RSA was found to decrease with an increase in time of irradiation, but solvent to sample ratio did not have much impact on the DPPH RSA. This result was contrary to the results derived from extraction of polyphenols from *Camellia oleifera* fruit hull given by Zhang et al.[65] However, prolonged exposure to heat owing to increase irradiation time could have resulted in breakdown of antioxidant compounds.[66] Decrease in solvent concentration did not show any significant change in DPPH activity till 75% concentration. However,

further decrease in solvent concentration resulted in the decrease in DPPH RSA (Fig. 6.4(b)). Antioxidant activity increased slightly with increasing solvent to sample ratio from 1:10 to 1:15 (Fig. 6.4(c)). Beyond this level, the antioxidant activity of extract slightly decreased, which indicated that a greater antioxidant activity of extract could be achieved if the moderate solvent to sample ratio was maintained. Similar results were also reported by Zhang et al.[65]

Table 6.3 Box–Behnken design (BBD) with observed response for DPPH RAS and ABTS RAS.

Run No.	Uncoded (coded) process variables			Responses			
	Solvent concentration	Solvent-Sample ratio	Irradiation time	DPPH RSA (%)		ABTS RSA (mg-trolox equivalent/ g dry weight)	
	(%, X_1)	(w: w, X_2)	(min, X_3)	Actual	Predicted	Actual	Predicted
1	100(1)	15(0)	10(1)	66.60±2.26	64.44	6.04±1.18	4.81
2	75(0)	15(0)	7(0)	78.58±1.68	79.60	19.61±1.06	18.10
3	75(0)	15(0)	7(0)	78.98±1.04	79.68	18.31±0.64	18.10
4	50(-1)	15(0)	10(1)	52.95±0.73	53.07	11.44±0.82	10.01
5	50(-1)	15(0)	4(-1)	84.06±2.19	86.22	12.28±1.21	13.52
6	75(0)	20(1)	4(-1)	88.41±3.24	85.54	20.86±1.84	18.99
7	100(1)	10(-1)	7(0)	77.57±2.37	76.86	17.99±1.13	17.36
8	50(-1)	10(-1)	7(0)	69.51±3.04	66.52	15.65±0.71	15.22
9	75(0)	15(0)	7(0)	87.08±2.46	89.68	17.02±1.42	18.10
10	75(0)	20(1)	10(1)	60.68±1.73	59.85	9.63±1.32	10.43
11	100(1)	20(1)	7(0)	71.60±3.35	74.59	12.86±0.63	13.30
12	75(0)	10(-1)	4(-1)	78.52±1.45	79.35	20.99±0.74	20.19
13	75(0)	10(-1)	10(1)	52.81±2.56	55.68	10.35±1.49	12.21
14	75(0)	15(0)	7(0)	75.02±3.04	79.68	18.58±1.05	18.10
15	50(-1)	20(1)	7(0)	78.43±1.67	79.14	15.67±0.68	16.30
16	75(0)	15(0)	7(0)	78.35±1.16	79.68	17.00±1.28	18.10
17	100(1)	15(0)	4(-1)	80.78±1.82	80.66	16.42±1.35	17.85

All the responses are mean ± SD of three replicates. Numbers in bracket are coded values of process variables.

Table 6.4 ANOVA for DPPH RSA and ABTS RAS of GPR extract

Parameters	DF	DPPH RSA			ABTS RSA		
		SS	F value	*p* value	SS	F value	*p* value
Model	9	1668.37	10.29	0.0028	267.04	9.68	0.0034
X_1	1	16.83	0.93	0.3659	0.37	0.12	0.7368
X_2	1	53.64	2.98	0.1280	4.43	1.44	0.2684
X_3	1	1218.43	67.65	< 0.0001	136.80	44.63	0.0003
X_1^2	1	19.76	1.10	0.3297	44.06	14.37	0.0068
X_2^2	1	42.01	2.33	0.1706	1.92	0.63	0.4545
X_3^2	1	169.09	9.39	0.0182	46.50	15.17	0.0059
X_1X_2	1	55.42	3.08	0.1228	6.63	2.16	0.1848
X_1X_3	1	71.72	3.98	0.0862	22.72	7.41	0.0297
X_2X_3	1	1.02	0.056	0.8191	0.085	0.028	0.8726
Lack of Fit	3	46.14	0.77	0.5680	16.54	4.49	0.0904
Pure Error	4	79.93			4.91		
R^2		0.9297			0.9256		
Adjusted R^2		0.8394			0.8300		
CV (%)		5.73			11.42		

DF: degree of freedom; SS: sum of squares; CV: coefficient of variation

6.3.7 Effect of process variables on ABTS radical scavenging activity

ABTS RSA is significantly affected linearly by irradiation time followed by quadratic term of irradiation time and solvent concentration at 99% level (Table 6.4). Also interaction term of solvent concentration and irradiation time had significant effect at 95% level. However, other terms did not show significant effect on ABTS RSA. The effect of solvent to sample ratio and solvent concentration on ABTS RSA is given in Fig. 6.5(a). ABTS RSA was found to increase with decrease in solvent concentration; but, a further decrease in solvent concentration resulted in the reversal of this trend. However, solvent to sample ratio did not have much of an impact on the ABTS RSA. This result was contrary to the results derived for extraction of phenolic compounds from grape seed by Hong et al.[36] They observed higher extraction of polyphenolic compounds, when solvent polarity was modified by addition of water in the solvent.

Combine effect of solvent concentration and irradiation time had a significant impact on ABTS RSA. Increasing in solvent concentration and irradiation time, ABTS RSA found to be increased (Fig 6.5(b)). The effect of solvent to sample ratio and time on ABTS RSA are shown in Fig. 6.5(c). With respect to solvent to sample ratio, ABTS RSA antioxidant activity did not have any significant effect, but antioxidant activity showed a sharp decrease with increase in irradiation time. However, their combine effect showed the positive impact on ABTS RSA. This could be attributed to the fact that prolonged exposure to heat owing to increase irradiation time could have resulted in breakdown of antioxidant compounds as described earlier.

6.3.8 Optimization of microwave assisted extraction conditions

The process variables viz., solvent concentration, solvent to sample ratio and irradiation time was numerically optimized to find out the conditions for getting the best results from MAE extraction. Optimization was done for obtaining maximum DPPH RSA and ABTS RSA. The optimal conditions of MAE for antioxidant extract obtained were found as solvent concentration of 70.79, solvent to sample ratio of 20:1 and irradiation time of 4.73 minutes. For the validation and adequacy of the model equation, a verification experiment was carried out under the optimized conditions mentioned above. The predicted antioxidant activity of extract was 85.98% DPPH RSA and 19.23 mg-trolox equivalent/g dry weight (ABTS RSA), which was consistent with the practical antioxidant activity of 85.02% DPPH RSA and 19.68 mg-trolox equivalent/g dry weight (ABTS RSA) of extract. The strong correlation between the real and predicted results confirmed that the response model was adequate to reflect the expected optimization.

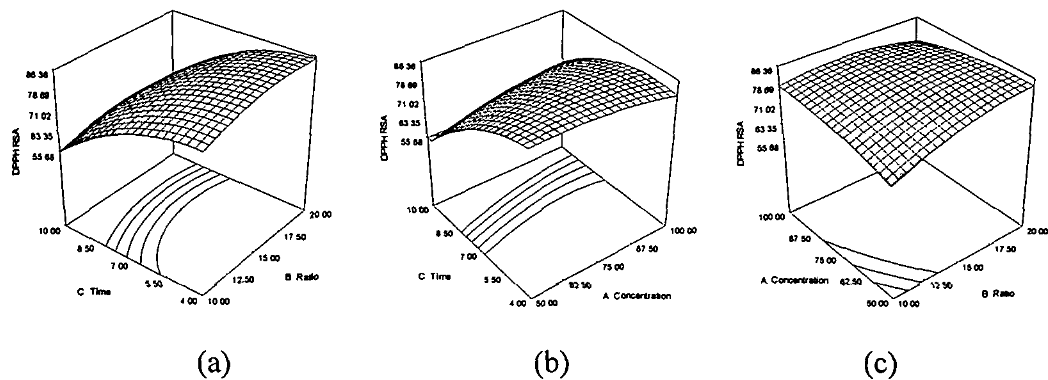


Fig. 6.4. DPPH RSA during microwave irradiation of GPR as function of (a) solvent to sample ratio and irradiation time; (b) solvent concentration and irradiation time; (c) solvent concentration and solvent to sample ratio

