ASSESSMENT OF CHITOSAN AS PRESERVATIVE ON SELF LIFE AND MAJOR NUTRIENT CONTENTS ON FRUITS AND VEGETABLES

MUST. ALIMA RAHMAN

DEPARTMENT OF AGRICULTURAL CHEMISTRY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
DHAKA -1207

JUNE, 2017

ASSESSMENT OF CHITOSAN AS PRESERVATIVE ON SELF LIFE AND MAJOR NUTRIENT CONTENTS ON FRUITS AND VEGETABLES

A Thesis
By

MUST. ALIMA RAHMAN

Reg. No. 16-07582

Submitted to the Department of Agricultural Chemistry
Sher-e-Bangla Agricultural University, Dhaka
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE (M.S.)
IN
AGRICULTURAL CHEMISTRY

Semester: Jan-June, 2017

APPROVED BY:

……

Supervisor
(Dr. Mohammed Ariful Islam)
Professor
Department of Agricultural Chemistry
Sher-e-Bangla Agricultural University,
Dhaka-1207

……

Co-supervisor
(Mohammad Zahirul Islam Mollah)
Senior Scientific Officer
Institute of Radiation and Polymer TechnologyBangladesh Atomic Energy Commission,
Ganakbari, Savar, Dhaka-1349

……

(Dr. Md. Sirajul Islam Khan)
Chairman
Examination Committee

DEPARTMENT OF AGRICULTURAL CHEMISTRY
Sher-e-Bangla Agricultural University

Sher-e-Bangla Nagar, Dhaka-1207

Ref.No:Date:

CERTIFICATE

This is to certify that the thesis entitled "**ASSESSMENT OF CHITOSAN AS PRESERVATIVE ON SELF LIFE AND MAJOR NUTRIENT CONTENTS ON FRUITS AND VEGETABLES''** submitted to the department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (M.S.)** in **AGRICULTURAL CHEMISTRY**, embodies the results of a piece of *bona fide* research work carried out **MUST. ALIMA RAHMAN**, Registration No. 16-07582, under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma in any other institution.

I further certify that any help orsources of information received during the course of this investigation have duly been acknowledged.

Dated:

Dhaka, Bangladesh

(Dr. Mohammed Ariful Islam)
Professor
Department of Agricultural Chemistry
Sher-e-Bangla Agricultural University,
Dhaka-1207

Supervisor

ACKNOWLEDGEMENTS

The author first wants to articulate her enormous wisdom of kindness to the Almighty Allah for His never ending blessing, protection, regulation, perception and assent to successfully complete of research and prepare thesis.

The author would like to express her deepest sense of gratitude to her research supervisor, Dr. Md. Ariful Islam, Professor, Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, for his scholastic guidance, innovative suggestion, constant supervision and inspiration, valuable advice and helpful criticism in carrying out the research work and preparation of this manuscript.

The author also grateful to her respected Co-Supervisor Mohammad Zahirul Islam Mollah, Senior Scientific Officer, Institute of Radiation and Polymer Technology, Atomic Energy Research Establishment, Bangladesh Atomic Energy Commission, Ganakbari, Savar, Dhaka-1349, for constant encouragement, cordial suggestions, constructive criticisms and valuable advice during the research period and preparing the thesis.

The author would like to express her deepest respect and boundless gratitude to Dr. Ruhul Amin Khan, Director, Institute of Radiation and Polymer Technology, Atomic Energy Research Establishment, Bangladesh Atomic Energy Commission, Ganakbari, Savar, Dhaka-1349, for constant encouragement,

cordial suggestions, constructive criticisms and valuable advice during the research period and preparing the thesis.

The author would like to express her deepest respect and boundless gratitude to Abu Hena Mostofa Kamal, Scientific Officer, Institute of Radiation and Polymer Technology, Atomic Energy Research Establishment, Bangladesh Atomic Energy Commission, Ganakbari, Savar, Dhaka-1349, for constant encouragement, cordial suggestions, constructive criticisms and valuable advice during the research period and preparing the thesis.

The author would like to express her deepest respect and boundless gratitude to Dr. Md. Sirajul Islam Khan, Associate Professor and Chairman, Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207 and all the respected teachers of the Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka-1207, for the valuable teaching, sympathetic co-operation and inspirations throughout the course of this study and suggestions and encouragement to research work.

The author would like to express her cordial thanks to the departmental staffs for their active help during the experimental period.

The author expresses her unfathomable tributes, sincere gratitude and heartfelt indebtedness from his core of heart to her father Md. Azizur Rahman, mother Fozilatun Nesa, whose blessings, inspirations,

sacrifices and moral supports opened the gate and paved to way of her higher study.

The Author

Abstract

An experiment was conducted in the laboratory of the Bangladesh Atomic Energy Commission in Ganakbari, Savar, Dhaka and the laboratory of the Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka-1207 during September 2017 to March 2018 to assessment of Chitosan as preservative on self life and major nutrient contents on fruits and vegetables. Prawn shells and some selected fruits and vegetables were collected from different markets near the Dhaka city. In this study, fruits and vegetables were collected from 3 markets of Bank Town Bazar, Savar Bazar, Ganakbari Bazar. At first some selected fruits and vegetables were divided into 2 or 3 groups. Among them 1 group for control and other group for treatment. Chitosan was applied on treatment groups by deep coating method. The factorial experiment was laid out with three replications [Samples are BC= Banana Control, BT_1= Banana Treatment 1, BT_2= Banana Treatment 2, PC= Papaya Control, PT_1= Papaya Treatment 1, TC= Tomato Control and TT_1= Tomato Treatment 1; T_1= 500 ppm Chitosan and T_2= 1000 ppm Chitosan]. Color index shows a significant difference ($p \leq 0.05$) among the varieties. In case of color index and weight loss the significant difference were higher in Tomato and Papaya than Banana, while EC and pH levels were remains similar. In case of Total Suspended Solids (TSS) and Total Dissolved Solids (TDS), percentages of TSS and TDS were also remaining similar. In case of Total Viable Count (TVC) or Total Bacterial Count and Total Fungal

Count (TFC) or Yeast & Mold Count (YMC) the significant difference is higher in control of Banana, Tomato and Papaya than treatment-1 and treatment-2 of Banana, Tomato and Papaya. In case of Nitrogen (N), Phosphorus (P) and Potassium (K) content, percentages of Nitrogen and Potassium content were remaining similar or high, while Phosphorus (P) content was remaining similar. This study points out that 500 ppm chitosan is sufficient for storage of tomatoes, papayas and bananas at low temperature and highhumidity.This study shown that coating with chitosan and a chitosan derivative can be employed to extend the shelf life and to improve quality offruits and vegetables by delaying ripening, reducing weight loss and reducing microbial growth in fruits and vegetables. From the experiment, it can be concluded that 500 ppm dose of chitosan is more effective compared to that of 1000 ppm dose of chitosan in Banana fruits. Therefore, it suggests that the use of Chitosan as preservative on self life could be an effective approach to extend the shelf life and to improve quality of fruits and vegetables by delaying ripening, reducing weight loss and reducing microbial growth in fruits and vegetables.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ABBREVIATION	FULL WORD
%	Percent
^{0}C	Degree Celsius
DMRT	Duncan's Multiple Range Test
e.g.	As for example
et al	and others
i.e.	that is
K	Potassium
N	Nitrogen
P	Phosphorus
pH	Hydrogen ion concentration
ppm	Parts Per Million
SAU	Sher-e-Bangla Agricultural University
GDP	Gross Domestic Product
CDC	Center for Disease Control
PLA	Polylactic acid
PHAs	Polyhydroxyalkanoates
PHB	Polyhydroxybutyrate
PBS	Polybutylene succinate
PE	Polyethylene
CFU's	Colony Forming Units
O_2	Oxygen
g	Gram
mg	Miligram
CAS	Controlled Atmosphere Storage
MAP	Modified Atmosphere

	Packaging
EC	Electrical Conductivity
TSS	Total Suspended Solid
TDS	Total Dissolved Solid
µm	Micro Meter
PDA	Potato Dextrose Agar
PCA	Plate Count Agar
ml	Mili Liter
TVC	Total Viable Count
TFC	Total Fungal Count
YMC	Yeast and Mold Count

CHAPTER 1
INTRODUCTION

DEDICATED TO MY BELOVED PARENTS

Bangla and favora ture sector DP) and en uge portion vest preser due to poo past decade lms which ove food p ation and to reduce the use of chemical preservatives such as plastic. Abundant naturally occurring polymers – as starch, collagen, gelatin, alginate, cellulose and chitin represent attractive candidates as natural preservatives. Biologically active bio-molecules such as chitosan and its derivatives have a significant potential in the food industry because of probable microbial contaminations associated with food products and the increasing concerns in relation with the negative environmental impact of conventional packaging materials. Chitosan offers real potential for applications in the food industry due to its particular

physico-chemical properties, short time biodegradability, biocompatibility with human tissues, antifungal activities, and non-toxicity. Furthermore, chitosan exhibits high antimicrobial activity against pathogenic and spoilage micro-organisms, including both Gram-positive and Gram-negative bacteria. Chitosan is also quite unique bio-based polymer: its intrinsic properties are so singular and valuable that chitosan possesses no actual petrochemical equivalent. Thus, chitosan-based films have attracted serious attention in food preservation and packaging technology. Consequently, the inherent characteristics of chitosan make it exploitable directly for itself. Chitosan is a linear, semi-crystalline polysaccharide composed of (1-4)-2-acetamido-2-deoxy-b-D-glucan (N-acetyl D-glucosamine) and (1-4)-2-amino-2-deoxyb- D-glucan (D-glucosamine) units. As such, chitosan is not extensively present in the environment however; it can be easily derived from the partial deacetylation of a natural polymer: the chitin. As functional materials, chitin and chitosan offer a unique set of characteristics: biocompatibility, biodegradability to harmless products, physiological inertness, antibacterial properties, heavy metal ions chelation, gel forming properties hydrophilicity, and remarkable affinity to proteins. The aim of the present research work is to assess the effect of chitosan (an organic preservative collected from Shrimp) on self life and major nutrient contents of some selected fruits and vegetables. To achieve this objective, some selected and available fruits and vegetables in Bangladesh will be treated with chitosan to-

- know the preservative effect of chitosan,

- study the effect of chitosan on the major nutrient contents.

- identify the microbial effects.

CHAPTER 2

REVIEW OF LITERATURE

This study is about chitosan and its preservative effect on the quality of a horticultural produce. This review will cover topics on postharvest changes in bananas, papayas and tomatoes in particular, of chitosan.

2.1. Preservatives:

A preservative is a substance or a chemical that is added to products such as food, beverages, pharmaceutical drugs, paints, biological samples, cosmetics, wood, and many other products to prevent decomposition by microbial growth or by undesirable chemical changes. In general, preservation is implemented in two modes, chemical and physical. Chemical preservation entails adding chemical compounds to the product. Physical preservation entails processes such as refrigeration or drying (Erich Lück 2002). Preservative food additives reduce the risk of food borne infections, decrease microbial spoilage, and preserve fresh attributes and nutritional quality. Some physical techniques for food preservation include dehydration, UV-C

radiation, freeze-drying, and refrigeration. Chemical preservation and physical preservation techniques are sometimes combined.

2.2. Chemical Food Preservatives

Preservatives are commonly used to preserve food that is susceptible to damage. This substance is an ingredient that is added to the diet to prevent or inhibit the growth of fungi, bacteria, or microorganisms. Thus, the process of fermentation (decomposition), acidification or decomposition due to microorganism activity can be prevented so that the shelf life is relatively longer. But it is not uncommon for manufacturers to use it on relatively long-lasting foods with the aim of extending the storage period or improving texture. The most common preservatives sold in the market and used to preserve various foods are benzoates, which are generally present in sodium benzoate or more soluble potassium benzoate. Benzoate is often used to preserve various foods and beverages such as fruit juice, soft drinks, ketchup, chili sauce, jam and jelly, sweets, soy sauce and etc. The use of preservatives in food should be appropriate both types and dosage. A preservative may be effective in preserving certain foods, but it is ineffective in preserving other foods because they have different properties so that the destructive microbes that are inhibited by their growth are also different.Foods which requires a long shelf life often contain chemical food preservatives, which are effective at preventing bacterial growth. Conversely, there are concerns that prolonged use of some chemicals for this purpose pose potential heath problems.

Nitrates - Sodium nitrate is an antimicrobial additive commonly found in cured meats, including hot dogs, bacon, ham, corned beef, deli meats and smoked fish. Some experts say that this preservative can cause the formation of "nitrosamines," which are cancer-causing chemicals. Studies have found a link between consuming cured meats and nitrite, and cancer in humans.

Benzoates - This preservative can be found in some teas, coffees and fruit juices. Banned in Russia, this chemical is believed to prompt skin rashes, asthma, allergies

and even brain damage. Benzoic acid is often found in salad dressings, ketchup and some soft drinks. Initially, the FDA deemed sodium benzoate was a safe chemical to add to foods. This decision is now being questioned because there may be a link between sodium benzoate and the chemical "benezene," a known carcinogen.

Sorbates - Sorbic acid is used to keep foods such as salad dressing, cakes and cheeses preserved.Potassium sorbate kills yeast, bacteria and fungus, and is added to dried fruits and meats, baked goods, wines, yogurt and cheese. This non-toxic preservative, when used inmoderation, is often considered safe in foods, although some experts have expressed concern about possible allergic side effects to prompt skin rashes.

Sulphites - Found in canned clams, relishes, pie crust, beer, dried citrus fruit beverage mixes, wine and dried fruits. In the past, sulfites were used on vegetables and fruits that people generally ate raw to keep fresh produce crispy and colorful. The FDA putthe brakes on this practice in 1986 due to possible side effects that included diarrhea, nausea, hives, shortness of breath andbrain damage in some cases death.

Caramel - A broadly-used food additive, caramel coloring has been in use for decades. It can be found in many processed foods and drinks, such as potato chips, doughnuts, ice cream, bread, candy, soft drinks, beer, frozen pizza, vinegar, cookies and dark liquors, to name a few. This common additive has been named as a contributor to vitamin B6 deficiencies as well as cancer.

At present, some food producers still use the preservatives that are prohibited for use in food and harmful to health. For examples are Borax and Formalin. Borax is an antiseptic and germ killer, therefore widely used as an anti-fungal, wood preservative and for antiseptic ingredients in cosmetics.

The use of Borax is often unintentional because it is unknowingly contained in the making of meatball, wet noodles, and many traditional foods. In addition there is also a lot of Formalin in use to preserve food such as in tofu and wet noodles. Formalin is actually an ingredient for preserving corpses and organs and is very dangerous to health therefore formalin use is strictly prohibited.

2.3. List of ChemicalPreservatives

The following list outlines all preservatives that we should steer clear from. The list also includes nitrates and sulphites (particular types of preservatives) that should also be avoided.

Table 1: List of Chemical Preservatives with E number.

E number	Chemical compound	Used as
200	Sorbic acid	preservative
201	Sodium sorbate	preservative
202	Potassium sorbate	preservative
203	Calcium sorbate	preservative
210	Benzoic acid	preservative
211	Sodium benzoate	preservative
212	Potassium benzoate	preservative
213	Calcium benzoate	preservative
216	Propylparaben or Propyl-p-hydroxy-benzoate	preservative
218	Methylparaben or Methyl-p-hydroxy-benzoate	preservative
220	Sulphur dioxide	preservative
221	Sodium sulphite	preservative
222	Sodium bisulphite	preservative

223	Sodium metabisulphite	preservative
224	Potassium metabisulphite	preservative
225	Potassium sulphite	preservative
228	Potassium bisulphite	preservative
234	Nisin	preservative
235	Natamycin or Pimaricin	preservative
242	Dimethyl dicarbonate	preservative
249	Potassium nitrite	Preservative, colour fixative
250	Sodium nitrite	Preservative, colour fixative
251	Sodium nitrate	Preservative, colour fixative
252	Potassium nitrate	Preservative, colour fixative
280	Propionic acid	Preservative
281	Sodium propionate	Preservative
282	Calcium propionate	Preservative
283	Potassium propionate	Preservative
385	Calcium disodium ethylenediaminetetraacetate or calcium disodium EDTA	Preservative, antioxidant
1105	Lysozyme	enzyme ,preservative

2.4. Public awareness of food preservation

Public awareness of food preservatives is uneven.Americans have a perception that food-borne illnesses happen more often in other countries. This may be true, but the occurrence of illnesses, hospitalizations, and deaths are still high. It is estimated by the Center for Disease Control (CDC) that each year there are 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths linked to food-borne illness.