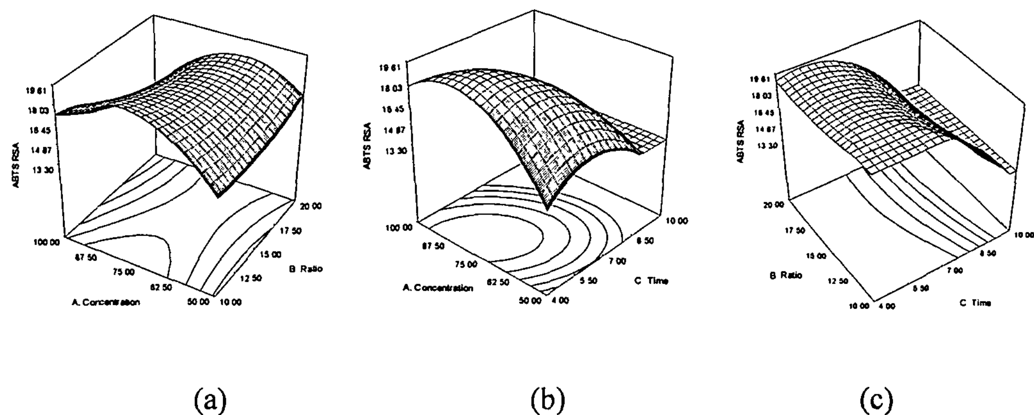


Fig. 6.5. ABTS RSA during microwave irradiation of GPR as function of (a) solvent concentration and solvent to sample ratio (b) solvent concentration and irradiation time and (c) solvent to sample ratio and irradiation time

6.3.9 FTIR spectroscopy of untreated GPR powder and MAE treated residue

Optimized value of solvent concentration (70.79% ~71%) and solvent to sample ratio (20:1) were taken for MAE at different irradiation time (4, 7 & 10 min.). FTIR spectra for the untreated GPR powder and MAE treated residue obtained at these three different conditions were recorded by FTIR spectrometer (Fig. 6.6). The broadband around 3420–3440 cm^{-1} is characteristic for hydroxyl group indicates the presence of phenolic compounds and the peak intensity get reduced with increasing extraction time. The observed absorption band at 1628 cm^{-1} indicates N–H bend, shows the presence of primary amines in untreated GPR powder and MAE residue treated for 4 min. However, this peak intensity was fully reduced with 7 and 10 min MAE treatment. Absorption at 2920-2930 cm^{-1} is characteristics of C-H stretching in aldehydes. Strong band in the region (1200-1000 cm^{-1}) confirms the presence of esters, aldehydes, ketones, lactones, carboxylic acids, amides, alcohols. A band at 1400 cm^{-1} is the characteristic for C-C stretch ring, which shows the presence of aromatic compounds in untreated GPR and this peak is absent in all MAE treated samples. Absorption at 1105 cm^{-1} is the most characteristics of ethers *i.e.* C-O stretching in CO- C group. Strong band at 1195 cm^{-1} and another at 1401 cm^{-1} gives the presence of phenols. Appearance of strong band at 1628 cm^{-1} is due to C=O stretching which indicates the presence of quinines and amides. Absorption around 1750–1735 (s) could be due to C=O stretch caused by esters and saturated aliphatic groups. Absorption at around 700 cm^{-1} may be due to carbohydrate. Samples treated with MAE showed gradual reduction in peak intensities at 4, 7 and 10 minutes respectively, indicating release of antioxidant and cellular components into the surrounding solvents, hereby resulting in absence from the spectra of the residual cellular matter. FTIR study revealed that, the extraction was higher with increase in irradiation time, but antioxidant activity of extract was lower with increase in irradiation time. This could be attributed to loss of antioxidant activity of extract due to prolonged exposure to heat caused by irradiation.

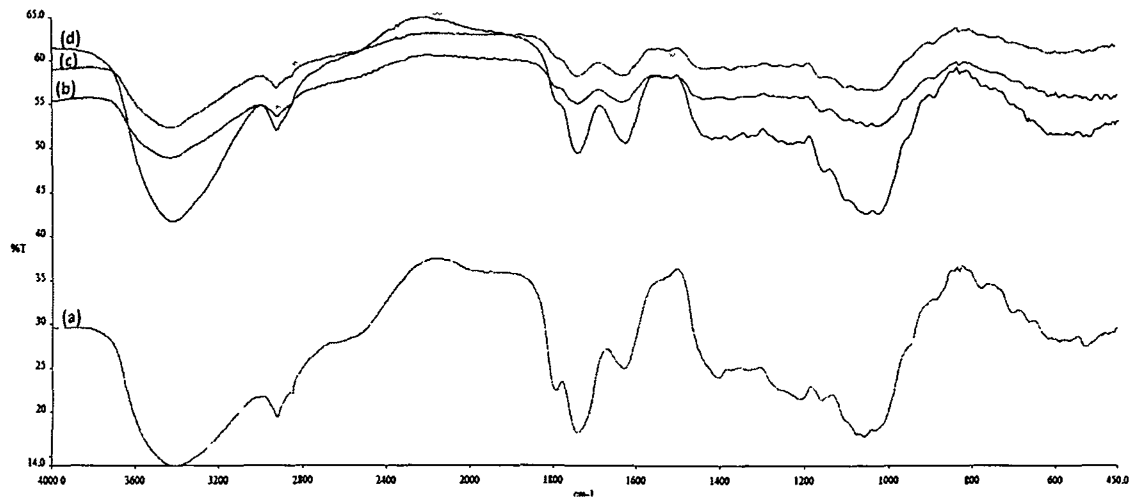


Fig. 6.6. FTIR spectra of GPR obtained from (a) control (untreated GPR powder) (b) MAE treated residue at 70% concentration, 20:1 ratio and 7 min (c) MAE treated residue at 70% concentration, 20:1 ratio and 10 min (d) MAE treated residue at 70% concentration, 20:1 ratio and 4 min

6.3.10 Microscopic observation of untreated GPR powder and MAE treated residue

The morphological changes in the sample during microwave assisted extraction were studied by observing the samples under light microscope. The micrographs of the untreated GPR cross-sectional slices and that of treated slices of GPR irradiated at the optimized condition (Fig. 6.7). There was no visible destruction in the cells for the untreated samples, as shown in Fig. 6.7(a) for exocarp and Fig. 6.7(c) for mesocarp. After microwave bombardment, there was visible change in the texture of the mesocarp (Fig. 6.7(d)) and breakage in the cells structure in the exocarp in (Fig. 6.7(b)) was observed, This could be attributed to the absorption of microwave energy by the water in the cells and parenchyma, resulting in sudden rise in temperature and internal pressure rise.[67] In MAE, the exocarp of samples was immensely changed and destroyed

in Fig. 6.7(b). The higher efficiency could be attributed to action of microwave irradiation, which produces the disruptions of tissues and cell walls leading to a greater contact area between solid and liquid phase, better access of solvent to valuable components.[68] The microscopic imaging confirmed the bursting of cells and disruption in the cellular parenchyma.

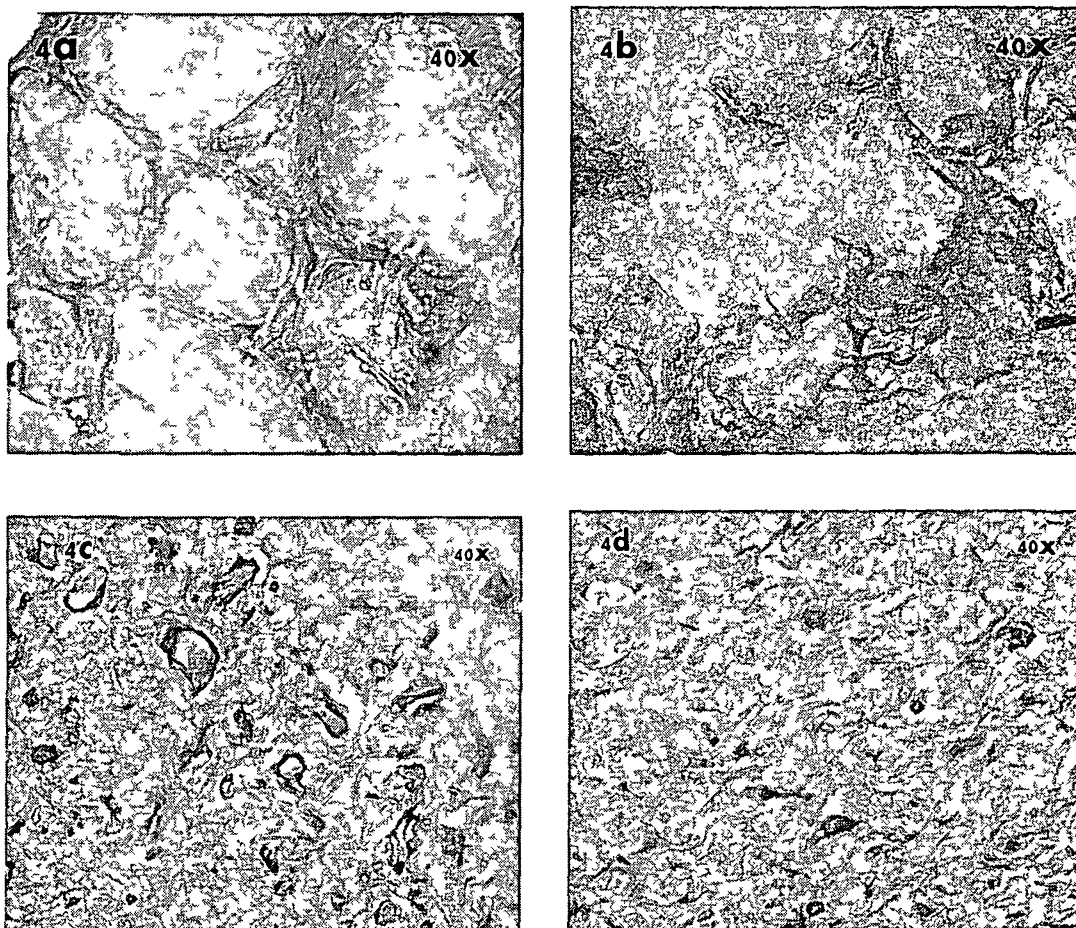


Fig. 6.7. Light microscope images of (a) untreated GPR exocarp, (b) sample after MAE (exocarp), (c) untreated GPR mesocarp, (d) sample after MAE (mesocarp)

6.4 Conclusion

The study shows that *L. plantarum* isolated from fermented bamboo shoots strongly inhibits the three test pathogens. This study will offer useful information for the improvement of *khorisa* production. This biometabolites could be incorporated in edible film and coating to control the growth of pathogenic microorganism on fruits, vegetables and other food products. The biometabolite might also be used in combination with established antibiotics, and could prove useful in combating emerging drug resistant species of enteropathogens.

MAE results evinced that irradiation time was the major factors which affected the antioxidant activity of extract obtained from GPR. The optimum extraction parameters were obtained and the predicted values for antioxidant activity of extracts were well consistent with the experimental ones. The extraction of antioxidant extract using MAE method used lesser solvent and decreased extraction time compared to conventional solvent extraction methods. The extract exhibited significant DPPH RSA and ABTS RSA. The information obtained from this study would be valuable for further exploitation and application of this resource.

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Chapter 7

Development of antimicrobial and antibrowning edible coating and its effect on bamboo shoot quality

7.1 Introduction

Edible film and coatings can be used to preserve fresh cut fruits and vegetables, providing a selective barrier to moisture, oxygen and carbon dioxide, improving mechanical and textural properties. Further addition of antioxidants and antimicrobial compounds, impart microbial barriers, avoiding volatile loss, etc.[1] Edible biological components, viz., polysaccharides, proteins and lipids or a mixture of these were successfully used for the preparation of coatings and films.[2-3] Plasticizers are added in coating mixture to improve the flexibility, and reduce brittleness of coating by reducing the internal hydrogen bonds between polymer chains and increasing intermolecular spaces.[4] Owing to their hydrophilic nature, the incorporation of a lipid substance to the coating mix might be necessary in order to improve water vapor barrier properties.[5-6]

Edible films and coatings might also serve as carriers of food additives by incorporating antioxidant, antibrowning and antimicrobials agents, colourants, flavours, nutrients, and spices into them. [1,7-10] The edible films prepared from single film-forming polymer displays good properties in some aspects but are poor in others. An alternative promising strategy to improve the properties of edible films and coatings is through blending of biopolymers. Composite or blend films are usually composed of two or three biopolymers and are prepared by varying methods.[11-13]

Most of study highlighted the use of biodegradable materials in place of petro-based plastics with similar properties and low in cost. Starch is the most important polysaccharide polymer that is used to develop biodegradable films because it has capability of forming a continuous matrix and it is a renewable and abundant resource. Starch-based films exhibit physical characteristics similar to synthetic polymers, such as being transparent, odorless, tasteless, semi-permeable to carbon dioxide and resistant to oxygen passage.[14] However, starch exhibits several disadvantages such as a strong hydrophilic character and poor mechanical properties. This can be overcome with the addition of carboxymethyl cellulose (CMC) in starch which helps to lower the water vapor permeability and increasing the mechanical strength.[15] Alginate has potential use in films or as a coating component because of its unique colloidal and gel-forming

properties. However, the incorporation of calcium help reduces their water vapor permeability and making alginate films water insoluble.[16] The composite film prepared from these materials could be the best film having improved barrier and mechanical properties compared to individual biopolymers.

Bamboo shoots are highly nutritious and potentially rich sources of dietary fibers, antioxidants, amino acids, minerals, vitamins and low in calories. The protein content of the shoots is also high, and contains fewer amounts of fats; however, it is rich in essential fatty acids.[17-19] Bamboo shoot is a significant item of consumption in the home, while it is not significant in commercial terms due to short shelf life. Bamboo shoots deteriorate rapidly during transportation and storage which is a serious post harvest problem for traders. Enzymatic browning also poses a serious problem during the storage of post harvest bamboo shoots. Edible coatings might have the potential to inhibit the rapid quality degradation of harvested bamboo shoots. The present study was focused to develop composite film from alginate, starch and carboxymethyl cellulose with added antibrowning and antimicrobial agents and to examine the effect of coating on bamboo shoot quality.

7.2 Materials and methods

7.2.1 Materials

Potato starch, sodium alginate and carboxymethyl cellulose (CMC) were provided by HiMedia laboratories (India). Glycerol, calcium carbonate (analytical grade) were purchased from Merck (India) and refined sunflower oil (Dhara, India) from local market. Fresh bamboo shoots (*Bambusa balcooa*) and *Garcinia pedunculata* Roxb. were collected from Nagaon district of Assam (India). *Lactobacillus* strains were collected from the Department of Food Engineering and Technology, Tezpur University, Assam (India). These strains were isolated and identified in the laboratory from fermented bamboo shoot product (*khorisa*) of Assam, India.

7.2.2 Preparation of antioxidant extract

Antioxidant compounds were extracted from *Garcinia pedunculata* Roxb. using microwave assisted extraction. In preliminary experiments the extraction process was optimized at 180 Watt microwave power. The optimized values recorded were, solvent concentration of 70.79%, solvent to sample ratio of 20:1 and irradiation time of 4.73 min. Under these optimized conditions, the antioxidant activity of extract was found to reach upto 85.98% DPPH radical scavenging activity and 19.23% ABTS radical scavenging activity. The extract was filtered through cellulose acetate syringe filters and stored at 4 °C for further use.

7.2.3 Preparation of antimicrobial extract

Biometabolites were extracted and purified from a *lactobacillus* strain isolated from fermented bamboo shoot of Assam and it was assessed for antimicrobial activity for three indicator strains of food contaminating microbes' viz., *Escherichia coli*, *Streptococcus aureus* and *Bacillus cereus*. In preliminary study, submerged fermentation technique was used for the fermentation of biometabolite, and was purified by liquid-liquid extraction with chloroform. Minimum lethal dose concentration (LD_{min}) assay of the chloroform extract were reported as 27 mg/ml, 1.68 mg/ml and 1.68 mg/ml for *E. coli, S. aureus and B. cereus* respectively. The cell free purified supernatant was stored at 4 °C and used as antimicrobial agent during this study.

7.2.4 Preparation and standardization of film

The blend film composed of 6 different combinations (w/w) of alginate, starch and CMC varied at 15% to the amount of starch. The proportion of alginate and starch were taken in the ratio of 2:0(F_1), 2:1(F_2), 1:1(F_3), 1:1.5(F_4), 1:2(F_5), 0:2(F_6). The weight of total solid matter in all film forming solution was kept constant. Starch was mixed with distilled water (100 ml) and agitated by magnetic stirrer (500 rpm) for 30 min at 95 °C for complete gelatinization. In a separate vessel, alginate solution was prepared by dissolving alginate in distilled water (100 ml) and heated at 70 °C and

stirred until the solution became clear (~30 min). CMC (15% w/w starch) was solubilized in distilled water (50 ml) at 75 °C for 10 min.[15] The starch, alginate and CMC solutions were mixed together and stirred at 75 °C for 10 min for proper mixing, stability and keeping property of the suspension. Glycerol was added (40 ml/ 100 g of solid matter) to the suspension as a plasticizer for decreasing brittleness of the film.

Suspensions were then cooled to 40 °C and then kept under vacuum (600 mm Hg) for 24 h to release all air bubbles. Then, the suspension was poured into a Teflon casting tray resulting in thin films, and then dried at 60 °C in an oven to cast the films. Dry films were peeled intact from the casting surface. The films were then immersed in 2 % w/w $CaCl_2$ solution for 10 min and dried at 40 °C for 6 h. $CaCl_2$ has been reported to increase strength of films, likely due to the development of crosslinks between the carboxyl groups in the film molecules and the calcium ions.[14]

The prepared films were conditioned following ASTM D618-05 method.[20] For all tests the prepared films were conditioned at 55±1% RH and 20±1 °C in a desiccator containing a saturated solution of $Mg(NO_3)_2.6H_2O$ for 72 h, or till further tests. All measurements were performed in three replicates. Based on mechanical and barrier properties of film, the best film was selected and the proportions of alginate, starch and CMC of selected film were used for the formulation of coating solution.