The increasing demand for ready-to-eat fresh food products has led to challenges for food distributors regarding the safety and quality of their foods. Artificial preservatives meet some of these challenges by preserving freshness for longer periods of time, but these preservatives can cause negative side-effects as well. Sodium nitrite is a preservative used in lunch meats, hams, sausages, hot dogs, and bacon to prevent botulism. It serves the important function of controlling the bacteria that cause botulism, but sodium nitrite can react with proteins, or during cooking at high heats, to form carcinogenic N-nitrosamines.It has also been linked to cancer in lab animals.The commonly used sodium benzoate has been found to extend the shelf life of bottled tomato paste to 40 weeks without loss of quality. However, it can form the carcinogen benzene when combined with vitamin

C.Many food manufacturers have reformed their products to eliminate this combination, but a risk still exists.Consumption of sodium benzoate may also cause hyperactivity. For over 30 years, there has been a debate about whether or not preservatives and other food additives can cause hyperactivity. Studies have found that there may be increases in hyperactivity amongst children who consume artificial colorings and benzoate preservatives and who are already genetically predisposed to hyperactivity, but these studies were not entirely conclusive. Hyperactivity only increased moderately, and it was not determined if the preservatives, colorings, or a combination of the two were responsible for the increase.

2.5.Bio-based polymers

Bio-based polymers are materials which are produced from renewable resources. The terms bio-based polymers and biodegradable polymers are used extensively in the literature, but there is a key difference between the two types of polymers. Biodegradable polymers are defined as materials whose physical and chemical properties undergo deterioration and completely degrade when exposed to microorganisms, carbon dioxide (aerobic) processes, methane (anaerobic processes), and water (aerobic and anaerobic processes). Bio-based polymers can be biodegradable (e.g., polylactic acid) or nondegradable (e.g., biopolyhethylene). Similarly, while many bio-based polymers are biodegradable (e.g., starch and polyhydroxyalkanoates), not all biodegradable polymers are bio-based (e.g., polycaprolactone).

Bio-based polymers still hold a tiny fraction of the total global plastic market. Currently, biopolymers share less than 1% of the total market. At the current growth rate, it is expected that biopolymers will account for just over 1% of polymers by 2015 (Doug 2010).

The worldwide interest in bio-based polymers has accelerated in recent years due to the desire and need to find non-fossil fuel-based polymers. As indicated by ISI Web of Sciences and Thomas Innovations, there is a tremendous increase in the number of publication citations on bio-based polymers and applications in recent years(Chen and Martin 2012).

Bio-based polymers offer important contributions by reducing the dependence on fossil fuels and through the related positive environmental impacts such as reduced carbon dioxide emissions. The legislative landscape is also changing where bio-based products are being favored through initiatives such as the *Lead Market Initiative* (European Union) and *BioPreferred* (USA). As a result, there is a worldwide demand for replacing petroleum-derived raw materials with renewable resource-based raw materials for the production of polymers.

The first generation of bio-based polymers focused on deriving polymers from agricultural feedstocks such as corn, potatoes, and other carbohydrate feedstocks. However, the focus has shifted in recent years due to a desire to move away from food-based resources and significant breakthroughs in biotechnology. Bio-based polymers similar to conventional polymers are produced by bacterial fermentation processes by synthesizing the building blocks (monomers) from renewable resources, including lignocellulosic biomass (starch and cellulose), fatty acids, and organic waste. Natural bio-based polymers are the other class of bio-based polymers which are found naturally, such as proteins, nucleic acids, and polysaccharides (collagen, chitosan, etc.). These bio-based polymers have shown enormous growth in recent years in terms of technological developments and their commercial applications. There are three principal ways to produce bio-based polymers using renewable resources:

1. Using natural bio-based polymers with partial modification to meet the requirements (e.g., starch)
2. Producing bio-based monomers by fermentation/conventional chemistry followed by polymerization (e.g., polylactic acid, polybutylene succinate, and polyethylene)
3. Producing bio-based polymers directly by bacteria (e.g., polyhydroxyalkanoates).

In this paper, an overview of bio-based polymers made from renewable resources and natural polymers derived from plant and animal origins is presented. The review will focus on the preparation, properties, applications, and future trends for bio-based polymers. This paper discusses the use of renewable resources such as lignocellulosic biomass to create monomers and polymers that can replace petroleum-based polymers, such as polyester, polylactic acids, and other natural bio-based polymers, which are presented in Figure 2.1.

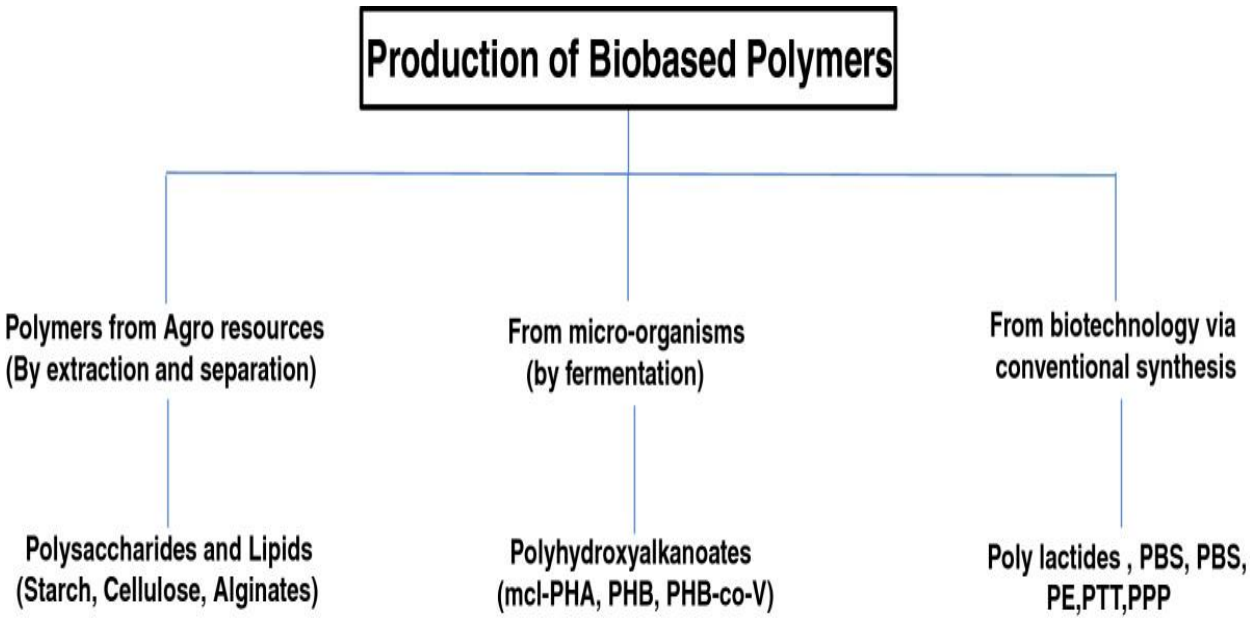


Figure 2.1: Most common categories of bio-based polymers produced by various processes. From Luc and Eric (2012).

2.5.1. Polylactic acid

Polylactic acid (PLA) has been known since 1845 but not commercialized until early 1990. PLA belongs to the family of aliphatic polyesters with the basic constitutional unit lactic acid. The monomer lactic acid is the hydroxyl carboxylic acid which can be obtained via bacterial fermentation from corn (starch) or sugars obtained from renewable resources. Although other renewable resources can be used, corn has the advantage of providing a high-quality feedstock for fermentation which results in a high-purity lactic acid, which is required for an efficient synthetic process. l-lactic acid or d-lactic acid is obtained depending on the microbial strain used during the fermentation process.

PLA can be synthesized from lactic acid by direct polycondensation reaction or ring-opening polymerization of lactide monomer. However, it is difficult to obtain high molecular weight PLA via polycondensation reaction because of water formation during the reaction. Nature Works LLC (previously Cargill Dow LLC) has developed a low-cost continuous process for the production of PLA (Erwin et al. 2007). In this process, low molecular weight pre-polymer lactide dimers are formed during a condensation process. In the second step, the pre-polymers are converted into high molecular weight PLA via ring-opening polymerization with selected catalysts.

Depending on the ratio and stereochemical nature of the monomer (l or d), various types of PLA and PLA copolymers can be obtained.

Table 2: Application of PLA and their blends in various fields

Polymer	Applications
PLGA/PGA	Ovine pulmonary valve replacement
PLA/chitosan PLA/PLGA/chitosan PLA	Drug carrier/drug release
PLGA and copolymers	Degradable sutures
PLA/HA composites	Porous scaffolds for cellular applications
PLA-CaP and PLGA-CaP	Bone fixation devices, plates, pins, screws, and wires, orthopedic applications
PDLLA	Coatings on metal implants
PLA/PLGA	Use in cell-based gene therapy for cardiovascular diseases, muscle tissues, bone and cartilage regeneration, and other treatments of cardiovascular and neurological conditions
PLA and PLA blends	Packaging films, commodity containers, electrical appliances, mobile phone housings, floor mats, automotive spare parts
PLA	Textile applications

2.5.2. Polyhydroxyalkanoates

Polyhydroxyalkanoates (PHAs) are a family of polyesters produced by bacterial fermentation with the potential to replace conventional hydrocarbon-based polymers.

PHAs occur naturally in a variety of organisms, but microorganisms can be employed to tailor their production in cells. Polyhydroxybutyrate (PHB), the simplest PHA, was discovered in 1926 by Maurice Lemoigne as a constituent of the bacterium *Bacillus megaterium* (Lemoigne 1923).

PHA can be produced by varieties of bacteria using several renewable waste feedstocks. A generic process to produce PHA by bacterial fermentation involves fermentation, isolation, and purification from fermentation broth. A large fermentation vessel is filled with mineral medium and inoculated with a seed culture that contains bacteria. The feedstocks include cellulosics, vegetable oils, organic waste, municipal solid waste, and fatty acids depending on the specific PHA required. The carbon source is fed into the vessel until it is consumed and cell growth and PHA accumulation is complete. In general, a minimum of 48 h is required for fermentation time. To isolate and purify PHA, cells are concentrated, dried, and extracted with solvents such as acetone or chloroform. The residual cell debris is removed from the solvent containing dissolved PHA by solid-liquid separation process. The PHA is then precipitated by the addition of an alcohol (e.g., methanol) and recovered by a precipitation process (Kathiraser et al. 2007).

More than 150 PHA monomers have been identified as the constituents of PHAs (Steinbüchel and Valentin 1995). Such diversity allows the production of bio-based polymers with a wide range of properties, tailored for specific applications. Poly-3-hydroxybutyrate was the first bacterial PHA identified. It has received the greatest attention in terms of pathway characterization and industrial-scale production. It possesses similar thermal and mechanical properties to those of polystyrene and polypropylene (Savenkova et al. 2000). However, due to its slow crystallization, narrow processing temperature range, and tendency to ‘creep’, it is not attractive for many applications, requiring development in order to overcome these shortcomings (Reis et al. 2008). Several companies have developed PHA copolymers with typically 80% to 95% (*R*)-3-hydroxybutyric acid monomer and 5% to 20% of a second monomer in order to improve the properties of PHAs. Some specific examples of PHAs include the following:

- Poly(3HB): Poly(3-hydroxybutyrate)
- Poly(3HB-*co*-3HV): Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate), PHBV

- Poly(3-HB-*co*-4HB): Poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate)
- Poly(3HB-*co*-3HH): Poly(3-hydroxyoctanoate-*co*-hydroxyhexanoate)
- Poly(3HO-*co*-3HH): Poly(3-hydroxyoctanoate-*co*-hydroxyhexanoate)
- Poly (4-HB): Poly(4-hydroxybutyrate).

The copolymer poly(3HB-*co*-3HV) has a much lower crystallinity, decreased stiffness and brittleness, and increased tensile strength and toughness compared to poly(3HB) while remaining biodegradable. It also has a higher melt viscosity, which is a desirable property for extrusion and blow molding (Hanggi 1995).

The first commercial plant for PHBV was built in the USA in a joint venture between Metabolix and Archer Daniels Midland. However, the joint venture between these two companies ended in 2012. Currently, Tianan Biologic Material Co. in China is the largest producer of PHB and PHB copolymers. Tianan's PHBV contains about 5% valerate which improves the flexibility of the polymer. Tainjin Green Biosciences, China, invested along with DSM to build a production plant with 10-kton/year capacity to produce PHAs for packing and biomedical applications (DSM press release 2008).

Table 3: Application of PHAs and their blends in various fields

PHA polymer type	Applications
P(3HB), P(3HB-*co*-3HHX) and blends	Scaffolds, nerve regeneration, soft tissue, artificial esophagus, drug delivery, skin regeneration, food additive
mcl-PHA/scl-PHA	Cardiac tissue engineering, drug delivery, cosmetics, drug molecules
P(4HB) and P(3HO)	Heart valve scaffolds, food additive
P(3HB-*co*-4HB), P(3HB-*co*-3HV)	Drug delivery, scaffolds, artificial heart values, patches to repair gastrointestinal tracts, sutures
PHB, Mirel P103	Commodity applications, shampoo and cosmetic bottles, cups and food containers

2.5.3. Polybutylene succinate

Polybutylene succinate (PBS) is an aliphatic polyester with similar properties to those of PET. PBS is produced by condensation of succinic acid and 1,4-butanediol. PBS can be produced by either monomers derived from petroleum-based systems or the bacterial fermentation route. There are several processes for producing succinic acid

from fossil fuels. Among them, electrochemical synthesis is a common process with high yield and low cost. However, the fermentation production of succinic acid has numerous advantages compared to the chemical process. Fermentation process uses renewable resources and consumes less energy compared to chemical process. Several companies (solely or in partnership) are now scaling bio-succinate production processes which have traditionally suffered from poor productivity and high downstream processing costs. Mitsubishi Chemical (Japan) has developed biomass-derived succinic acid in collaboration with Ajinomoto to commercialize bio-based PBS. DSM and Roquette are developing a commercially feasible fermentation process for the production of succinic acid 1,4-butanediol and subsequent production of PBS. Myriant and Bioamber have developed a fermentation technology to produce monomers.

Table 4: Applications of PBS and their blends

Polymer type	Applications
PBS/PLA blend	Packaging films, dishware, fibers, medical materials
PBS and blends	Drug encapsulation systems
PBS/starch	Barrier films
PBS and copolymers	Industrial applications
PBS ionomers	Orthopedic applications

2.5.4. Bio-polyethylene

Polyethylene (PE) is an important engineering polymer traditionally produced from fossil resources. PE is produced by polymerization of ethylene under pressure, temperature, in the presence of a catalyst. Traditionally, ethylene is produced through steam cracking of naphtha or heavy oils or ethanol dehydration. With increases in oil prices, microbial PE or green PE is now being manufactured from dehydration of ethanol produced by microbial fermentation. The concept of producing PE from bioethanol is not a particularly new one. In the 1980s, Braskem made bio-PE and bio-PVC from bioethanol. However, low oil prices and the limitations of the biotechnology processes made the technology unattractive at that time (de Guzman 2010).

Currently, bio-PE produced on an industrial scale from bioethanol is derived from sugarcane. Bioethanol is also derived from biorenewablefeedstocks, including sugar beet, starch crops such as maize, wood, wheat, corn, and other plant wastes through microbial strain and biological fermentation process. In a typical process, extracted sugarcane juice with high sucrose content is anaerobically fermented to produce ethanol. At the end of the fermentation process, ethanol is distilled in order to remove water and to yield azeotropic mixture of hydrous ethanol. Ethanol is then dehydrated at high temperatures over a solid catalyst to produce ethylene and, subsequently, polyethylene (Guangwen et al. 2007; Luiz et al. 2010).