7.2.5 Properties of film

7.2.5.1 Film thickness

Films were measured with hand-held micrometer (Alton M820-25, China) having a sensitivity of 0.01 mm. The ten pieces of films were stacked one above the other and average thickness was recorded. The average thickness of the coating formed around a shoot was also determined as the difference between the diameter of the shoot before and after coating at different points.[21]

7.2.5.2 Water vapour permeability (WVP)

WVP tests were carried out using the standard ASTM[20] with some modifications.[22] Glass beakers, with an average diameter of 2 cm and a·depth of 4.5 cm, utilized to determine WVP of films. Films were cut into discs with a diameter slightly larger than the diameter of the cup. After placing 3 g of anhydrous $CaSO_4$ in each cup, they were covered with edible films of varying composition. Relative humidity (RH) of 0% was maintained using anhydrous $CaSO_4$ in the cup. Each cup was placed in a desiccator containing saturated K_2SO_4 solution to maintain the RH of 97% at 25 °C. The desiccators were kept in an incubator at 25 °C. Cups were weighed every 24 h and changes in the weight of the cup were recorded as a function of time. Slopes were calculated by linear regression (weight change vs. time) and the water vapor transmission rate (WVTR) was defined as the slope (g/h) divided by the transfer area (m^2). WVP (g/ Pa h m) was calculated using Eq. (7.1).

$$WVP = [WVTR / P (R_1 - R_2) X] \quad (7.1)$$

Where, P is the saturation vapor pressure of water (Pa) at the test temperature (25 °C); R_1 is the RH in the desiccator; R_2 is the RH in the cup and X is the film thickness (m). Under these conditions, the driving force $[P (R_1-R_2)]$ is 3073.93 Pa.

7.2.5.3 Moisture absorption

The dried films of 20 mm × 20 mm were first conditioned at 0% RH ($CaSO_4$) for 24 h. The weight (W_o) of films were taken and they were conditioned in a desiccator containing $CaNO_3$ saturated solution at 20 °C to ensure a relative humidity of 55%. Each film was weighed at desired intervals (W_t) until the equilibrium state was reached.[15] The moisture absorption of the samples was calculated using Eq. (7.2).

$$\text{Moisture absorption } \% = [(W_t - W_o)/W_o] \times 100 \quad (7.2)$$

Where, W_t and W_o are the weights of the sample after t time at 55% RH and the initial weight of the sample, respectively.

7.2.5.4 Solubility in water

Solubility in water was defined as the percentage of the dry matter of film which is solubilized after 24 h immersion in water.[23] Film specimens were kept in a desiccator containing dry calcium sulphate ($CaSO_4$) till they reached constant weight. Afterwards, about 500 mg of each film were immersed in beaker containing 50 ml of distilled water at 23 °C for 24 h with periodical gentle manual agitation. The films were removed from the water and were placed back in the desiccator until they reached a constant weight to obtain the final dry weight of the film. The percentage of the total soluble matter (%TSM) of the films was calculated using using Eq. (7.3).

$$\%TSM = [(\text{Initial dry weight} - \text{Final dry weight}) / \text{Initial dry weight}] \times 100 \qquad (7.3)$$

7.2.5.5 Tensile properties

Length of elongation and strain to break (SB) of the films were measured by Kieffer Dough and Gluten Extensibility Rig (A/KIE) with the help of Texture Analyzer (TA-HDPlus, Stable Microsystems, UK). The thin strips (80 mm× 3 mm) were cut from each film and were used to analyze textural properties. The test had a tension mode with following settings. Pre-test speed of 2 mm/sec, test speed of 3 mm/sec, post test speed of 10 mm/sec, distance of 75 mm, trigger force of 10g was used and the probe was attached to a 5 kg load cell.

7.2.5.6 Film colour

CMC and starch based films were highly transparent, but the addition of alginate in the film forming solution imparted an amber colour to the developed films. The colour of the developed films was measured using a Hunter Lab colorimeter (Ultrascan VIS, Hunter Lab. Inc., USA) with reflectance mode, CIELab scale (L, a and b), D65 as illuminant and a 10° observer angle as a reference system. The colour measurements were expressed in terms of lightness L (L=0 for black and L=100 for white), and the chromaticity parameters a (green [−] to red [+]) and b (blue [−] to yellow [+]). In addition, the total colour change (dE) values were calculated (Eq. 7.4) from the Hunter

L, *a* and *b* scale measured with absorption mode and used to describe the colour change during coating of bamboo shoot.

$$dE = \sqrt{(L_o-L_t)^2 + (a_o-a_t)^2 + (b_o-b_t)^2} \quad (7.4)$$

Where, L_o, a_o, b_o are the initial colour measurements of fresh bamboo shoot cubes and L_t, a_t, b_t are the colour measurements of coated shoot.

7.2.5.7 Film surface characteristics

Surface morphology of the films was observed under a Scanning Electron Microscope (JEOL JSM 6390 LV, Singapore). Dried film samples were sputter coated with platinum and the images were taken at an accelerating voltage of 5 kV and magnification of 1000X.

7.2.5.8 Infrared spectroscopy

The infrared spectra for all the films were obtained with a FTIR spectrometer (PerkinElmer, USA). The equipment was operated with scanning range of 4000 –450 cm^{-1} and spectrum of 100. The films were ground to a fine powder and then sample (clear glassy disk) for FTIR analysis were prepared by mixing powdered sample with IR grade KBr using FTIR hand operated press at around 12,000 psi pressure.

7.2.5.9 Thermal properties

The thermal degradation properties of the films were determined by Thermo Gravimetric Analysis (TGA). Thermo gravimetric measurement was carried out on a Shimadzu TGA-50 thermogravimetric analyzer. Non-isothermal experiments were performed in the temperature range 25–900 °C at heating rates of 10, 20 and 40 °C min^{-1} on each sample. The average sample size was 10 mg and the nitrogen flow rate was 30 ml min^{-1}.

7.2.6 Effect of coating on bamboo shoot quality

7.2.6.1 Incorporation of antioxidant and antibacterial extracts in film forming solution

The proportions of alginate, starch and CMC of films having best mechanical and barrier properties were chosen for preparing the final basic coating formulation. Antioxidant extract (4 ml/ 100 ml) were incorporated[21] and antibacterial extracts was adjusted to 27 mg/ml in the film forming solution during the last 5 min of mixing. Film forming solution was coated on bamboo shoots for prevention of enzymatic browning and control of microbial degeneration.

7.2.6.2 Coating of bamboo shoot samples

Bamboo shoots were dipped in the coating solutions for 1 min and left to dry at room temperature for 30 min by hanging with constant air flow. Coated shoots were again dipped in the coating solution for another 10 s and dried again to a uniform coating layer. The uniform coating is achieved by turning the side of shoot during second dipping in film forming solution. All the samples were stored at room temperature (30±2 °C) and relative humidity of 64 ±3 % for 5 days.

7.2.6.3 Effects of coating on weight loss and surface colour

The effect of coating on weight loss and surface colour of bamboo shoot was tested at 12 h intervals for 5 days. Weight was recorded after removing the coating from shoots and weight loss was measured as the percentage weight loss from the original weight. The surface colour of bamboo shoot cubes was measured using a Hunter Lab colorimeter as discussed for film colour. The measurements were made in triplicate (three cubes per treatment taken from different trays) and each sample was scanned at four different regions of the shoot.

7.2.6.4. Effects of coating on surface microbial count

Surface microbial count of the uncoated (control) and coated (coating was removed at the time of analysis) bamboo shoots were observed for a period of 5 days after every 24 h. Both coated and uncoated bamboo shoot pieces (1 cm^3) were immersed in 90 ml sterile peptone water and vortexed for 2 min in a vortex shaker. Precisely 100 ml of this peptone water was taken and spread on plate count agar media and incubated for 36 h. Visible colonies were counted and cfu/cm^2 was calculated.

7.2.7 Statistical analysis

All the analyses were performed taking three replicates and data were reported as mean ±SD. Single factor ANOVA was used to determine the critical difference of means, and variance among the different samples were checked at significance level $P \leq 0.05$.[24]

7.3 Results and discussion

7.3.1 Effect of different combinations of alginate, starch and CMC on film properties

7.3.1.1 Film thickness and water vapour permeability

The thickness of the prepared films and the coatings formed around the bamboo shoots were 0.43± 0.08 mm and 0.15 ± 0.02 mm respectively. The water vapour permeability (WVP) values of the obtained films are shown in Table 7.1. The WVP value of pure alginate film (F_1) was 4.01×10^{-9} g /Pa h m, which reduced to 1.21×10^{-9} g /Pa h m for F_6 film. The addition of starch in alginate caused the significant decrease in WVP values ($P \leq 0.05$), resulting in better film resistance to water vapour transmission. However, pure starch-CMC (F_6) film had WVP of 4.06×10^{-9} g /Pa h m, which is slightly higher than the alginate film (F_1). It might be attributed to gelatinized starch with α–(1→6) glycosidic linkages, which resulted in much tightened structures, resulting in limited mobility even after plasticizing. The tightened structures could possibly have offered greater resistance to mass transfer.

Table 7.1. Properties of film prepared from different compositions of alginate, starch and CMC

Films	Water vapor permeability (g /Pa h m)	Moisture absorption (%)	Solubility in water (%)	Breakage strength (g)	Elongation capacity (mm)	Colour value		
						L	a	b
F_1 (2:0)	$4.01 \times 10^{-9\,a}$	18.29 ± 0.82^{a}	89.38 ± 2.65^{a}	273.2 ± 11.23^{a}	49.43 ± 2.45^{a}	63.16 ± 1.34^{a}	6.66 ± 0.31^{a}	11.59 ± 0.37^{a}
F_2 (2:1)	$3.13 \times 10^{-9\,b}$	15.67 ± 1.05^{b}	55.21 ± 1.57^{b}	411.5 ± 15.68^{b}	31.55 ± 1.73^{b}	67.19 ± 2.36^{b}	6.29 ± 0.30^{ab}	10.02 ± 0.25^{b}
F_3 (1:1)	$2.90 \times 10^{-9\,c}$	13.97 ± 1.23^{c}	53.56 ± 1.84^{b}	488.9 ± 9.42^{c}	22.23 ± 1.02^{c}	74.61 ± 1.39^{c}	6.22 ± 0.26^{b}	9.85 ± 0.13^{bc}
F_4 (1:1.5)	$1.26 \times 10^{-9\,d}$	11.64 ± 0.68^{d}	52.16 ± 1.65^{bd}	856.5 ± 10.59^{d}	21.02 ± 1.32^{c}	81.67 ± 2.67^{d}	5.74 ± 0.18^{c}	9.40 ± 0.26^{c}
F_5 (1:2)	$1.21 \times 10^{-9\,d}$	9.37 ± 1.12^{e}	40.00 ± 2.45^{c}	977.3 ± 17.42^{e}	20.76 ± 1.14^{c}	85.47 ± 1.73^{e}	4.39 ± 0.38^{d}	7.60 ± 0.63^{d}
F_6 (0:2)	$4.06 \times 10^{-9\,a}$	17.43 ± 0.45^{a}	50.91 ± 1.23^{d}	208.4 ± 12.42^{f}	14.62 ± 1.08^{d}	90.36 ± 2.69^{f}	1.66 ± 0.10^{e}	2.59 ± 0.12^{e}

All data are the mean ± SD of three replicates Mean followed by different letters in the same column differs significantly ($P \leq 0$ 05)

7.3.1.2 Moisture absorption and water solubility

The moisture absorption and water solubility patterns of film are shown in Table 7.1. The films F_1 and F_6 evinced the highest moisture absorption of 18.29% and 17.43% respectively and there is no significant difference in water absorption of both films. However, moisture absorption of alginate films decreased significantly with an increase in starch-CMC content ($P \leq 0.05$). The F_1 film had moisture absorption of 18.29% which reduced to 9.37% for F_5 film. The results are in line with the finding of Ghanbarzadeh, et al.[15] for starch/CMC blend film.

The water solubility of alginate film (F_1) was 89.38%, which was more compared to the starch-CMC blend film (F_6). It was also observed that, as the concentration of starch in the blend increased there was a marked decrease in the solubility of the films in water (Table 7.1). This is contrary to the findings of Rachtanapun & Tongdeesoontorn,[25] who reported that starch based films are highly hydrophilic in nature. In the present study, the decrease in solubility could be due to a more closed matrix, owing to addition of alginate and CMC, making the blend film less accessible to water. This result is comparable with the decrease in WVP with concomitant increase in the concentration of starch in the blend.

7.3.1.3 Tensile properties

Breakage strength and elongation of the film under stress conditions could be used to describe how the tensile properties of the film are related to the chemical structure. In this study, the films capacity for stretching could be described by elongation capacity, and breakage strength. It represents the film resistance to elongation and the amount of load it can handle. The end use handling properties and mechanical performance of the films are governed by these parameters. The breakage strength of film increased significantly ($P \leq 0.05$) with an increase in the amount of starch in the film composition and recorded the highest for F_5 film and lowest in F_1 and F_6 films.

However, increase in starch in the film composition produced an inverse effect on the film elongation capacity. This effect suggested that an increase in starch content resulted in films with more load capacity and stiffness and leading to a decrease in

elongation capacity (Table 7.1). This could be attributed to the presence of α-(1→6) glycosidic linkages. Films produced solely from starch-CMC had significantly lower tensile properties compared to the other blend films and alginate standalone film. Therefore, additions of CMC in the blend films did not have profound effect on the mechanical properties as a whole. The breakage strength and elongation capacity for pure starch-CMC (F_6), pure alginate (F_1) and alginate-starch-CMC (F_5) films were reported as 208.4 g and 14.62 mm, 273.2 g and 49.43 mm and 977.3 g and 20.76 mm respectively.

7.3.1.4 Film colour

The six different films produced varied in their colour, mostly due to the addition of alginate in the blend. Alginate left an amber colour in the films. Hunter colour data revealed that starch-CMC film (F_6) had the highest L value (90.36) and lowest for the F_1 film (Table 7.1). The L value of F_1 film was 63.16, which increased significantly to 85.47 for F_5 film after addition of starch in alginate. The concentration of alginate in the final films was responsible for the difference in the colour of the films. Redness (a value) and yellowness (b value) value of films decreased with concomitant increase in the starch content and it was least for the pure starch-CMC film.

7.3.1.5 Film surface characteristics

The scanning electron micrographs of outer surface for different film specimens are shown in Fig. 7.1. Overall, the surface demographics for all films showed uniform structures without cracks, pores or major disturbances. The micrograph for the six films was homogeneous and there were no signs of phase separation between the components. This indicated that the three polymers are physically compatible with each other. The film F_3 showed irregularity with large crystal like particles on it; however, some granules particles did appear in the F_2 and F_3 films. The crystal like particles in F_3 film might be due to gelatinized starch granules remaining on film after majority of internal starch polymers have been released.[21,26]

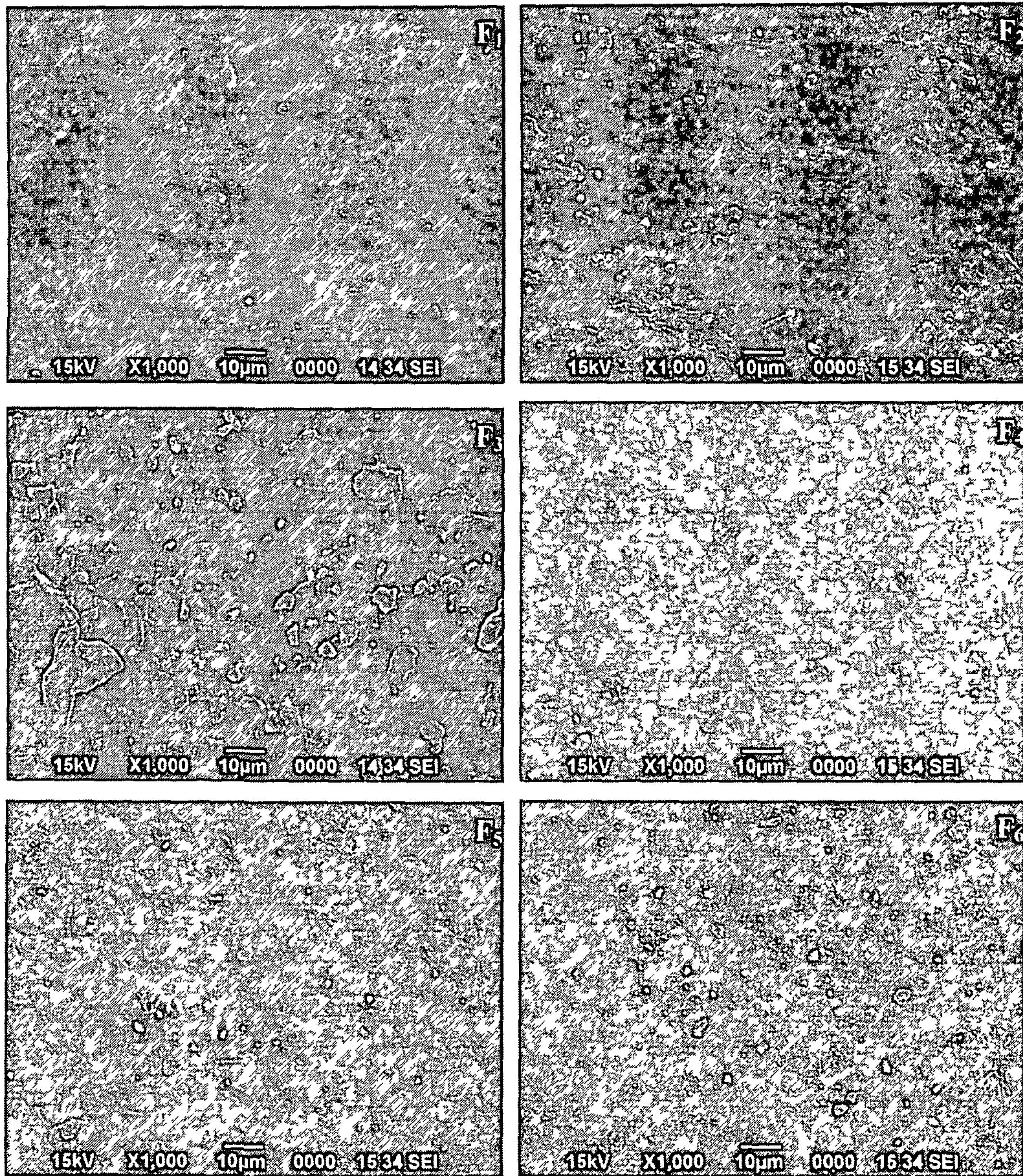

Fig. 7.1. Scanning electron micrographs of blended films

7.3.1.6 Infrared spectroscopy of films

The C-H stretching resulted in peaks for all films at around 2900 cm^{-1} as seen in the IR spectral graph (Fig. 7.2). Wideband was visible around 3400-3200 cm^{-1} and found in all the films and was caused by N-H stretching, to some extent O-H stretching and intra-intermolecular hydrogen bonds. The blend films exhibited peaks at around 1600 and 1400 cm^{-1} might be due to symmetric and asymmetric vibrations for COO^- group.[27-29] Change in concentration of starch content in the films however, resulted in a tiny gradual shift in the peaks towards a higher IR range. This suggests that symmetric and asymmetric vibrations of the C-O and C=O increased. When starch was added to the blend, the disarray in the intermolecular hydrogen bonds that were present between the carboxylic groups might have caused the above shift. In F_3 film prominent peaks were observed in the range of 1150 cm^{-1}, this peak might be originated from glycosidic linkages in polysaccharides and these bands were assigned to antisymmetric α-(1→4) stretching mode of glycosidic linkages.[30-32] However, these peaks were found in other film but they were less prominent. Overall there are less changes were observed in FTIR spectra of all the prepared film.