Bio-based polyethylene has exactly the same chemical, physical, and mechanical properties as petrochemical polyethylene. Braskem (Brazil) is the largest producer of bio-PE with 52% market share, and this is the first certified bio-PE in the world. Similarly, Braskem is developing other bio-based polymers such as bio-polyvinyl chloride, bio-polypropylene, and their copolymers with similar industrial technologies. The current Braskem bio-based PE grades are mainly targeted towards food packing, cosmetics, personal care, automotive parts, and toys. Dow Chemical (USA) in cooperation with Crystalsev is the second largest producer of bio-PE with 12% market share. Solvay (Belgium), another producer of bio-PE, has 10% share in the current market. However, Solvay is a leader in the production of bio-PVC with similar industrial technologies. China Petrochemical Corporation also plans to set up production facilities in China to produce bio-PE from bioethanol (Haung et al. 2008).

Bio-PE can replace all the applications of current fossil-based PE. It is widely used in engineering, agriculture, packaging, and many day-to-day commodity applications because of its low price and good performance. Table 5 shows applications of bio-PE in different fields where it can replace conventional PE.

Table 5: Application of bio-PE polymer and their blends

Polymer type	Applications
Bio-PE	Plastics bags, milk and water bottles, food packaging films, toys
Bio-PE and blends	Agricultural mulch films

2.6. Bio-based natural polymers

This group consists of naturally occurring polymers such as cellulose, starch, chitin, and various polysaccharides and proteins. These materials and their derivatives offer a wide range of properties and applications. In this section, some of the natural bio-based polymers and their applications in various fields are discussed.

2.6.1. Starch

Starch is a unique bio-based polymer because it occurs in nature as discrete granules. Starch is the end product of photosynthesis in plants - a natural carbohydrate-based polymer that is abundantly available in nature from various sources including wheat, rice, corn, and potato. Essentially, starch consists of the linear polysaccharide amylose and the highly branched polysaccharide amylopectin. In particular, thermoplastic starch is of growing interest within the industry. The thermal and mechanical properties of starch can vary greatly and depend upon such factors as the amount of plasticizer present. The T_g varies between −50°C and 110°C, and the modulus is similar to polyolefins (Jane 1995). Several challenges exist in producing commercially viable starch plastics. Starch's molecular structure is complex and partly nonlinear, leading to issues with ductility. Starch and starch thermoplastics suffer from the phenomenon of retrogradation - a natural increase in crystallinity over time, leading to increased brittleness. Plasticizers need to be found to create starch plastics with mechanical properties comparable to polyolefin-derived packaging. Plasticized starch blends and composites and/or chemical modifications may overcome these issues, creating biodegradable polymers with sufficient mechanical strength, flexibility, and water barrier properties for commercial packaging and consumer products (Maurizio et al. 2005).

Novamont is one of the leading companies in processing starch-based products (Li et al. 2009). The company produces various types of starch-based products using proprietary blend formulations.

Table 6: Application of starch and their blends in various fields

Polymer type	Applications
Starch	Orthopedic implant devices as bone fillers
Starch/ethylene vinyl alcohol/HA	Bone replacement/fixation implants,

starch/polycaprolactone blends	orthopedic applications
Starch/cellulose acetate blends with methylmethacrylate and acrylic acid	Bone cements
Modified starch	Food applications
Starch derivatives	Drug delivery
Thermoplastic starch	Packaging, containers, mulch films, textile sizing agents, adhesives

2.6.2. Cellulose

Cellulose is the predominant constituent in cell walls of all plants. Cellulose is a complex polysaccharide with crystalline morphology. Cellulose differs from starch where glucose units are linked by β-1,4-glycosidic bonds, whereas the bonds in starch are predominantly α-1,4 linkages. The most important raw material sources for the production of cellulosic plastics are cotton fibers and wood. Plant fiber is dissolved in alkali and carbon disulfide to create viscose, which is then reconverted to cellulose in cellophane form following a sulfuric acid and sodium sulfate bath. There are currently two processes used to separate cellulose from the other wood constituents (Yan et al. 2009). These methods, sulfite and pre-hydrolysis kraft pulping, use high pressure and chemicals to separate cellulose from lignin and hemicellulose, attaining greater than 97% cellulose purity. The main derivatives of cellulose for industrial purposes are cellulose acetate, cellulose esters (molding, extrusion, and films), and regenerated cellulose for fibers.

Cellulose is a hard polymer and has a high tensile strength of 62 to 500 MPa and elongation of 4% (Bisanda and Ansell 1992; Eichhorn et al. 2001). In order toovercome the inherent processing problems of cellulose, it is necessary to modify, plasticize, and blend with other polymers. The mechanical and thermal properties vary from blend to blend depending on the composition. The T_g of cellulosic derivatives ranged between 53°C and 180°C (Picker and Hoag 2002).

Eastman Chemical is a major producer of cellulosic polymers. FKuR launched a biopolymer business in the year 2000 and has a capacity of 2,800 metric ton/year of various cellulosic compounds for different applications (Doug 2010).

Table 7: Application of cellulose and their compounds in various fields

Polymer type	Applications

Cellulose esters	Membranes for separation
Carboxylated methyl cellulose	Drug formulations, as binder for drugs, film-coating agent for drugs, ointment base
Cellulose acetate fibers	Wound dressings
Hydroxyethyl cellulose	Spray for clothes polluted with pollen
Modified celluloses, cellulose whiskers, microfibrous cellulose	Barrier films, water preservation in food packing
Cellulose nanofibers	Textile applications
Cellulose particles	Chromatographic applications, chiral separations

2.6.3. Chitin and Chitosan

Chitin and chitosan are the most abundant natural amino polysaccharide and valuable bio-based natural polymers derived from shells of prawns and crabs. Currently, chitin and chitosan are produced commercially by chemical extraction process from crab, shrimp, and prawn wastes (Roberts 1997). The chemical extraction of chitin is quite an aggressive process based on demineralization by acid and deproteination by the action of alkali followed by deacetylated into chitosan (Roberts 1997). Chitin can also be produced by using enzyme hydrolysis or fermentation process, but these processes are not economically feasible on an industrial scale (Win and Stevens 2001). Currently, there are few industrial-scale plants of chitin and chitosan worldwide located in the USA, Canada, Scandinavia, and Asia (Ravi Kumar 2000).

Chitosan displays interesting characteristics including biodegradability, biocompatibility, chemical inertness, high mechanical strength, good film-forming properties, and low cost (Marguerite 2006; Virginia et al. 2011; Liu et al. 2012). Chitosan is being used in a vast array of widely varying products and applications ranging from pharmaceutical and cosmetic products to water treatment and plant protection. For each application, different properties of chitosan are required, which changes with the degree of acetylation and molecular weight. Chitosan is compatible with many biologically active components incorporated in cosmetic product composition (Ravi Kumar 2000). Due to its low toxicity, biocompatibility, and bioactivity, chitosan has become a very attractive material in such diverse applications as biomaterials in medical devices and as a pharmaceutical ingredient (Bae and Moo-Moo 2010; Ramya et al. 2012). Chitosan has application in shampoos, rinses, and permanent hair-coloring agents. Chitosan and its derivatives also have applications in

the skin care industry. Chitosan can function as a moisturizer for the skin, and because of its lower costs, it might compete with hyaluronic acid in this application (Bansal et al. 2011; Valerie and Vinod 1998; Hafdani and Sadeghinia 2011).

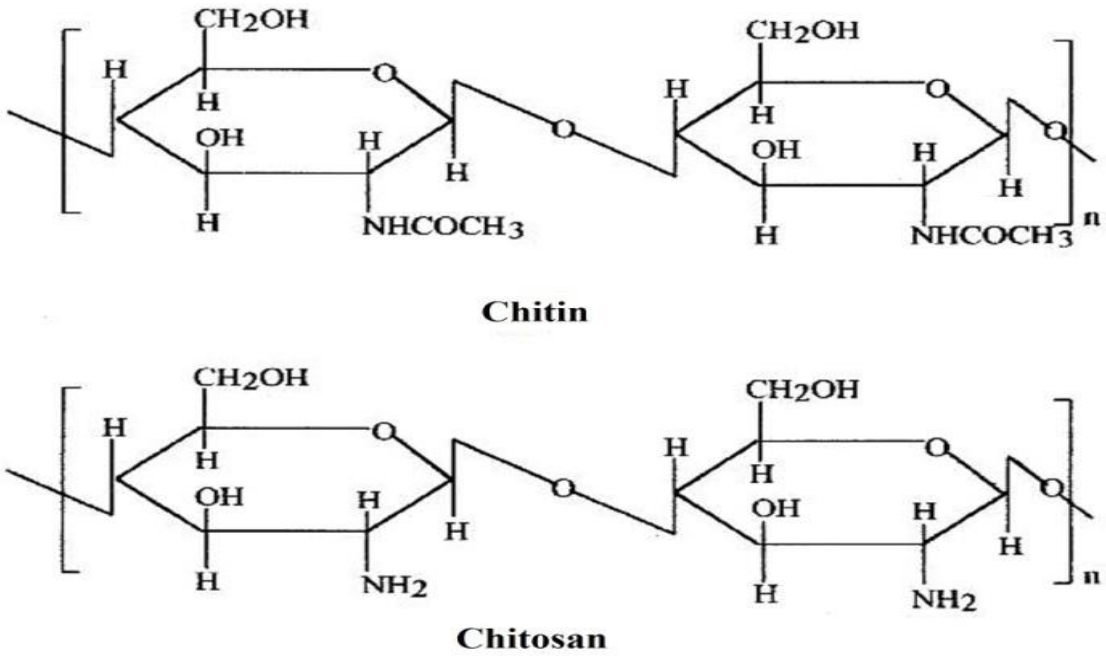


Figure 2.2: Structure of chitin and chitosan

2.6.4. Pullulan

Pullulan is a linear water-soluble polysaccharide mainly consisting of maltotriose units connected by α-1,6glycosidic units. Pullulan was first reported by Bauer (1938) and is obtained from the fermentation broth of *Aureobasidiumpullulans*. Pullulan is produced by a simple fermentation process using a number of feedstocks containing simple sugars (Bernier 1958; Catley 1971; Sena et al. 2006). Pullulan can be chemically modified to produce a polymer that is either less soluble or completely insoluble in water. The unique properties of this polysaccharide are due to its characteristic glycosidic linking. Pullulan is easily chemically modified to reduce the water solubility or to develop pH sensitivity, by introducing functional reactive groups, etc. Due to its high water solubility and low viscosity, pullulan has numerous commercial applications including use as a food additive, a flocculant, a blood plasma substitute, an adhesive, and a film (Zajic and LeDuy 1973; Singh et al. 2008; Cheng et al. 2011). Pullulan can be formed into molding articles which can resemble

conventional polymers such as polystyrene in their transparency, strength, and toughness (Leathers 2003).

Pullulan is extensively used in the food industry. It is a slow-digesting macromolecule which is tasteless as well as odorless, hence its application as a low-calorie food additive providing bulk and texture. Pullulan possesses oxygen barrier property and good moisture retention, and also, it inhibits fungal growth. These properties make it an excellent material for food preservation, and it is used extensively in the food industry (Conca and Yang 1993). In recent years, pullulan has also been studied for biomedical applications in various aspects, including targeted drug and gene delivery, tissue engineering, wound healing, and even in diagnostic imaging medium (Rekha and Chrndra 2007). Other emerging markets for pullulan include oral care products (Barkalow et al. 2002) and formulations of capsules for dietary supplements and pharmaceuticals (Leathers 2003), leading to increased demand for this unique biopolymer.

2.6.5. Collagen and Gelatin

Collagen is the major insoluble fibrous protein in the extracellular matrix and in connective tissue. In fact, it is the single most abundant protein in the animal kingdom. There are at least 27 types of collagens, and the structures all serve the same purpose: to help tissues withstand stretching. The most abundant sources of collagen are pig skin, bovine hide, and pork and cattle bones. However, the industrial use of collagen is obtained from nonmammalian species (Gomez-Guille et al. 2011). Gelatin is obtained through the hydrolysis of collagen. The degree of conversion of collagen into gelatin depends on the pretreatment, function of temperature, pH, and extraction time (Johnston-Banks 1990).

Collagen is one of the most useful biomaterials due to its biocompatibility, biodegradability, and weak antigenicity (Maeda et al. 1999). The main application of collagen films in ophthalmology is as drug delivery systems for slow release of incorporated drugs (Rubin et al. 1973). It was also used for tissue engineering including skin replacement, bone substitutes, and artificial blood vessels and valves (Lee et al. 2001).

The classical food, photographic, cosmetic, and pharmaceutical applications of gelatin is based mainly on its gel-forming properties. Recently in the food industry, an increasing number of new applications have been found for gelatin in products in line with the growing trend to replace synthetic agents with more natural ones (Gomez-Guille et al. 2011). These include emulsifiers, foaming agents, colloid stabilizers, biodegradable film-forming materials, and microencapsulating agents.

2.6.6. Alginates

Alginate is a linear polysaccharide that is abundant in nature as it is synthesized by brown seaweeds and by soil bacteria (Draget et al. 1997). Sodium alginate is the most commonly used alginate form in the industry since it is the first by-product of algal purification (Draget 2000). Sodium alginate consists of α-*l*-guluronic acid residues (G blocks) and β-*d*-mannuronic acid residues (M blocks), as well as segments of alternating guluronic and mannuronic acids.

Although alginates are a heterogeneous family of polymers with varying content of G and M blocks depending on the source of extraction, alginates with high G content have far more industrial importance (Siddhesh and Edgar 2012). The acid or alkali treatment processes used to make sodium alginate from brown seaweeds are relatively simple. The difficulties in processing arise mainly from the separation of sodium alginate from slimy residues (Black and Woodward 1954). It is estimated that the annual production of alginates is approximately 38,000 tons worldwide (Helgerud et al. 2009).

Alginates have various industrial uses as viscosifiers, stabilizers, and gel-forming, film-forming, or water-binding agents (Helga and Svein 1998). These applications range from textile printing and manufacturing of ceramics to production of welding rods and water treatment (Teli and Chiplunkar 1986; Qin et al. 2007; Xie et al. 2001). The polymer is soluble in cold water and forms thermostable gels. These properties are utilized in the food industry in products such as custard creams and restructured food. The polymer is also used as a stabilizer and thickener in a variety of beverages, ice creams, emulsions, and sauces (Iain et al. 2009).

Alginates are widely used as a gelling agent in pharmaceutical and food applications. Studies into their positive effects on human health have broadened recently with the

recognition that they have a number of potentially beneficial physiological effects in the gastrointestinal tract (Peter et al. 2011; Mandel et al. 2000). Alginate-containing wound dressings are commonly used, especially in making hydrophilic gels over wounds which can produce comfortable, localized hydrophilic environments in healing wounds (Onsoyen 1996). Alginates are used in controlled drug delivery, where the rate of drug release depends on the type and molecular weight of alginates used (Alexnader et al. 2006; Goh et al. 2012). Additionally, dental impressions made with alginates are easy to handle for both dentist and patient as they fast set at room temperature and are cost-effective (Onsoyen 1996). Recent studies show that alginates can be effective in treating obesity, and currently, various functional alginates are being evaluated in human clinical trials (Georg et al. 2012).

2.7. Current status and future trends

The use of bio-based feedstocks in the chemical sector is not a novel concept. They have been industrially feasible on a large scale for more than a decade. However, the price of oil was so cost-effective, and the development of oil-based products created so many opportunities that bio-based products were not prioritized at the time. Several factors, such as the limitations and uncertainty in supplies of fossil fuels, environmental considerations, and technological developments, accelerated the advancement of bio-based polymers and products. It took more than a century to evolve the fossil fuel-based chemical industry; however, the bio-based polymer industry is already catching up with fossil fuel-based chemical industry, which has augmented in the last 20 years. Thanks to advancements in white biotechnology, the production of bio-based polymers and other chemicals from renewable resources has become a reality. The first-generation technologies mainly focused on food resources such as corn, starch, rice, etc. to produce bio-based polymers. As the food-versus-fuel debate ascended, the focus of technologies diverted to cellulose-based feedstocks, focusing on waste from wood and paper, food industries, and even stems and leaves and solid municipal waste streams. More and more of these technologies are already in the pipeline to align with the abovementioned waste streams; however, it may take another 20 years to develop the full spectrum of chemicals based on these technologies (Michael et al. 2011).