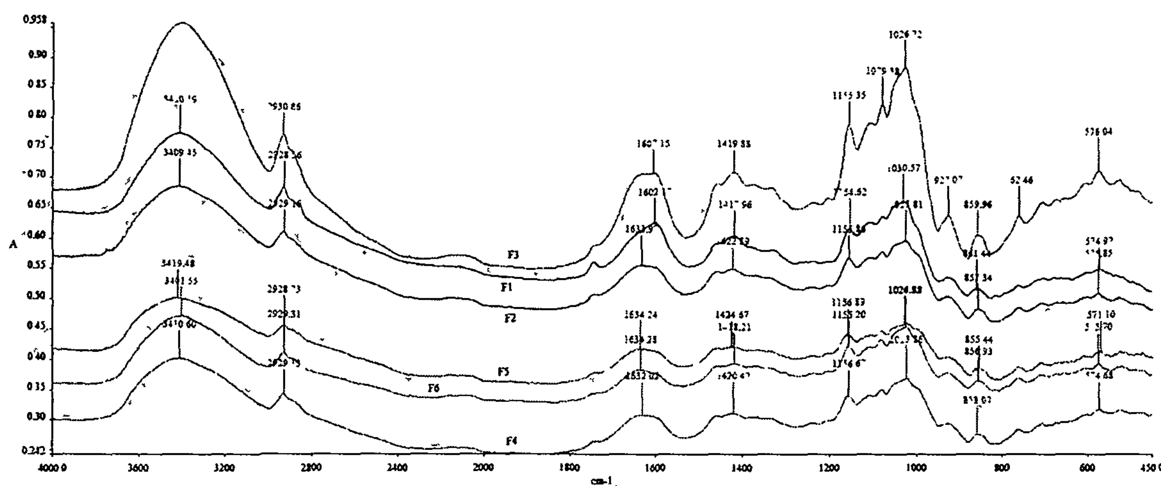

Fig. 7.2. FTIR spectra of films and individual polymers

7.3.1.7 Thermal stability assessment by TGA

The curves of thermo gravimetric analysis (TGA) for the films with the varying concentrations are shown in Fig. 7.3. A gradual loss of weight was observed in all films till about 200°C which could be attributed to loss of free and bound moisture from the films. A relatively low decomposition temperature of about 180°C was found for F_1 and F_6 films. The films F_2, F_3, F_4 and F_5 evinced similar thermal decomposition behavior above 200°C, and recorded sharp weight loss in the 250-350°C region. As the starch content in the films increased, the thermal stability also enhanced. However, CMC content in the blends helped to enhance for thermal stability of film. In case of pure CMC, mostly weight loss was observed in the temperature range of 300–500 °C.[33] It corroborates that the three polymers were greatly compatible with one another.

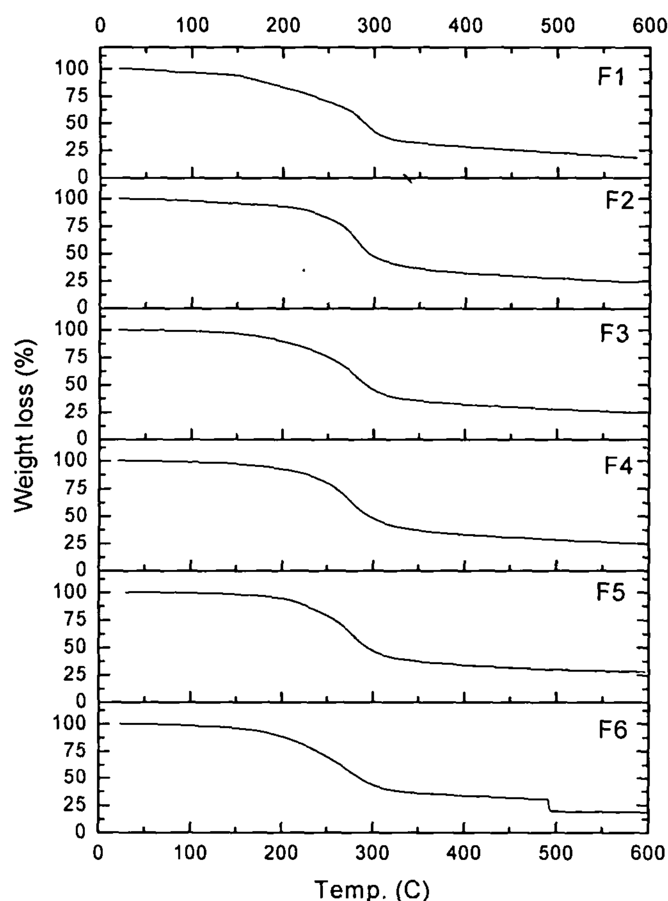

Fig. 7.3. Thermo gravimetric analyses curves of blended films.

7.3.1.8 Standardization of film

The film F5 with a polymer blend of alginate and starch (1:2) and 15% CMC equivalent to starch (w/w) was finally selected on the basis of better performance than the other blended films. WVP, moisture absorption and water solubility of F_5 film were reported as 1.21×10^{-9} g /Pa h m, 9.37% and 40% respectively and it was ahead of the other films. Tensile properties of F_5 film were acceptable and gave the highest breakage strength of 977.30 g. Colours value revealed the lightness of film, which is acceptable for coating purposes. Surface of F_5 film was smooth and uniform, without cracks, pores or major disturbances. Thermal stability of this film was more and decomposition behavior started above 200°C. Considering the suitability of film with respect to different film properties, same film forming solution of F_5 film was selected for coating of bamboo shoot.

7.3.2 Effect of coating on bamboo shoot quality

Film forming solution of selected film (F_5) was incorporated with the antioxidant and antimicrobial extract and same was used for coating of bamboo shoots. The effect of coating on bamboo shoot was studied as discussed below.

7.3.2.1 Weight loss

The percentage of weight loss in bamboo shoots as a function of time, with and without the coating is shown in Fig. 7.4. Weight loss of around 20% was reported in uncoated sample and around 13% in case of coated sample at the end of 5th day. Coating of shoot reduces the weight loss by 7%; this might be attributed to low WVP of film, which restricted the surface moisture loss from the bamboo shoot. Reduction of weight loss during storage by application of starch coating on tomatoes was reported by Das et al.[21] which corroborates the present findings.

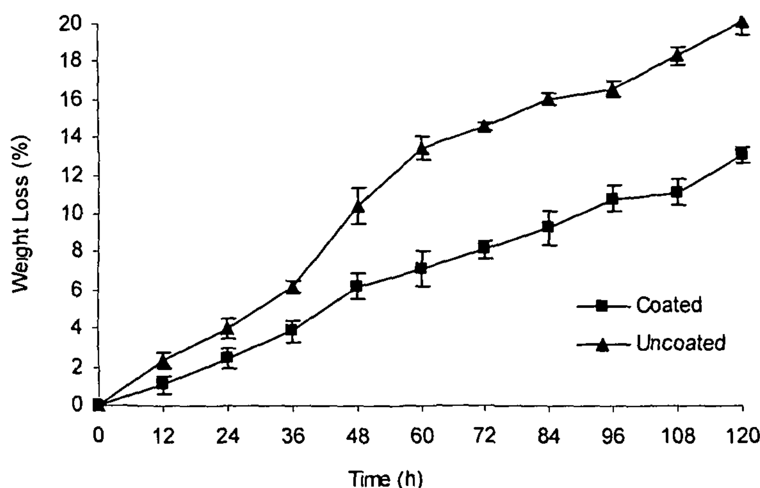

Fig. 7.4. Weight loss in coated and uncoated bamboo shoot during storage

7.3.2.2 Surface colour

The change in colour and L values of coated and uncoated bamboo shoot as a function of time, are given in Fig. 7.5. The lightness values decreased significantly for all coated and uncoated samples during storage. Control samples presented the lowest L values at the end of storage. Despite the addition of antioxidant extract on the film, the coated samples showed a gradual decrease in the L values after the 36th h. However, it recorded better results than the uncoated samples. The dE value of uncoated shoot increased exponentially with time; however, in case of coated shoot very less changes were observed upto 48th h and afterwards the value increased slowly. It has been reported that polysaccharides have good gas barrier properties, and thereby help in decreasing the respiration rate and eventually limiting metabolic activities. This helps to delay enzymatic browning. The use of alginate, other polysaccharides and antioxidants help to limit or delay browning of fruits; however, sometime the amber colour of the alginate solution imparts lower L value of fruits. Results of present study are in line with finding of several research works.[1,16,34]