Challenges that need to be addressed in the coming years include management of raw materials, performance of bio-based materials, and their cost for production. Economy of scale will be one of the main challenges for production of bio-based monomers and bio-based polymers from renewable sources. Building large-scale plants can be difficult due to the lack of experience in new technologies and estimation of supply/demand balance. In order to make these technologies economically viable, it is very important to develop (1) logistics for biomass feedstocks, (2) new manufacturing routes by replacing existing methods with high yields, (3) new microbial strains/enzymes, and (4) efficient downstream processing methods for recovery of bio-based products.

The current bio-based industry focus is mainly on making bio-versions of existing monomers and polymers. Performance of these products is well known, and it is relatively easy to replace the existing product with similar performance of bio-versions. All the polymers mentioned above often display similar properties of current fossil-based polymers. Recently, many efforts are seen towards introducing new bio-based polymers with higher performance and value. For example, Nature Works LLC has introduced new grades of PLA with higher thermal and mechanical properties. New PLA-tri block copolymers have been reported to behave like thermoplastic elastomer. Many developments are currently underway to develop various polyamides, polyesters, polyhydroxyaloknates, etc. with a high differentiation in their final properties for use in automotive, electronics, and biomedical applications.

The disadvantage of some of the new bio-based polymers is that they cannot be processed in all current processing equipment. There is vast knowledge on additive-based chemistry developed for improving the performance and processing of fossil fuel-based polymers, and this knowledge can be used to develop new additive chemistry to improve the performance and properties of bio-based polymers (Ray and Bousmina 2005). For bio-based polymers like PLA and PHA, additives are being developed to improve their performance, by blending with other polymers or making new copolymers. However, the additive market for bio-based polymers is still very small, which makes it difficult to justify major development efforts according to some key additive supplier companies.

The use of nanoparticles as additives to enhance polymer performance has long been established for petroleum-based polymers. Various nano-reinforcements currently

being developed include carbon nanotubes, graphene, nanoclays, 2-D layered materials, and cellulose nanowhiskers. Combining these nanofillers with bio-based polymers could enhance a large number of physical properties, including barrier, flame resistance, thermal stability, solvent uptake, and rate of biodegradability, relative to unmodified polymer resin. These improvements are generally attained at low filler content, and this nano-reinforcement is a very attractive route to generate new functional biomaterials for various applications.

Even though new bio-based polymers are produced on an industrial scale, there are still several factors which need to be determined for the long-term viability of bio-based polymers. It is expected that there will be feedstock competition as global demand for food and energy increases over time. Currently, renewable feedstocks used for manufacturing bio-based monomers and polymers often compete with requirements for food-based products. The expansion of first-generation bio-based fuel production will place unsustainable demands on biomass resources and is as much a threat to the sustainability of biochemical and biopolymer production as it is to food production (Michael et al. 2011). Indeed the European commission has altered its targets downwards for first-generation biofuels since October 2012, indicating its preference for non-food sources of sugar for biofuel production (EurActiv.com 2012). Several initiatives are underway to use cellulose-based feedstocks for the production of usable sugars for biofuels, biochemicals, and biopolymers (Jong et al. 2010).

2.8. Postharvest changes in fruits and vegetables

2.8.1. Physiological changes

When a fresh horticultural produce is harvested, the processes oflife continue in a modified form. The crop can no longer replace foodmaterials or water, so it must draw on its stored reserves. When they areused up, the fruit or vegetable undergoes an ageing process leading tobreakdown and decay. It will eventually become unacceptable as foodbecause of this natural rot. The principal normal physiological processesleading to ageing are respiration and transpiration (FAO, 1989).Respiration uses stored starch or sugar as long as they last.Oxygen from the air breaks down carbohydrates into carbon dioxide andwater. This reaction produces energy in the form of heat. When the airsupply is restricted and the amount of available oxygen in

the environmentfalls to about 2 percent or less, fermentation instead of respiration occurs. Fermentation breaks down sugars to alcohol and carbon dioxide, and thealcohol produced causes unpleasant flavors in produce and promotespremature ageing. Poor ventilation of produce leads also to theaccumulation of carbon dioxide around the produce. When theconcentration of this gas rises between one and five percent in theatmosphere, it will quickly spoil the produce by causing bad flavors,internal breakdown, failure of fruit to ripen and other abnormal physiological conditions. Therefore, proper ventilation of produce isessential. Ripening occurs when the fruit is said to be mature. It isfollowed by ageing (often called senescence) and breakdown of the fruit.There are fruits which show a rapid rise and fall in respiration rateduring ripening and are said to be climacteric, for example tomato andmango. The non-climacteric fruits like pineapple, lime and grape do notshow such sharp rise and fall in respiration rate. Ethylene gas is producedin most plant tissues and is an important factor in initiating the ripeningprocess (Irtwange, 2006).Most fresh fruits and vegetables contain from 65 to 95 percentwater when harvested. Fresh produce continues to lose water afterharvest and it cannot be replaced. This causes shrinkage and loss ofweight. This high humidity level prevents moisture loss that may occur due to increased respiration and lowered transpiration. When the harvestedproduce loses 5 or 10 percent of its fresh weight, it begins to wilt and soonbecomes unusable. The rate at which water is lost from plant depends onthe difference between the water vapor pressure of the plant and thepressure of water vapor in the air. To keep water loss from fresh produceas low as possible, it must be kept in a moist atmosphere. Air flow helps toremove heat of respiration but must be controlled to prevent moisture loss(Elazar, 2004). Altering the relative humidity (RH) of the storageenvironment may also delay senescence. Perishable fruit and vegetableproducts should be maintained at RH levels of 90-95%.

2.8.2 Compositional Changes

Many changes in the composition of the fruits and vegetables may occur duringdevelopment, maturation and ripening on the plant. Some may continueafter, or start only at harvest. These changes being either desirable orundesirable, can take place as loss of chlorophyll and development ofother colored pigments like

carotenoids (yellow orange and red colors),anthocyanins and other phenolic compounds. Starch is converted tosugars which increase in fruits and vegetablesduring ripening. In most commoditiesstarch is used as a substrate for respiration. Acidity decreases withripening and senescence. Acids and sugars are important for development of fruit flavor. During ripening, softening occurs and polysaccharides suchas pectins, cellulose and hemicellulose are degraded by enzymes. Thereare changes in proteins, amino acids and lipids which may affect the flavorof the commodity. Development of flavor and aroma volatiles is veryimportant for acceptable eating quality. Loss of vitamins, especiallyascorbic acid (vitamin C), takes place during storage and thus adverselyaffects nutritional quality (Irtwange, 2006).

2.9. Post harvest treatments

Fruits and vegetables are living and continue to respireafter separation from the parent plant. Their post-harvest life depends onthe rate at which they use up their stored food reserves and their rate ofwater loss. When food and water reserves are exhausted, the producedies and decays. Holding them at their lowest safe temperature (0^0 or 32°F for temperate crops or 10-12^0 or 50-54°F for chilling sensitive crops)and relative humidity (60-90%) will enhance storage life by loweringrespiration rate, decreasing sensitivity to ethylene and reducing waterloss. Water loss results in shriveling and wilting, causing severe postharvest losses (Krochta and Mulder-Johnston, 1997).Chilling injury should be avoided, as they may fail to ripen (bananasand tomatoes), develop pits or sunken areas, show brown discoloration,increase susceptibility to decay, and develop offflavors(tomatoes). In general proper storage practices includetemperature control, relative humidity control, air circulation andmaintenance of space between containers for adequate ventilation, andavoiding incompatible product mixes (Shewfelt, 1990).

Sanitation is another essential factor, both to control pathogencontamination and food spoilage. Chlorine treatments (100 to 150 ppm Cl)can be used in wash water to help control pathogen buildup duringpacking operations. A rule of thumb is to use 1 to 2 ml of chlorine bleachper liter (1 to 2 ounces of chlorine bleach per 8 gallons of clean water).Walls, floors and packing equipment can also be cleaned usingquarternary

ammonium compounds labeled as safe for food processingequipment (Kupferman, 1990).

2.10. Do Colors in Fruits & Vegetables Play an Important Role?

The U.S. Department of Agriculture recommends eating at least five to nine servings per day of fruits and vegetables. However, the amount of vegetables you eat isn't the only thing you need to take into consideration. You also need to attempt to consume fruits and vegetables in a variety of different colors. This is because the different colors of fruits and vegetables indicate the different nutrients they contain. Consuming a rainbow of fruits and vegetables helps insure you get enough of the different nutrients you need for good health.

2.10.1. Red, Blue and Purple

Red, blue and purple fruits and vegetables usually contain anthocyanins, and red fruits and vegetables often contain lycopene as well. Anthocyanins have antioxidant properties that help limit damage caused to your cells by free radicals and may also lower your risk for heart disease, stroke, cancer, macular degeneration and memory problems. Lycopene may help lower your risk for cancer and heart disease. These brightly colored fruits and vegetables often also contain essential vitamins and minerals such as potassium, vitamin A, vitamin C and folate. Compounds in these fruits and vegetables also help keep your vision and immune system healthy and limit your risk for urinary tract infections.

2.10.2. White

White fruits and vegetables get their color from polyphenol compounds with antioxidant properties called anthoxanthins, which may help lower your risk for heart disease and cancer. Some white foods, like garlic, contain allicin, which may help lower your risk for high blood pressure, high cholesterol, cancer and heart disease. These foods may also be good sources of potassium, vitamin C, folate, niacin and riboflavin. A study published in November 2011 in "Stroke" found that consuming more white fruits and vegetables may lower your risk for strokes.

2.10.3. Orange and Yellow

The compounds that give orange and yellow fruits and vegetables their color are called carotenoids. Carotenoids may help improve your immune function and lower your risk for heart disease, vision problems and cancer. Beta-carotene is a carotenoid that your body uses to create vitamin A. Folate, potassium, bromium and vitamin C are also often found in orange and yellow fruits and vegetables.

2.10.4. Green

Chlorophyll gives green fruits and vegetables their color. Some of these fruits and vegetables also contain indoles, which may lower your risk for cancer, and lutein, which helps prevent problems with your vision. Other common nutrients in many of these fruits and vegetables include vitamin A, vitamin C, vitamin K and folate.

2.11. Classification of Salinity

Salt-affected soils are represented differently and appear on different taxonomical levels in different soil classification system (Abrol*et al*., 1998) which uptake by the plants. On the basis of electrical conductivity, the classifications are shown in the Table 8.

Table 8. Salinity classification

Salinity Class	***EC (dS/m)***
Non saline (S0)	<2
Slightly saline (S1)	2-4
Moderately saline (S2)	4-8
Saline (S3)	8-16
Highly saline (S4)	>16

2.12. pH Levels

The pH is a measure of the acidity or basicity. pH leads to changes in anion and cation exchange capacity of fruits and vegetables to a small extent (T.S. Umesha*et al*. 2012).

pH of fruits and vegetables can also be defined by acid rain and growing industrialization in this region. Acid rain results in changes in physico-chemical characteristics of fruits and vegetables due to cation exchange (P. Sharma *et al*. 2011).

Table 9. Classification of pH levels of the sample

pH range	***Type***
Less than 4.0	Strongly acidic
4.1 to 5.0	Acidic
5.1 to 6.8	Moderately acidic
6.9 to 7.0	Neutral
7.2 to 7.9	Moderate basic
8.0 to 8.9	Basic
More than 9.0	Strongly basic

2.13. Viable Counts

It is often necessary to determine how many live bacteria are actually in a sample, especially when measuringgrowth rates or determining disinfectant effectiveness. This involves the serial dilution of bacteria samplesand plating them on suitable growth media. We can also filter our samples through a membrane which weplace on a pad soaked with growth media. The plates are incubated until we see visible colonies, usually 18-24 hours. The colonies we see growing on the plate are considered to have started from one viable bacterialunit. Because bacteria are usually not found as individuals, the colony we see may have started from a singlecell or a group of cells. The results are reported as colony forming units (CFU's).There are several methods commonly used for plate counting bacteria: pour plate, overlay plate, and surfacecount. For the pour method the bacterial sample is suspended in molten agar that is just barelywarm enough to keep the agar from setting up. It is then poured into an empty Petri dish or poured in a thinlayer on another agar surface. The advantages of these methods are that the colonies stay small and compact.We can count plates with a lot higher concentration because the colonies will not be touching one another.The main disadvantage is the difficulty in keeping the agar hot enough to keep it from setting up until we pourit and cool enough to not heat shock or kill bacteria.

2.14. Tomato composition and post harvest treatment

Tomato (Lycopersicumesculentum) is a warm-season crop. In2005, Mexico, United States and Turkey were the world's leadingproducers of tomatoes according to the FAOSTAT database. Worldtomato production was 125 Mt in 2005, of which 83 Mt came from developing countries. India produced about 7.6 Mt in 2005(http://faostat.fao.org).

Tomatoes were ranked highest in a comparison of crops and theircontribution of nutrients to the diet (Wills 1981). Tomatoes also providepotassium, iron, phosphorus, and some B vitamins, and are a good sourceof dietary fiber. Ripe tomatoes are red in color because they containlycopene, an antioxidant. Lycopene is a pigment synthesized by plantsand microorganisms. It has twice the ability as that of beta-carotene and10 times that of alpha-tocopherol to quench singlet oxygen, which is ametastable state of molecular oxygen (O_2) responsible for oxidativereactions (Rao and Agarwal 1999).

Water comprises 90% of the fresh weight of tomato fruit; and thesize of the fruit is influenced by the availability of water to the plant. Thelarge amount of water also makes the fruit perishable. As the tomato fruitdevelops, starch decreases while carbohydrates such as sucrose andreducing sugars increase (Jones 1999). Sugars are mostly found in ripefruit; and starches are found mostly in unripe fruit (Wills 1981). In a ripetomato, solids form about 5-7% of the fruit. About half of the solidscomprise sugars and one eighth is acids. The main sugar in tomatoes isglucose. Citric acid is the main acid in tomato juice; and the pH of fruit is normally between 4.0 and 4.5. The pH of the fruit increases throughoutdevelopment.

Vegetables or fruits with natural coatings of wax have lowerrespiration rates than fruits without such protective barriers. Transpirationis the movement of water through the cellular tissue of a plant, andeventual evaporation of this water from plant surfaces. This movement ofwater is driven by the gradient existing between the tissue of the plant andthe humidity of the surrounding air (Ben-Yehoshua, 1987). Transpirationserves two purposes: first transpiration contributes to the lowering of thesurface temperature of the plant's tissues by evaporation. The secondfunction of transpiration relevant to post harvest is the maintenance ofturgidity of the plant's

tissues and fruits. As much as 90% of the watermoving into a plant can be lost through transpiration. Plants havetherefore developed specialized tissue structures for preventing moistureloss. When a fruit is removed from the plant, the replenishing watersource, the soil, is cut off and turgor is altered. The speed at whichdamage from loss of turgidity occurs depends on the characteristics of thecommodity, including its rate of respiration, size, and state of maturity.

Respiration produces water and heat, both of which directly affecttranspiration. The metabolic water produced through respiration remains within the fruits' tissue; however, the carbon dioxide lost to the air throughopen stomata can result in weight loss of harvested fruits. Heat generatedduring increased respiration after harvest may also contribute to weightloss of a fruit. The heat lost to the environment contributes to increasedevaporation of water.

Water losses from transpiration may also be affected by the stageof fruit maturity. In general, climacteric fruits have increased transpirationat very early (pre-climacteric) stages. Increased transpiration also occursat the beginning of the climacteric phase.

Fruits and vegetables have colored pigments. The green coloredchlorophyll pigments are contained within the chloroplast. This pigmentmay also be lost through photo degradation, which occurs whenchlorophyll molecules are bleached by light and oxygen. This processoccurs during ripening and senescence. Carotenoids are pigments withcolors ranging from yellow to orange red. The important pigments intomatoes are the lycopene and the beta carotene (Jones 1999).

Texture is imparted by components of plant tissue and its cell walls.The cellular walls are made up of cellulose fibers which are held togetherby cement like substance called pectin. These cells take up water, whichgenerates a hydrostatic pressure, giving rise to the crisp texture of vegetable and fruit products. After harvest several factors affect thetexture of fruit and vegetable products. First, turgor pressure, and hencecrisp texture is altered. Turgor pressure change results from decreasedtranspiration and respiration. Because additional water can not move intothe plant cells, and water still is being continually lost from the plant'ssurface, wilting occurs. Softening of fruits and vegetables is brought aboutby enzymatic dehydration of the pectin holding adjacent cells together. Asthe fruit begins to senesce and proceed to an overripe stage,

the pectin isbeing changed into pectic acid by the enzyme pectinase. Pecticacidimparts the characteristic mushy texture to overripe fruit (Whitaker, 1996).

Tomato is a short duration crop and it gives high yield; it isimportant from economic point of view and hence area under its cultivationis increasing day by day. It is not uncommon to hear that farmers indeveloping countries like India dump cartloads of tomato on the streets.

Excess production results in a crash in tomato prices, with prices slumpingto 50 paise a kilo (one Canadian cent is equivalent to 42 paise), farmersare left with no choice. Ever since the Agreement on Agriculture of theWorld Trade Organization began to be debated in the country, increasingagricultural productivity and improving food quality are being consideredas the only solution for farmers. There is good scope for increased utilization of tomatoes but this can be obtained only by adopting suitablestorage techniques to avoid losses. Simple, low cost technologies can be more appropriate for smallvolume, limited resource commercial operations, for farmers involved indirect marketing, for home gardeners, as well as for handlers indeveloping countries. Local conditions for small-scale handlers mayinclude labor surpluses, lack of credit for investments in post harvesttechnology, unreliable electric power supply, lack of transport, storagefacilities and/or packaging materials, as well as a host of other constraints.

Bruising, moisture loss, chilling injury, compositional changes, overripening, softening and decay are caused by harvesting at impropermaturity, rough handling, inadequate cooling and temperaturemaintenance and lack of sorting. Utilizing improved post harvest practicesoften results in reduced food losses, improved overall quality and foodsafety, and higher profits for growers and marketers (Talukder et al., 2003;Kitinoja and Kader, 1995)

Depending on the market and production area, tomatoes areharvested at stages of maturity ranging from mature-green stage throughfull-ripe. There are six stages of tomato fruit development during ripening(for red fruited cultivars): mature green, breaker, turning, pink, light-red, and red. Fruit change from green to red, due to the conversion ofchloroplasts, which contain chlorophyll to chromoplasts, which have red oryellow carotenoids. Greenhouse-grown tomatoes are generally harvestedat various stages of maturity after mature green stage. Losses can occurby improper packing

through dropping, compression, vibration andpuncture (Walker1992). The fruits are sorted, graded and packed in liddedcartons or plastic crates that can be stacked in a cold room for precooling.

Tomatoes are routinely palletized and cooled to 20 °C (68 °F) for ripeningor to 12 °C (53.6 °F) for storage. Optimal storage temperatures depend onthe maturity stage of the tomatoes. Optimal conditions for ripening are 19to 21 °C (66 to 70 °F) with 90 to 95% RH.

According to Kader (1993) tomatoes are classed as highly perishablecrops. Mature-green tomatoes can be stored at18-220 C at 90-95% RH for1-3 weeks and firm-ripe ones at 13-150 C at 90-95% RH for 4-7 days.Chilling injury can occur at 7-100 C in ripe fruits and at 130 C in maturegreen tomatoes, developing poor color and alternaria rot.

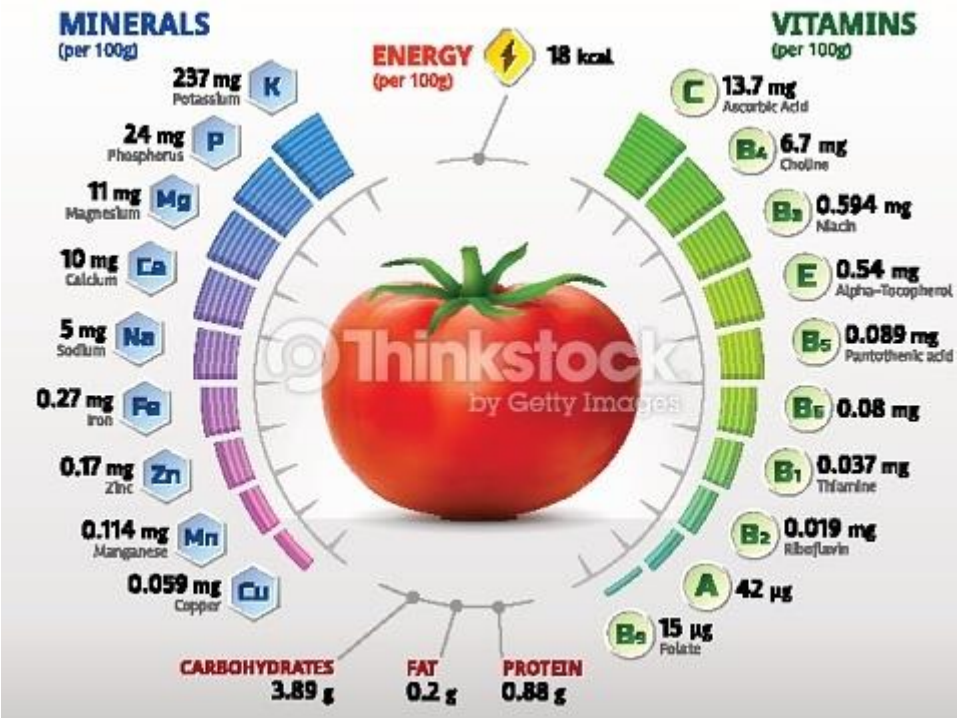


Figure 2.3: Composition of Tomato, Kader (1993)

2.15. Banana composition and post harvest treatment

Banana is one of the widely grown and consumed fruits due to their distinct aroma and taste, in all parts of the world. It is the staple food and economic life line for many

countries. It is cheap source of carbohydrate and rich source of potassium, calcium, antioxidants and other micronutrients. The sugar rich and low-fat bananas have varied uses as infant food, functional food, dessert, carbohydrate based staple food and many more diversified food/feed uses (Agunbiade et al., 2006; Aparicio-Saguilán et al., 2007; Aurore et al., 2009; Mohapatra et al., 2009). But this fruit is highly perishable owing to its high water content and is susceptible to many diseases, especially fungal infection. Being a climacteric fruit, it produces enough ethylene bringing about rapid changes in physico-chemical properties, such as colour, texture, aroma, chemical composition, respiration rate and senescence. The climacteric phase is characterised by enhanced ethylene production, higher oxygen consumption, starch to sugar conversion, chlorophyll degradation and relocation of the micro and macro nutrients between the pulp and other plant parts (Marriott et al., 1981). The harvesting standard varies from place to place, season, transport distance and the end use of the fruit. For local use, the fruits may be harvested at fully matured stage; for short distance transport, the fruits may be harvested at 90% maturity level and for long distance transport, the fruits may even be harvested at a maturity level of 75%. Again, the fruits are used for table purpose or processed for value addition. For processing, fully matured and yellow banana is preferred. In the later case, usually, green banana is used which has yet to reach the climacteric stage. In the cooler season, the fruits may be harvested after 105 days of flowering, but during hot season, the fruits can be harvested between 98 to 115 days (Robinson, 1996). After harvest, bunches are properly cushioned and transported to the warehouse. Mechanical damage to bananas during transport results in crown browning caused by enzymatic oxidative degradation of phenolic compounds by polyphenol oxidase. This can be avoided by dehanding the fruits under water, applying vacuum, waxing and application of antioxidants like thiourea and potassium aluminiumsulphate (Ismail et al., 2004).

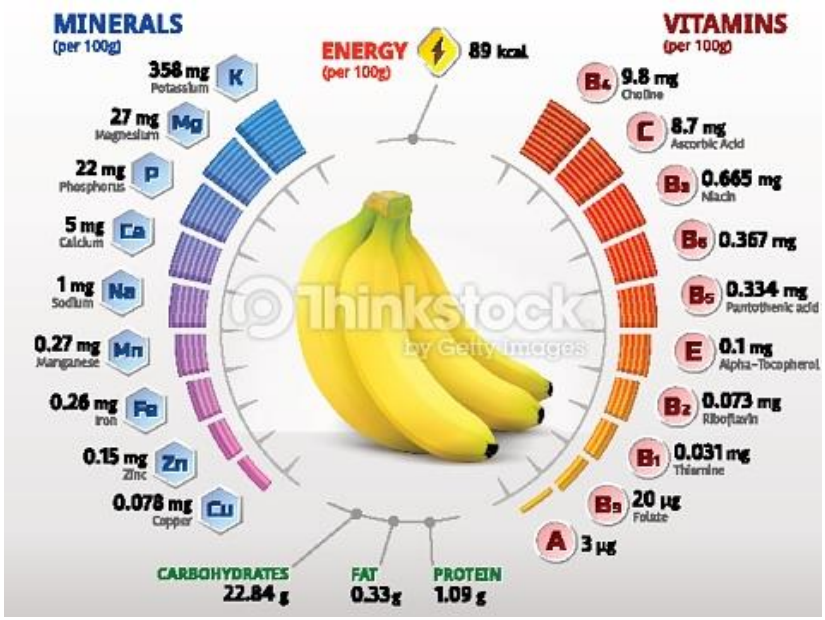


Figure2.4: Composition of Banana, (Ismail et al., 2004).

2.16. Papaya composition and post harvest treatment

A green papaya fruit has been reported by Duke (1996), which (per 100 g) provides 26 calories, 92.1 g H2O, 1.0 g protein, 0.1 g fat, 6.2 g total carbohydrate, 0.9 g fiber and 0.6 g ash. USDA National Nutrient database recorded an orange-freshed papaya (per 100 g) contained 39 calories, 88.8 g H2O, 0.61 g protein, 0.14 g fat, 9.81 g total carbohydrate, 1.8 g fiber, 0.61 g ash (Table.1). Additionally, Oyoyede (2005) tested the chemical profile of unripe pulp of *carica papaya* and reported papaya fruit was very rich in carbohydrate (42.28% starch, 15.15% sugar) but low levels of fat. Papaya fruit also contains high levels of vitamin C (51.2 mg/100g), vitamin A precursors including β-carotene (232.3 µg/100g), and β-cryptoxanthin (594.3 µg/100g), as well as magnesium (19.2-32.7 mg/100g), which has been reported by Wall (2006). The papaya seeds contain balance-nutrients which consists of protein (24.3%), fatty oil (25.3%) and total carbohydrate (32.5%). Although it contains significantly high level ofunsaturated fatty acids, papaya seeds seem not to be good oil seeds. In some tropical countries, papaya leaves are used as food sources, which can be cooked by stir-fry. The papaya leaves (per 100 g), were reported by Duke (1996), contains 74 calories, 77.5 g H2O, 7.0 g. protein, 2.0g fat, 11.3 g total carbohydrate, 1. 8 g fiber, 2.2 g ash, 344 mg Ca, 142 mg P, 0.8 mg Fe, 16 mg Na, 652 mg K, 11,565 ug β-carotene equivalent, 0.09 mg thiamine, 0.48 mg riboflavin, 2.1 mg niacin, and

140 mg ascorbic acid, as well 136 mg vitaminE.

Recent years, papaya latex and its commercial products have been widely applied in baking and beverage industries, pharmacy and new chemicals synthesis. There are four major components including papain (EC. 3.4.22.2), chymopapain (EC. 3.4.22.6), caricain (EC. 3.4.22.30), glycylendopeptidase (EC. 3.4.22.25), and papaya lipase (EC. 3.1.1.3)

Figure 2.5: Composition of Papaya, Oyoyede (2005)

Table 10. Composition of Papaya

Nutrient	**Units**	**Value per 100 grams**
Proximates		
Water	g	88.83
Energy	kcal	39
Energy	kj	163
Protein	g	0.61
Total lipid (fat)	g	0.14
Ash	g	0.61
Carbohydrate, by difference	g	9.81
Fiber, total dietary	g	1.8
Sugars, total	g	5.90
Minerals		
Calcium, Ca	mg	24
Magnesium, Mg	mg	10
Potassium, K	mg	257
Sodium, Na	mg	3
Zinc, Zn	mg	0.07
Vitamins		

Vitamin C, total ascorbic acid	mg	61.8
Niacin	mg	0.338
Pantothenic acid	mg	0.218
Folate, total	mcg	38
Folate, food	mcg	38
Folate, DFE	mcg_DFE	38
Choline, total	mg	6.1
Vitamin A,IU	IU	1094
Vitamin A, RAE	mcg_RAE	55
Virtamin E (alpha-tocopherol)	mg	0.73
Vitamin K	mcg	2.6
Lipids		
Fatty acids, toital saturated	g	0.043
Fatty acids, total monosaturated	g	0.038
Fatty acids, total polysaturated	g	0.031
Other		
Carotene, beta	mcg	276
Cryptoxanthin, beta	mcg	761
Lutein+Zeaxanthin	mcg	75

2.17. Extending shelf life of fruits and vegetables

The aim of applying postharvest technology to fruits and vegetablesis to maintain quality and to reduce losses between harvest andconsumption. Most often hi-tech practices are not suitable for small-scale farmers in developing countries for the simple reason of economies ofscale (Kader, 1992). Limitations faced by small-scale handlers mayinclude labor surpluses, lack of credit for investments in postharvesttechnology, unreliable electric power supply, lack of transport, storagefacilities and/or packaging materials and other constraints. There aresimple postharvest technologies that may meet the requirements of smallscalefood handlers. Despite good management practices, some producerequire treatment to prevent spoilage especially by pathogenicmicroorganisms. This is done by hot water dipping, chilling, exclusion ofoxygen and use of chemical or bio pesticides (Shewfelt, 1986). The majorfactors responsible for extending the shelf life of fruits and vegetablesinclude: careful harvesting so as not to injure the product, harvesting atoptimal horticultural maturity for intended use, and good sanitation(Moleyar and Narasimham, 1994; Lee et al., 1996).

The delay in deterioration due to senescence is the main goal in thepreservation of fresh produce, as senescence accounts for the majority ofpost-harvest losses (Lee et

al., 1996). Control measures taken to minimizeproduction of ethylene following harvest include storage in a modifiedatmosphere at optimal low temperatures (just above the chilling orfreezing injury threshold) and oxidizing the ethylene by various chemical and physical means. Successful control of both product respiration andethylene production and perception by MAP can result in a fruit orvegetable product of high organoleptic quality; however, control of theseprocesses is dependent on temperature. Controlled or modifiedatmosphere storage should be used as a supplement to, and not as asubstitute for, proper temperature and relative humidity management.

2.18. Modified atmospheric storage

Ripening can be checked by using controlled atmosphere storage (CAS)and/or active or passive modified atmosphere packaging (MAP) or withedible coatings. A modified atmosphere can be defined as one that iscreated by altering the normal composition of air (78% nitrogen, 21%oxygen, 0.03% carbon dioxide and traces of noble gases) to provide anoptimum atmosphere for increasing the storage length and quality offood/produce (Moleyar and Narasimham 1994; Phillips 1996). In all casesan atmosphere of low oxygen (1-5%) and high carbon dioxide is created tohelp reduce the respiration rate of fruits and vegetables and depressethylene production, thus preventing ripening (Lee et al., 1996). Reducingthe rate of respiration by limiting O2 prolongs the shelf life of fruits and vegetables by delaying the oxidative breakdown of the complex substrateswhich make up the product. However, at extremely low O2 levels (<1%), anaerobic respiration can occur, resulting in tissue destruction and theproduction of off-flavors and off-odors (Zagory, 1995), as well as thepotential for growth of food borne pathogens such as Clostridiumbotulinum (Austin et al., 1998). Therefore, the recommended percentageof O2 in a modified atmosphere for fruits and vegetables for both safetyand quality falls between 1 and 5%. CO2 can inhibit ethylene action aswell as autocatalytic production of ethylene by climacteric products suchas apples and tomatoes (Lee et al., 1996). The rate of respiration of a fruitor vegetable is inversely proportional to the shelf life of the product; ahigher rate decreases shelf life.

CHAPTER 3

MATERIALS AND METHODS

The fruits (Banana) and vegetable (Tomato and Papaya) samples were collected from different markets of Dhaka City and carried to the laboratory of the Bangladesh Atomic Energy Commission in Ganakbari, Savar, Dhaka for analysis of Chitosan as preservative on self life and major nutrient contents of fruits and vegetables during September 2017 to March 2018. From the collection of samples to the final analysis, all way required a number of processes which are described below.

3.1 Study area

The study area included major three markets of Dhaka City and garden of the Bangladesh Atomic Energy Commission in Ganakbari, Savar, Dhaka. The area of

Dhaka City is about 270 sq km, located at 23.42° North latitude and 90.22° East longitude with an elevation of 4 meter from the sea level.