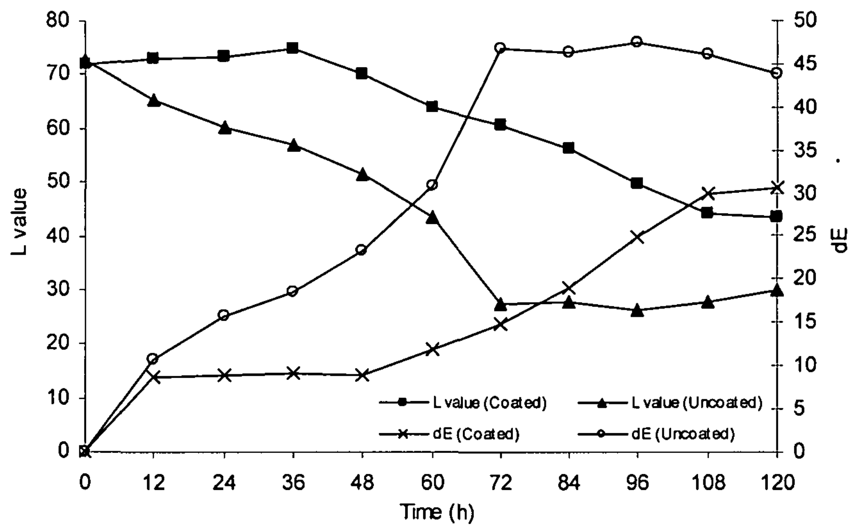

Fig. 7.5. Effects of coating on surface colour of bamboo shoot

7.3.2.3 Surface microbial count

The microbial count increased in the uncoated samples during storage from 4.60 to 6.20 log cfu/cm^2 (Fig. 7.6). However, in the coated samples the surface microbial load dropped significantly ($P \leq 0.05$) from 4.64 to 2.34 log cfu/cm^2. This might be due to addition of the antimicrobial agent in film forming solution, as this antimicrobial agent earlier tested proved effective against *E. coli, S. aureas* and *B. ceres*. An antimicrobial agent incorporated film declined the microbial growth and kept the surface microflora in control. Polysaccharide films also provide good barrier against entry of microbes through the film.[21] The microbial barrier properties of the F_5 film contributed to decline in the surface microbial load of coated bamboo shoots might be due to use of polysaccharides and addition of antimicrobial metabolites.

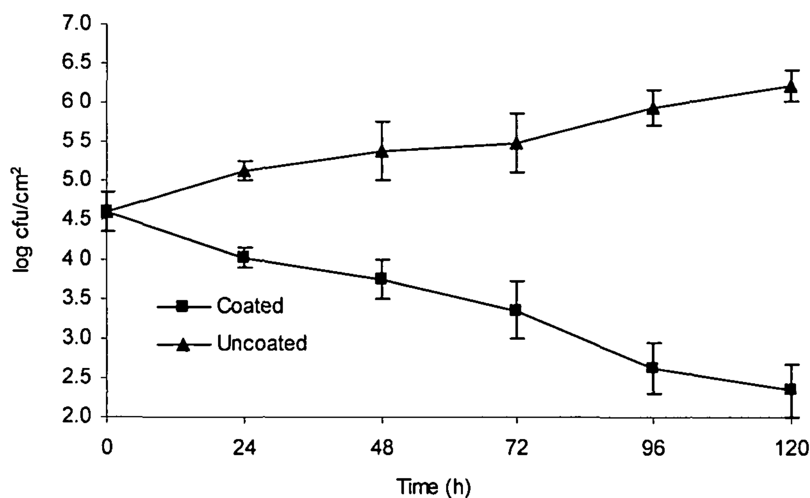

Fig. 7.6. Effects of coating on surface microbial count of bamboo shoots

7.4 Conclusion

Alginate, starch, and CMC were successfully employed to fabricate the edible film. The F_5 film prepared from alginate and starch (1:2) blended with added CMC (15% of starch) evinced lower water vapor permeability, moisture absorption, water solubility and elongation capacity; however, breakage strength were reported high with less yellowness and lighter film. Surface characteristics showed the uniformity of film as well as more thermal stability. Addition of starch and CMC in alginate imparted an important influence on the barrier and mechanical properties of the resulted composite films. Coating of bamboo shoot resulted in less water loss, lowered the colour change (dE) value and reduction in surface microbial count, which could be attributed to incorporation of antioxidant and antibacterial extracts in film forming solution.

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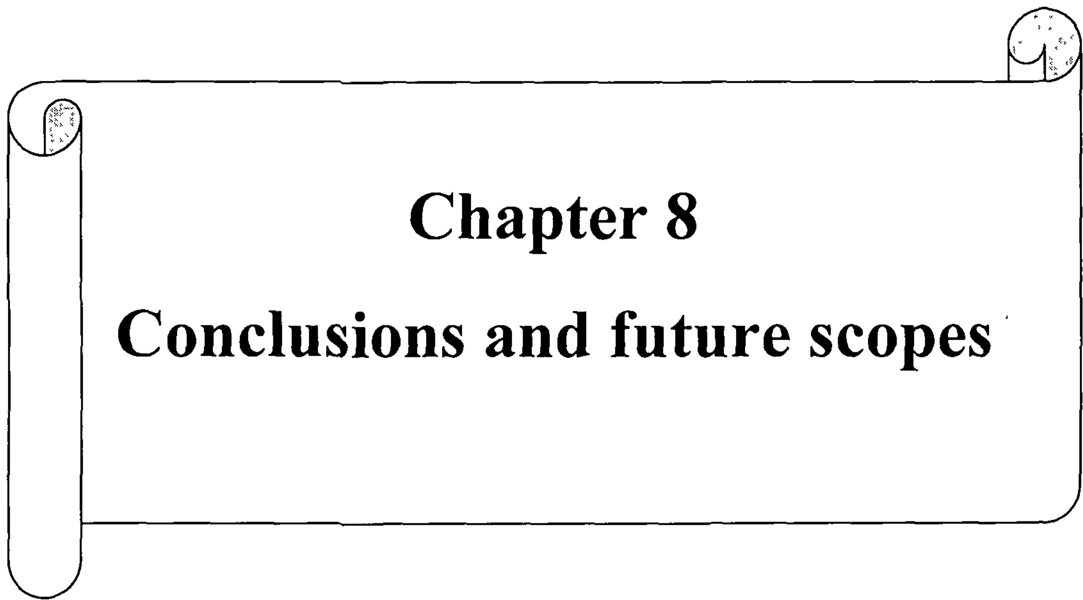

Chapter 8

Conclusions and future scopes

8.1 Conclusions

The present study was undertaken and investigated under five clearly focused objectives. The nutritional potential of edible bamboo shoots of Assam was studied and further evaluated the effect of blanching temperature and time on physicochemical properties of bamboo shoot. The optimization of osmotic dehydration process of bamboo shoot and its mass transfer kinetics has been examined. The effect of centrifugal force and pulsed vacuum on osmotic dehydration of bamboo shoot at optimized process condition were studied and compared. In addition, the physicochemical and microbial changes during the fermentation of bamboo shoots were examined in the process of making *khorisa*. The antimicrobial biometabolites were extracted from *Lactobacillus plantarum*. It was isolated form fermented bamboo shoot product of Assam (*khorisa*). Microwave assisted extraction process was optimized for the extraction of antioxidant extract from *Garcinia pedunculata* Robx. fruit. The edible films were prepared with varying compositions of alginate, starch and carboxymethyl cellulose. The films were tested for their mechanical, thermal and barrier properties. The effect of edible coating coupled with antimicrobial and antibrowning agents on bamboo shoot quality was also investigated.

The salient findings of the thesis are summarized below:

I. **Effect of blanching temperature and time on physicochemical properties of bamboo shoot**

- Out of the four species of bamboo the shoot of *Bambusa balcooa* found to be relatively rich in protein, vitamin C, total phenolics and antioxidant activity.
- The blanching time and temperature have significantly influenced on the nutrient like protein, carbohydrate and reducing sugars but less on ash and crude fiber.
- Maximum losses were reported at 95 °C for 20-30 min and minimum at 75 °C for 5-10 min of blanching.
- Retention of ascorbic acid was higher at 75 °C which gradually reduced at 85 and 95 °C.

- Maximum loss of phenolics was observed at 95 °C for 30 min (38.70% retention) of blanching and highest retention of phenolics was reported at 75 °C for 5 min (88.11% retention).
- The significant loss of DPPH free radical scavenging activity of bamboo shoot was found as the temperature and time of the blanching increased.
- Blanching resulted in soft texture and decrease in lightness and increase in greenness and yellowness were observed.