3.2. Collection of Samples:

Prawn shells and some selected fruits and vegetables werecollected from different markets near the Dhaka city.In this study, fruits and vegetables were collected from 3 markets of Bank Town Bazar, Savar Bazar, Ganakbari Bazar.

3.3. Preparation of samples:

The collected prawn shells samples were transported to the laboratory of the Bangladesh Atomic Energy Commission in Ganakbari, Savar, Dhaka and air dried. After drying in air, the prawn shells will be converted into chitosan through deprotenization, demineralization and deacetylation processes. After preparing Chitosan, applying on fruits and vegetables; and these samples were later used for various chemical analysis in the laboratory of the Department of Agricultural Chemistry of sher-e- Bangla Agricultural University.

3.4. Preparation of Chitosan:

(a) The collected waste prawn shell was washed with hot water (70^0 C) and then dried in an oven at 105^0C for 72 hours. Dried prawn shellwas ground and then deproteinized with 1 N NaOH solutionat boiling temperature (100^0 C) for 4 hours (prawn shell:NaOH = 1:16, w/v) and demineralinzed with 1 N HClsolution at boiling temperature (100^0 C) for 4 h (chitin:HCl = 1:13,w/v). The mixture was then washed with distilled water,filtered to neutralize and dried at 105^0 C in an oven for24 hours. Prepared chitin is an intermediate product of chitosan.

(b) Chitosan was obtained by deacetylation of chitin using 1N NaOH (chitin:NaOH = 1:20, w/w, Solid state ratio) at 100^0 C for 4 hours.After this process, solid was separated from the alkali and was extensively washed with distilled water to removetraces of NaOH (neutralization was confirmed using Litmus paper). The

resultant solid was dried in a vacuumoven at 50^0 C for 24 hours. Chitosan was extracted in this wayfrom prawn shell waste. At last solid chitosan was converted into solution by 2% acetic acid.

Figure3.1:Preparation of Chitosan

3.5. Application of Chitosan:

At first some selected fruits and vegetables were divided into 2 or 3 groups. Among them 1 group for control and other groups for treatment. Chitosan was applied on treatment groups by deep coating method.

3.6. Name of the Experiment:

After application of Chitosan, samples were used in different experiment to the laboratory of the Bangladesh Atomic Energy Commission in Ganakbari, Savar, Dhaka for analysis of Chitosan as preservative on self life of fruits and vegetables.

3.6.1. Color Index:

Skin color development was assessed visuallyand a color index was calculated on scale from 1 (green) to 5 (full color). To do so, skin color of individual fruit was scored on a ripening scale of:

0 = green

1 = breaker

2 = <25% color change

3 = 25-30% color change

4 = >50% color change but <75% color change

5 = full color

The color index (CI) was calculated using the following formula:

$$CI\ (\%) = \frac{\sum(\text{color scale} \times \text{No. of corresponding fruits or vegetables})}{(\text{The highest scale} \times \text{No. of the total fruits or vegetables})} \times 100$$

Note: Color Index was recorded at days 0, 10, 20 and 30.

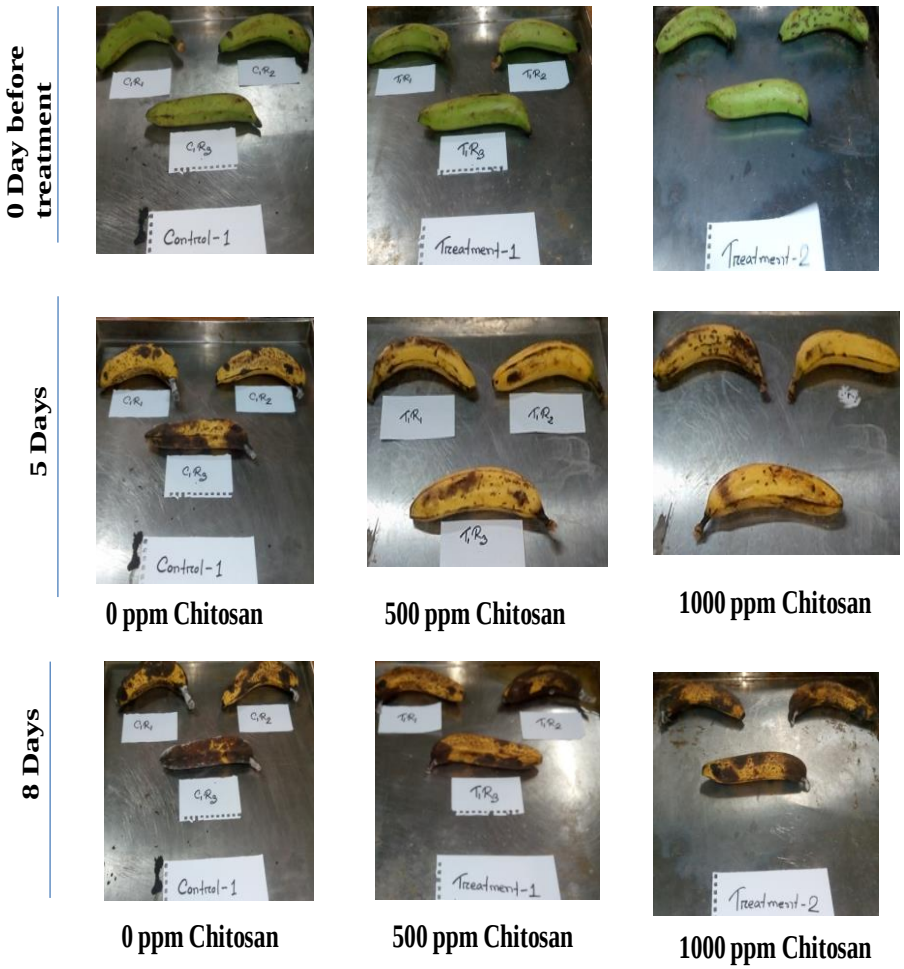


Figure 3.2. Color changes in Banana

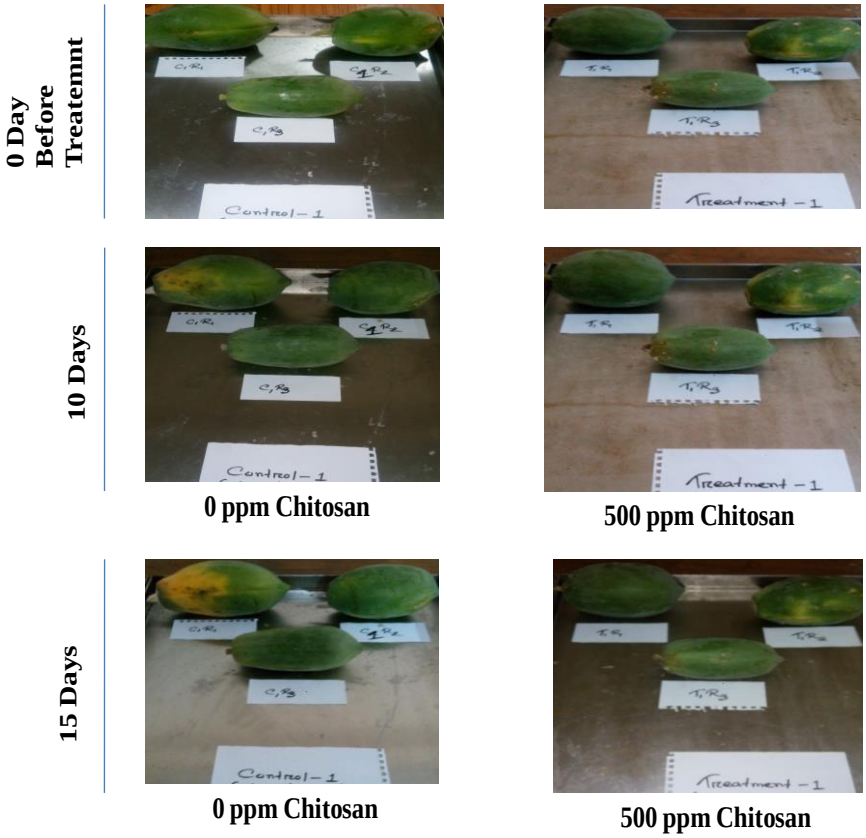


Figure3.3. Color changes in Papaya

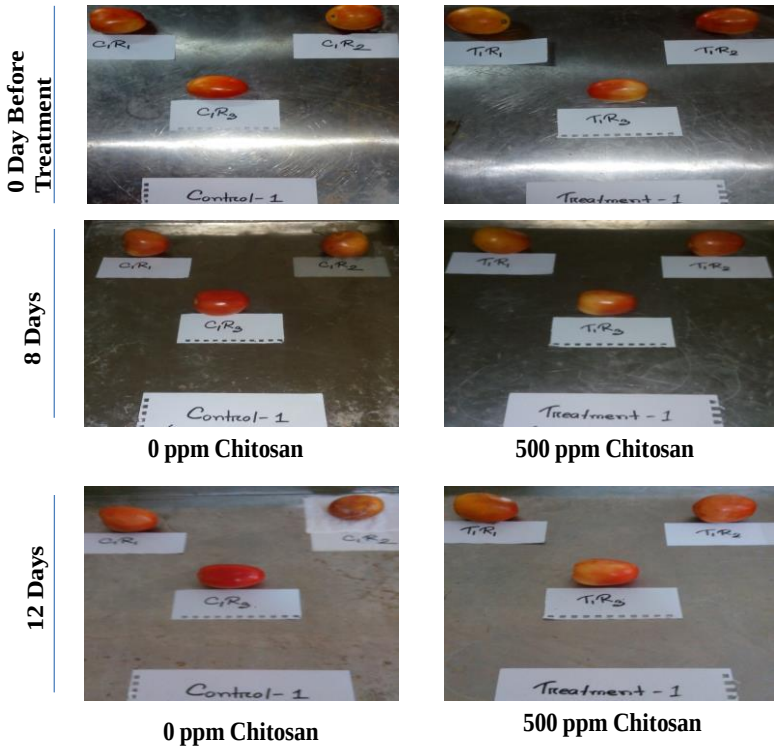


Figure3.4.Color changes in Tomato

3.6.2. Weight Loss:

The weight loss was measured, fruits or vegetables were taken out from each samples and weighed per 3 days. The weight loss was calculated by the following formula:m_1

$$Weight\ loss\ (\%) = \frac{\mathrm{m0} - \mathrm{m1}}{\mathrm{m0}} \times 100$$

Where, m_o = the initial weight

m_1 = the weight measured during storage

3.6.3. Electrical Conductivity:

At first, take 20g sample and add 50mL distilled water. Stir the mixture for half an hour to ensure complete dispersion of the sample. Left the conical flask in rest to decant the sample and the electrode of the EC meter is deep into the overlying water. Take care that the electrode does not touch the underlying sample. Record the reading.

3.6.4. pH:

At first, take 20g sample and add 50mL distilled water. Stir the mixture for half an hour to ensure complete dispersion of the sample. Left the conical flask in rest to decant the sample and the electrode of the p^H meter is deep into the overlying water. Take care that the electrode does not touch the underlying sample. After stabilization, take the reading.

Figure 3.5.Determination of pH and EC of fruits and vegetables

3.6.5. Total Suspended Solid:

Oven dry a WN-1 filter paper at 110^0 C temperature for about 2-3 hours and record the weight after cooling. Take 20g sample and add 50mL distilled water. Mixed very well and filter with WN-1filter paper. After filtration, again oven dry the filter paper at 110^0 C temperature for about 2-3 hours and record the weight after cooling.

Calculation:

$$TSS\ (\%\ w/w) = [(W_d - W_e) \div E] \times 100$$

Where, W_d = Oven dry weight of the filter paper with suspended materials

W_e = Oven dry weight of the empty filter paper

E = Weight of the sample taken for filtration

3.6.6. Total Dissolved Solid:

Oven dry a empty conical flask at 110^0 C temperature for about 2-3 hours and record the weight after cooling. Take 20g sample and add 50mL distilled water. Mixed very well and filter with WN-1filter paper. After filtration, again oven dry the conical flask at 110^0 C temperature for about 2-3 hours and record the weight after cooling.

Calculation:

$$TDS\ (\%\ w/v) = [(W_d - W_e) \div E] \times 100$$

Where, W_d = Oven dry weight of the conical flask with dissolved materials

W_e = Oven dry weight of the empty conical flask

E = Volume of the water taken for filtration

3.7. For Microbial Growth Analysis

3.7.1. Principle:

Bactria and fungus are prokaryotic organisms. They are generally in order of 1-2 μm long and 0.5-1.0 μm in diameter. For identification of bacterial and fungal colony need to culture bacteria and fungus in culture media. This cultured process involves several steps such as preparation of nutrient agar, which support bacterial and fungal growth and it is a solidifying agent which support the surface growth of bacteria and fungus which are clearly appearance in naked eye. Sterilization is another step that need to avoid contamination of the equipment's. The next step, agar is placed on Petridish which is known as petriplate. For decreasing concentration of bacteria and fungus in sample successively dilution is needed called serial dilution. To isolate bacterial colonies and appearance clearly serial dilution must be needed. Then sample is placed on the surface of the agar and the base is covered by lid for gaseous exchange. After that, the bacterial and fungal colony is counted.

Equipment's:

- Petridishes
- Beaker
- Conical flask
- Volumetric flask
- Autoclave

- Laminar air flow chamber
- Inoculating needle
- Pipettes
- Testubes with cork
- Blade
- Raping paper
- Ethyl alcohol
- Hand gloves
- Musk
- Apron
- Rack
- Incubator
- L-shaped glass
- Physiological water.

3.7.2. Successive order of procedure for bacterial growth culture:

For the identification of bacteria and fungus, the following successive processes were done:

- Preparation of nutrient agar.
- Preparation of physiological water.
- Preparation of Sterilization
- Preparation of serial dilution.
- Preparation of petriplates.
- Counting of colony.

3.7.2.1. Preparation of nutrient agar:

For preparing bacterial culture medium, at first materials for bacterial growth were weighted and were taken in following amounts:

Table 11. Preparation of nutrient agar

Nutrient materials	**Amount**
Potato Dextrose Agar	19.5gm for 500mL
Plate Count Agar	11.75gm for 500mL
Water	1000mL

Then these are taken into two different500ml conical flask and 500ml water was added into the conical flask and stirred until the ingredients were completely dissolved. Then the conical flask was placed into autoclave for sterilizing the medium.

3.7.2.2. Preparation of physiological water

9 mL of 0.9% physiological water were taken into the testube (10 testubes).Then the testube was placed into autoclave for sterilizing the medium.

3.7.2.3. Preparation of sterilization:

Sterilization:

Sterilization is the process of rendering a medium or material free of all forms of life. Sterilization in an autoclave is most effective when the organisms are either contacted by the steam directly or are contained in small volume of aqueous (primarily water) liquid under these conditions, steam at a pressure at about 15 atmosphere pressure and 121^0c will kill all organisms and endow- spores in about 15 minutes.Autoclave is used to sterilize culture media, instruments dressing, intravenous equipment, applicators,

solutions, syringes, transfusion equipment and numerous other items to can with stand high temperature and pressure.

Requirements:

- Autoclave machine.
- Physiological water.
- The materials or items which we want to sterilize.
- Raping Paper.
- Cotton.

Figure 3.6: Autoclave (Sterilization machine)

Procedure:

1. It was ready by loading requiring amount of water. The item such as petridishes,Beaker, Conical flask, volumetric flask, Pipettes, Testubes with cork, Raping paper, and freshly prepared culture media were loaded in the autoclave containers of the autoclave.
2. The autoclave door was closed & locked.
3. The autoclave was set for 30 mins with selecting a slow of exhaust.
4. The autoclave temperature was set to 121℃ .
5. The autoclave was started by pushing the start button.
6. After half hours when the sterilization process was completed, the autoclave was stopped& when the pressure in the chamber read 0, the door was opened carefully.
7. The containers was uptake carefully from autoclave.
8. Then the sterilize items were kept in the laminar air flow chamber. If the laminar air flow chamber are not remain in our laboratory then the chamber made of glass into which a spirit lamp was burned for 30 mins was used in place of modern laminar air flow chamber. In ancient period this process was flowed when modern laminar air flow chamber was not invented.

Figure 3.7: Laminar air flow chamber.

3.7.2.4. Preparation for serial dilution:

Serial Dilution:

To ensure the colony counts by which process the original inoculums is diluted several times that process is called serial dilution.

$$\mathrm{DF} = \frac{\text{Initial concentration of the solution}}{\text{Final concentration of the solution}}$$

Requirements:

- Test tubes (sterilized), & test tube stand
- Sterilized water
- Laminar air flow chamber
- Pipettes (sterilized)
- Soil
- Balance
- Glass rod (sterilized)

Procedure:

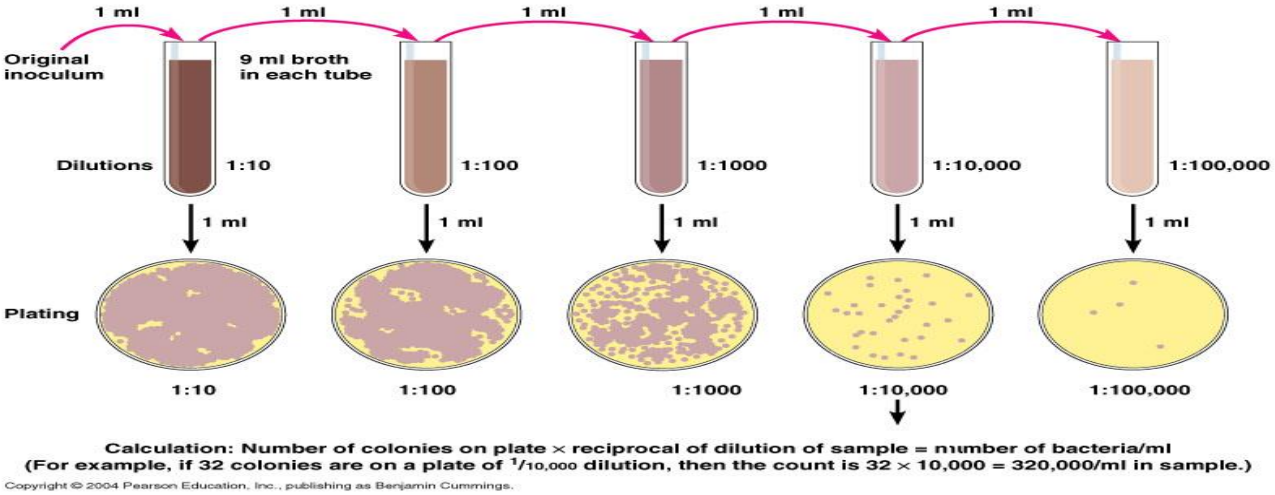
1. At first it was decided that the serial dilution would be done six (6) times.
2. Then by measuring 10 gm. of collecting fruits or vegetables sample with a balance, it was taken into beaker.
3. Some 90 mL water added into the beaker with a pipette. Than mixture of 100 mL solution and with a glass rod the suspension was mixed up.
4. When all the soil was mixed thoroughly with water, it was replaced into a test tube by washing the beaker with sterilized water carefully up to the mark of 100 mL with a pipette. The test tubes were kept on the test tube stand.
5. This solution diluted 1/100 or 1:99.

6. The test tube was marked by 10^2 which indicates the dilution factor 0.01. That means the solution was diluted 100 times. It means 10^2 dilutions.

7. Then 10 test-tube was taken and every test-tube was taken 1 ml at solution was poured into another test tube by a pipette and then dilution was performed by adding 9 ml of physiological sterilized water with the pipette. This solution dilutes 1:100 of original sample.

8. Then the same process was applied for making the serial dilution from 10^3 to 10^4, to 10^5,and 10^6.

9. In this process one pipette was used for one particular step. ***Caution:*** Not one pipette used different steps.



3.7.2.5. Preparation of petriplates:

Petriplates: When nutrient agar medium are pour out to Petridish then it is termed as petriplate. A petriplate are through in an incubator to prevent to contamination are there the base are comes in upper position and lid are goes into lower position.

Preparation

1. Sterilized agar was used for preparation petriplates.
2. Sterilized petridishes and medium was taken into the laminar air flow chamber and media was spreader equally into all petridishes.
3. Petridish are covered with lid.
4. After solidifying the agar, petridishes were taken for culturing bacteria.

Figure3.8. Incubator

3.7.2.6. Counting of colony:

Plate count:

The most frequently used method of measuring bacterialpopulations is the plate counts. Plate counts assume that each live bacterium grows and divides to produce a single colony. This is not always true because bacteria frequently grow linked in chains or as clumps. Therefore a colony often results, not from a single bacterium but from short segments of a chain or from a bacterium clump to reflect this reality, plate counts are often reported as colony forming units (CFU). When a plate count is performed, it is important that only a limited number of colonies develop in the plate. When too many colonies are present, some cells are overcrowded and do not develop. This condition causes in accuracies in the count. Generally only plates with 25-250 colonies are counted.

1. Counting bacterial colonies on agar plates is a simple and effective method for determining the number of viable bacteria in a sample.

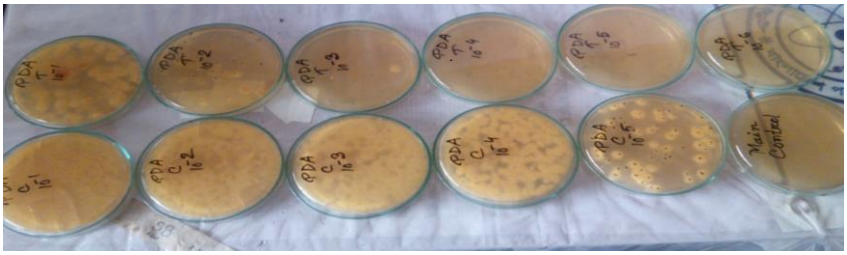
2. This method relies on the growth of a bacterial cell in an agar plate to form a visible colony, only living or viable bacterial cells will be counted. If the total cell count is required, please use a counting chamber (Haemocytometer).

Number of colony:

After 24 hours it was observed that colonies formed on the surface of petriplate.

MICROBIAL CONDITION OF BANANA

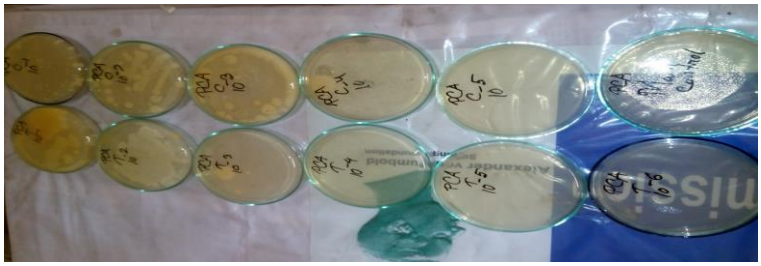
Bacterial Growth of Banana



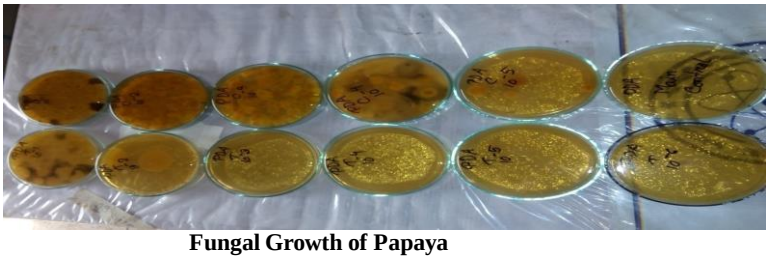
Fungal Growth of Banana

Figure3.9.Microbial condition of Banana

MICROBIAL CONDITION OF PAPAYA



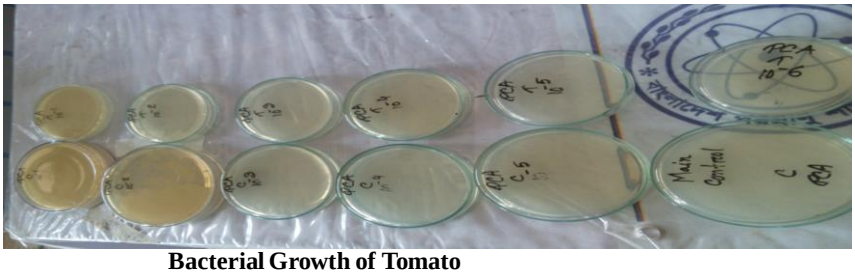
Bacterial Growth of Papaya



Fungal Growth of Papaya

Figure3.10.Microbial condition of Papaya

MICROBIAL CONDITION OF TOMATO



Bacterial Growth of Tomato

Fungal Growth of Tomato

Figure 3.11.Microbial condition of Tomato

3.8. Analysis of different chemical constituents in fruits and vegetables samples

After application of Chitosan, dried in an oven at 60-70ºC and then samples were used in analysis of different chemical constituents in fruits and vegetables samples to the laboratory of the Department of Agricultural Chemistry of sher-e- Bangla Agricultural University.

3.8.1 Grinding

The samples after oven dried were ground in a Wiley Hammer Mill, passed through 40 mesh screens, mixed well and stored in plastic vials.

3.8.2 Digestion

Exactly 1g oven-dried samples of wheat plant were taken in digestion tube. About 10 mL of concentrated percloric acid in a digestion tube and left to stand for 20 minutes and then

transferred to a digestion block and continued heating at 100^0C. The temperature was increased to 365^0C gradually to prevent frothing (50^0C steps) and left to digest until yellowish color of the solution turned to whitish color. Then the digestion tubes were removed from the heating source and allowed to cool to room temperature. About 40 mL of de-ionised water was carefully added to the digestion tubes and the contents filtered through Whatman no. 40 filter paper into a 100 mL volumetric flask and the volume was made up to the mark with de-ionised water. The samples were stored at room temperature in clearly marked containers.

Figure 3.12.Digestion of fruits and vegetables samples

3.9. Chemical Analysis:

Chemical analysis (N, P and K) was done by the following methods-

3.9.1. Total Nitrogen:

Total Nitrogen of the soil was determined by Micro- Kjeldahl's method following H_2SO_4 acid digestion as suggested by Jackson, 1962.

3.9.2. Available Phosphorus (P):

Available Phosphorus was determined by Molybdophosphoric blue colour method in sulfuric acid system. It is measured by Spectrophotometer at 882 nm wavelength.

Figure 3.13.Determination of Phosphorus in fruits and vegetables

3.9.3. Available Potassium (K^+):

Available Potassium (K^+) was extracted from the samples by 1N NH_4OAc(pH-7) solution followed by measurement of extractable K+ by Flame emission spectrophotometer (Model: Jenway, PEP-7) at 769 nm wave length using Potassium filter, as outlined by Jackson, 1973.

3.10. Statistical Analysis:

Means of three replicates of each control and treatmentwerecalculated. Statistical analysis will be performed by Microsoft Excel and SPSS window version 17.0 (SPSS Inc., Chicago, USA) to determine the statistical significance of the different parameters.Duncan's multiple range test (DMRT) at 5% level of probability will be used to test the difference between means of individual treatments (Gomez and Gomez, 1984).

CHAPTER 4

RESULTS AND DISCUSSION

The present experiment was carried out in order to find out the effect of chitosan on different fruits and vegetables. The data obtained on different parameters are discussed in this chapter. The analytical results of different fruits and vegetables and their statistical analysis have been given in appendices.

4.1. Color Index:

Significantly *($p \leq 0.05$)* high percentage of weight loss (50%, 22.22%, 50%) has found in the treatment-1 of BANANA, TOMATO and control of PAPAYA respectively. The lowest percentage of weight loss (45.83%, 0%, 16.66%) was observed with the sample of control and treatment-1 in BANANA, TOMATO and PAPAYA respectively. Fig. 4.1 shows that percentage of color index significantly *($p \leq 0.05$)* varied among the control and treatment-1 in different fruits and vegetables. Percentage of color index among different fruits and vegetables is in the order of,

For BANANA: Banana treatment-2 and Banana treatment-1>Banana Control.For PAPAYA: Papaya Control > Papaya treatment-1.For TOMATO: Tomato treatment-1> TomatoControl.

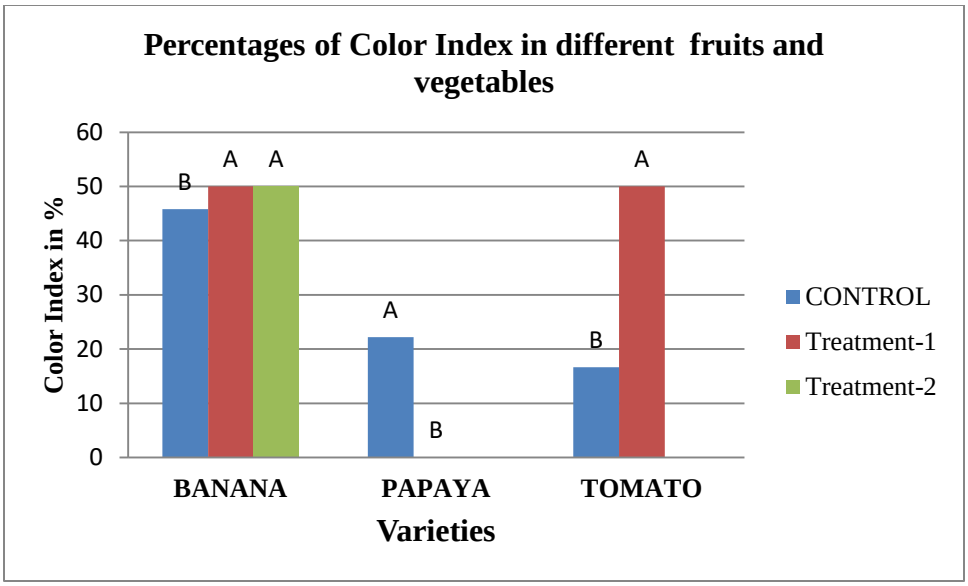


Figure4.1. Percentages of color index in different fruits and vegetables with three replications (color index were recorded at days 0, 5 and 10).

From the result, it can be said that there is a significant difference ($p \leq 0.05$) among the varities in case of color index. The significant difference is higher in Tomato and Papaya than Banana. In case of Banana and Tomato , if percentages of color index is high , it is beneficial. In case of Papaya , if percentages of color index is low , it is beneficial. As, Banana and Tomato are sold to the customer shown by its color; so, color index of Banana and Tomatoplay significant role. But in case of Papaya is not sold in to the customer shown by its color. Fig. 4.1 shows that percentage of color index significantly ($p \leq 0.05$) varied among the control and treatment-1 in different fruits and vegetables.From the result, it can be said that, CHITOSAN played an effective role as preservative. So, we can use CHITOSAN as natural preservative which can play a vital role in our agriculture.

4.2.Weight Loss

Significantly ($p \leq 0.05$) high percentage of weight loss (16.5667%, 12.9033%, 12.7867%) has found in the control of BANANA, PAPAYA and TOMATO respectively. The lowest percentage of weight loss (15.81%, 7.3967%, 9.49%) was observed with the sample of treatment-1 in BANANA, PAPAYA and TOMATO respectively. Fig. 4.2 shows that percentage of weight loss significantly ($p \leq 0.05$) varied among the control and treatment-1 in different fruits and vegetables. Percentage of weight loss among different fruits and vegetables is in the order of,

For BANANA: Banana Control >Banana treatment-2 >Banana treatment-1.For PAPAYA: Papaya Control >Papaya treatment-1.For TOMATO: Tomato Control > Tomato treatment-1.