II. Optimization of osmotic dehydration process, its mass transfer kinetics and effect of centrifugal force and pulsed vacuum on osmotic dehydration of bamboo shoot

- The optimum values for temperature, time, and salt concentration of osmotic dehydration process were found as 37 °C, 129 min, and 12.10 % respectively.
- The effective diffusivity of water as well as solute was dependent on osmotic solution temperature.
- The effective water and solute diffusivity values were in the range of 3.36×10^{-9} to 10.20×10^{-9} m^2/s and 3.20×10^{-9} to 25.31×10^{-9} m^2/s for the temperature range of 25-40 °C.
- Textures of bamboo shoot got softer with an increase in temperature of osmotic dehydration.
- At higher osmotic dehydration temperature, reduction in brightness of shoot, change in colour (dE) value and also damage to the cell structure were observed.
- The increase in water loss was observed with application of vacuum pulse up to 400 mm Hg, and then it decreased continuously with further increase of vacuum pressure to 600 mm Hg.
- The application of centrifugal force during osmotic dehydration enhanced water loss, reduction in solid gain with less change in colour and having softer texture.
- Vacuum pressure up to a certain level (400 mm Hg) brought more water loss compared to that of the centrifugal force and unlike with solid gain.

- The best levels of centrifugal force and vacuum pressure were recorded were 2800 rpm and 400 mm Hg respectively.

III. Influence of fermentation on bamboo shoot quality with and without addition of *Garcinia pedunculata* Roxb. fruit

- Minor changes in fat, protein, crude fibre, ash and vitamin C were recorded but carbohydrates concentration was dropped markedly during fermentation of bamboo shoot.
- Acidity increased 3-4 times and pH drop was observed from initial values of 6.40 to 4.52 and 4.09 for batch-1 and batch-2 respectively.
- There was an exponential increase in total phenols level with fermentation time, however, antioxidant activity also got doubled.
- A sharp decrease in the reducing sugars content was recorded during initial 8 days of fermentation and there after it became slow.
- The total plate count, yeast and mould count and lactic acid bacteria count decreased with progressive fermentation of bamboo shoot.
- HPLC analysis of organic acids evinced the presence of oxalic acid, tartaric acid, formic acid, pyruvic acid and lactic acid in fresh bamboo shoot.
- HPLC analysis of carbohydrates showed the presence of raffinose, sucrose, glucose, galactose and inositol in fresh bamboo shoot.
- The fermented bamboo shoot (*khorisa*) added with *Garcinia pedunculata* Roxb. fruit was found superior than the *khorisa* without *Garcinia pedunculata* Roxb. in terms of nutritional contents.

IV. Extractions of antimicrobial biometabolites and antioxidant extract from different biological sources

- *L. plantarum* isolated from fermented bamboo shoot evinced the strong antibacterial activity against *Staphylococcus aureus.*
- The yield of biometabolite was found to be the highest in the chloroform fraction followed by hexane and lowest in petroleum ether fraction.

- Cell-free crude supernatants (CFCS) and cell-free purified supernatants (CFPS) of chloroform fraction showed antimicrobial activity against all the three test pathogens (*E. coli, S. aureus* and *B. cereus*).

- HPLC analysis confirmed that the lactic acid is the main organic acid present in the CFCS with small amount of tartaric and formic acid.

- The minimum lethal dose concentration of CFPS chloroform fraction were recorded as 27 mg/ml for *E. coli*, 1.68 mg/ml for *S. aureus* and 1.68mg/ml for *B. cereus*

- Microwave assisted extraction (MAE) results evinced that irradiation time was the major factors which affected the antioxidant activity of extract obtained from *Garcinia pedunculata* Roxb. fruit.

- The optimal conditions of MAE for antioxidant extract obtained were found as solvent concentration of 70.79%, solvent to sample ratio of 20:1 and irradiation time of 4.73 min.

- The extraction of antioxidant extract using MAE method used lesser solvent and required less extraction time compared to conventional solvent extraction methods.

V. Development of antimicrobial and antibrowning edible coating and its effect on bamboo shoot quality

- Alginate, starch, and carboxymethyl cellulose (CMC) were successfully employed to fabricate the edible film.

- Addition of starch and CMC in alginate imparted an important influence on the barrier and mechanical properties of the resulted composite films.

- The F_5 film prepared from alginate and starch (1:2) blend with added CMC (15% of starch) showed better mechanical and barrier properties compared to other films.

- The surface characteristics showed the smooth and uniform film and thermal decomposition took place above 200°C.

- Coating of shoot reduced the weight loss by 7% and brightness of shoots was also consitant.

- The incorporation of antioxidant and antibacterial extracts in coating solution helps in lowering the browning of bamboo shoots, and also successfully inhibited surface microbial load.

Therefore, above stated salient findings conclude that the processing e.g. blanching, osmotic dehydration and fermentation have significant influence on bamboo shoot quality. Low temperature blanching could be better option for retention of micro nutrients. Enhancement of osmotic dehydration process resulted, with application of vacuum pressure and centrifugal force. Addition of *Garcinia pedunculata* Roxb. in bamboo shoot enhanced the fermentation and quality of fermented product (khorisa). The application of antimicrobial biometabolites and antioxidant extract in edible coating evinced better retention of bamboo shoot quality.

8.2 Future scopes of present investigation

- Osmotic dehydration might be combined with other drying methods.
- Khorisa potential could be further explored by analyzing its amino acids, aroma active compounds, phenolic profile, antioxidant compounds etc.
- Some other active agent might be incorporated in edible coating to enhance the quality of bamboo shoot.

List of Publications

Journal Publications

1. **Badwaik, L. S.**, Choudhury, M., Dash, K. K., Borah, P.K. and Deka, S. C. Osmotic dehydration of bamboo shoots enhanced by centrifugal force and pulsed vacuum using salt as osmotic agent, *Journal of Food Processing and Preservation*, DOI: 10.1111/jfpp.12186 **(Available Online)**

2. **Badwaik, L. S.**, Borah, P.K. and Deka, S. C. Antimicrobial and enzymatic antibrowning film used as coating for bamboo shoot quality improvement, *Carbohydrate Polymers,* 103, 213-220, 2014.

3. **Badwaik, L. S.**, Borah, P.K., Borah, K., Das, A.J., Deka, S. C. and Sharma, H.K. Influence of fermentation on nutritional compositions, antioxidant activity, total phenolic and microbial load of bamboo shoot, *Food Science and Technology Research*, 20(2), 255-262, 2014.

4. **Badwaik, L. S.**, Dash, K.K., Choudhury, M., Borah, P.K. and Deka, S. C. Mass transfer kinetics, and changes in texture, color and cell structure during osmotic dehydration of bamboo shoots, *Engineering in Agriculture, Environment and Food* **(Under Review).**

5. **Badwaik, L. S.**, Borah, P.K. and Deka, S. C. Optimization of microwave assisted extraction of antioxidant extract from *Garcinia Pedunculata* Roxb., *Separation Science and Technology* **(Under Review).**

6. **Badwaik, L. S.**, Borah, P.K. and Deka, S. C. Production and purification of anti-bacterial biometabolites from wild type lactobacillus, isolated from fermented bamboo shoot, *Applied Biochemistry and Biotechnology* **(Communicated).**

7. **Badwaik, L. S.**, Gitanjali, G. and Deka, S. C. Influence of blanching on nutritional components, ascorbic acid, total phenolics, antioxidant activity, colour and texture of bamboo shoot, *Food Science and Nutrition* **(Communicated).**

Book Chapter

1. **Badwaik, L. S.**, Borah, P.K. and Deka, S. C. Indigenous Fermented Foods Involving Acid Fermentation in *Indigenous fermented foods of South Asia.* V. K. Joshi ed., Chapter No. 8, CRC Press (Accepted).

Conference Presentations

1. **Badwaik, L. S.**, Choudhury, M., Dash, K. K., Borah, P. K., Sit, N., Seth D. and Deka S. C. "Effect of process parameters on osmotic dehydration of bamboo shoots using sodium chloride solutions" in 3rd International Conference on Food Technology (Incoftech-2013) at Indian Institute of Crop Processing Technology, Thanjavur, India, January 4-5, 2013.

2. **Badwaik, L. S.**, Borah, P. K. and Deka S. C. "Neutraceuticals potential of bamboo shoots and its fermented products of Assam region" in 18th International Conference on "Perspective and Challenges in Chemical and Biological Sciences: Innovation Cross Roads" (Post: ISCBC-2012) at IASST, Guwahati, India, January 28-30, 2012.

3. **Badwaik, L. S.**, Borah, K., Borah, P. K. and Deka, S. C. "Fermentation kinetics and biochemical analysis of fermented bamboo shoot product (Khorisa) of Assam" in 5th International Conference on Fermented Foods, Health Status and Social Well-being: Challenges and Opportunities (SASNET-2011) at CFTRI, Mysoor, India, December 15-16, 2011.

4. Borah, P. K., Borah, K., Choudhury, M. and **Badwaik, L. S.** "Investigating some varieties of bamboo shoots of Assam as a possible source of bioactive compounds" in National Seminar on Role of Bioactive Compounds in foods on Human health (BIOFOODS 2011) at Department of Food Engineering and Technology, Tezpur University, Tezpur, Assam, India, 14-16 November, 2011.

5. Borah, K., Borah, P. K., and **Badwaik, L. S.** "Status of bioactive compounds in fermented bamboo shoot product (Khorisa) of Assam" in National Seminar on Role of Bioactive Compounds in foods on Human health (BIOFOODS 2011) at Department of Food Engineering and Technology, Tezpur University, Tezpur, Assam, India, 14-16 November, 2011.