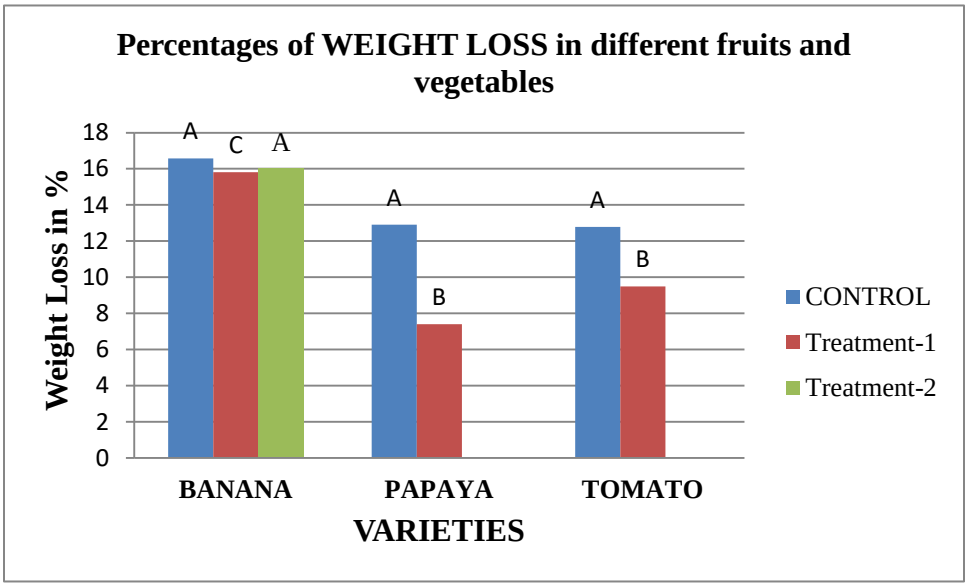


Figure4.2. Percentages of weight loss in different fruits and vegetables with three replications (weight loss were recorded at days 0, 3 and 6).

From the result, it can be said that there is a significant difference ($p \leq 0.05$) among the varieties in case of weight loss. The significant difference is higher in Tomato and Papaya than Banana. In case of weight loss, if percentages of weight loss is low then it is beneficial.As, Banana is not sold in kilogram but in hally; so, weight loss of Banana doesn't play significant role. But in case of Papaya and Tomato are sold in kilogram and the weight loss is less in treatments than control. Fig. 4.2 shows that percentage of weight loss significantly $(p \leq 0.05)$ varied among the control and treatment-1 in different fruits and vegetables.From the result, it can be said that, CHITOSAN played an effective role as preservative. So, we can use CHITOSAN as natural preservative which can play a vital role in our agriculture.

4.3.Electrical conductivity (EC)

Insignificantly (at 5% level) high level of Electrical conductivity (0.00022, 0.00088, 0.00024dS/m) has found in the control of BANANA, PAPAYA and TOMATO respectively. The lowest amount of electrical conductivity level (0.00021, 0.00087, 0.00023dS/m) was observed with the sample of treatment-2 and treatment-1 in BANANA, PAPAYA and TOMATO respectively. Fig. 4.3 shows that electrical conductivity level insignificantly (at 5% level) varied among the control, treatment-1 and treatment-2 in different fruits and vegetables. Level of Electrical conductivity among different fruits and vegetables is in the order of,

For BANANA: Banana Control > Banana treatment-1 > Banana treatment-2.
For PAPAYA: Papaya Control > Papaya treatment-1.
For TOMATO: Tomato Control > Tomato treatment-1.

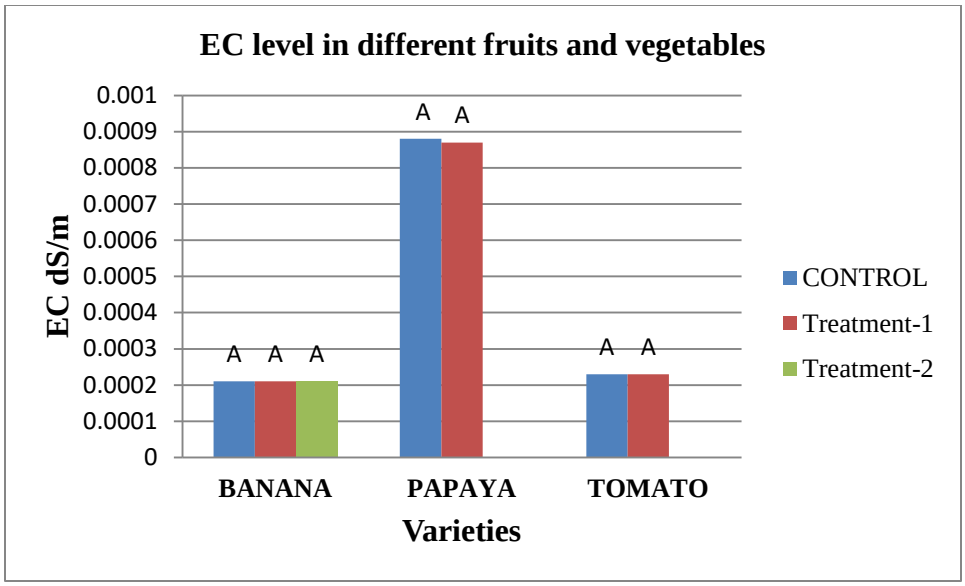


Figure4.3.EC level in different fruits and vegetableswith three replications (EC level were recorded at days 0, 3 and 6).

EC value presented in (Table8) shows that each and every control, treatment-1 and treatment-2 in different fruits and vegetables are non saline. In case of EC level, if EC levelis remaining similar, it is beneficial. Zaman and Bakri (2003) reported that coastal and south-east districts of Bangladesh are affected by salinity with EC values ranging between 4and 16dS/m which cover 3 million hectares of land. The changes in soil EC were significantly sensitive to the ripening stages of different fruits and vegetables on different levels of soil salinity. Nasiruddin and Rahman (1989) found 13 percent yield reduction of different fruits and vegetables due to the use of salt concentration of 4-8 (dSm-1). This may indicate that different fruits and vegetables might not affected by salinity as they have EC level of 0.00022, 0.00088 and 0.00024dS/m respectively (Fig. 4.3.).Fig. 4.3 shows that electrical conductivity level insignificantly (at 5% level) varied among the control, treatment-1 and treatment-2 in different fruits and vegetables.From the result, it can be said that, CHITOSAN played an effective role as preservative. So, we can use CHITOSAN as natural preservative which can play a vital role in our agriculture.

4.4.pH

pH level is insignificantly (at 5% level)more or lesshigh (4.877, 5.5533, 4.593) at the treatment-2, control and treatment-1 of BANANA, PAPAYA and TOMATO respectively. The lowest amount of pH level (4.6667, 5.35, 4.377) was associated with the sample of treatment-1, treatment-1 and control of BANANA, PAPAYA and TOMATO respectively. Fig. 4.4 shows that pH level insignificantly (at 5% level)varied among the control, treatment-1 and treatment-2 in different fruits and vegetables. pH among different Rice field is in order of

For BANANA: Banana treatment-2 > Banana control > Banana treatment-1.
For PAPAYA: Papaya Control > Papaya treatment-1.
For TOMATO: Tomato treatment-1 > Tomato control.

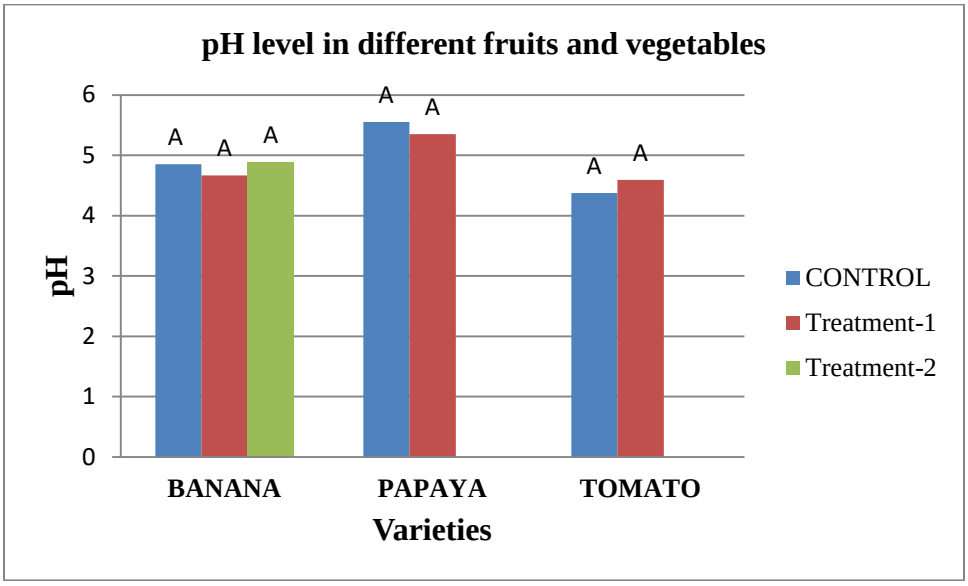


Figure4.4.pH level in different fruits and vegetableswith three replications (pH level were recorded at days 0, 3 and 6).

From the result, pH level of the different fruits and vegetables was acidic to moderately acidic in all the study fruits and vegetables. In case of pH level, if pH levelis remaining similar, it is beneficial. pH value from 4.5 to 5.2 in the Banana fruits which are slightly acidic to acidic in reaction. Uddin and Islam (1998) also found pH ranges 5.1 to 6.3 of the Papaya vegetables of Bangladesh. Rahmanet al. (2014) observed pH value of 4.3 to 4.9 in the Tomato vegetables in Bangladesh. BARC, (2005) reported that pH range of above values are optimum for the adequate availability of nutrients in the different fruits and vegetables. Thus it can be stated that all the fruits and vegetables have optimum pH range for adequate nutrient availability as the pH values ranged from 4 to 6. It also can be stated that among all of them fruits and vegetables is best at pH (Fig. 4.4.) for adequate nutrient supply.Fig. 4.4 shows that pH level insignificantly (at 5% level) varied among the control, treatment-1 and treatment-2 in different fruits and vegetables. So, it can be said that, CHITOSAN played an effective role as preservative. From the result, we can use CHITOSAN as natural preservative which can play a vital role in our agriculture.

4.5.Total Suspended Solids (TSS)

Total Suspended Solids (TSS)is insignificantly *(at 5% level)* more or lesshigh (3.564, 4.8123, 2.7963) at the treatment-2, treatment-1 and controlof BANANA, PAPAYA and TOMATO respectively. The lowest amount of Total Suspended Solids (TSS)(3.543, 4.7987, 2.6387) was associated with the sample of control, control and treatment-1 of BANANA, PAPAYA and TOMATO respectively. Fig. 4.5 shows that Total Suspended Solids (TSS) insignificantly *(at 5% level)*varied among the control, treatment-1 and treatment-2 in different fruits and vegetables. Total Suspended Solids (TSS) among different Rice field is in order of

For BANANA: Banana treatment-2 > Banana treatment-1> Banana control.

For PAPAYA: Papaya treatment-1> Papaya control.

For TOMATO: Tomato control> Tomato treatment-1.

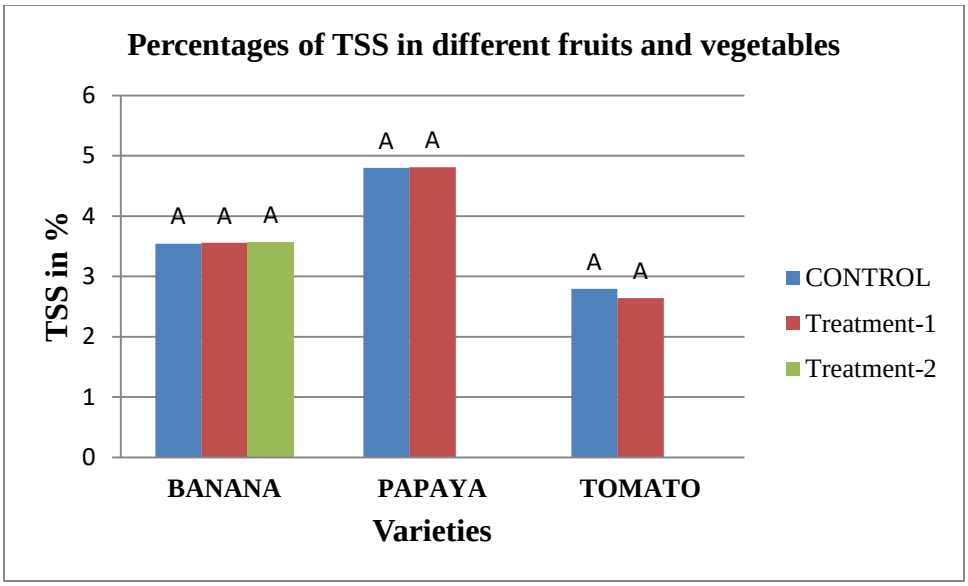


Figure4.5. Percentages of Total Suspended Solids (TSS) in different fruits and vegetableswith three replications (Percentages of Total Suspended Solids (TSS)were recorded at days 0, 3 and 6).

From the result, it can be said that there is ainsignificant difference (at 5% level) among the varieties in case of Total Suspended Solids (TSS). In case of Total Suspended Solids (TSS), if percentages of Total Suspended Solids (TSS) is remaining similar, it is beneficial. Fig. 4.5 shows that Total Suspended Solids (TSS) insignificantly (at 5% level) varied among the control, treatment-1 and treatment-2 in different fruits and vegetables.From the result, it can be said that, CHITOSAN played an effective role as preservative. So, we can use CHITOSAN as natural preservative which can play a vital role in our agriculture.

4.6.Total Dissolved Solids (TDS)

Total Dissolved Solids (TDS)is insignificantly *(at 5% level)* more or lesshigh (1.0553, 1.1953, 1.01267) at the control, treatment-1 and control of BANANA, PAPAYA and TOMATO respectively. The lowest amount of Total Dissolved Solids (TDS) (1.03867, 1.2453, 1.011) was associated with the sample of treatment-2, treatment-1 and treatment-1 of BANANA, PAPAYA and TOMATO respectively. Fig. 4.6 shows that Total Dissolved Solids (TDS) insignificantly *(at 5% level)* varied among the control, treatment-1 and treatment-2 in different fruits and vegetables. Total Dissolved Solids (TDS) among different Rice field is in order of

For BANANA: Banana control > Banana treatment-1 > Banana treatment-2.
For PAPAYA: Papaya control > Papaya treatment-1.
For TOMATO: Tomato control > Tomato treatment-1.

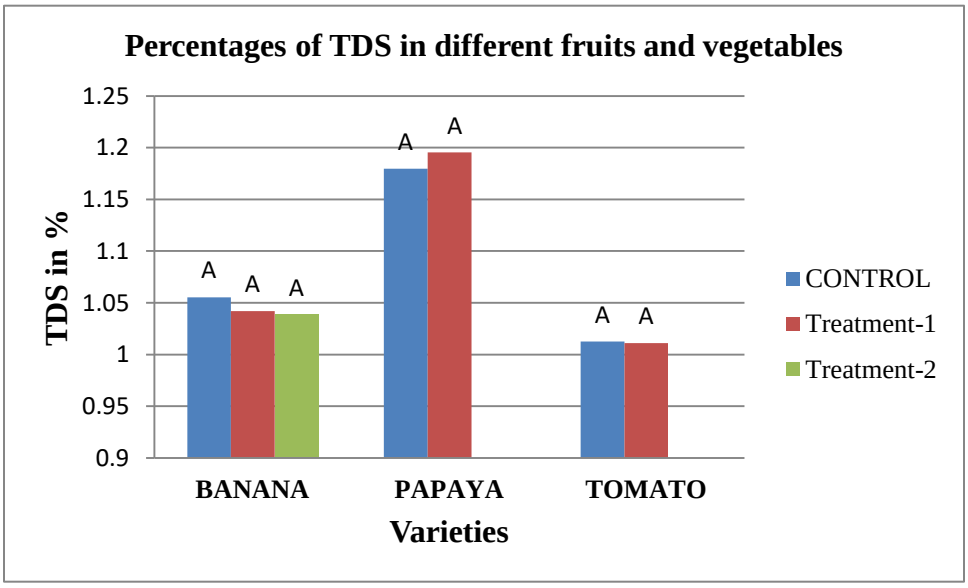


Figure4.6. Percentages ofTotal Dissolved Solids (TDS) in different fruits and vegetableswith three replications (Percentages ofTotal Dissolved Solids (TDS)were recorded at days 0, 3 and 6).

From the result, it can be said that there is ainsignificant difference (at 5% level) among the varieties in case of Total Dissolved Solids (TDS). In case of Total Dissolved Solids (TDS), if percentages of Total Dissolved Solids (TDS) is remaining similar, it is beneficial. Fig. 4.6 shows that Total Dissolved Solids (TDS) insignificantly (at 5% level) varied among the control, treatment-1 and treatment-2 in different fruits and vegetables.From the result, it can be said that, CHITOSAN played an effective role as preservative. So, we can use CHITOSAN as natural preservative which can play a vital role in our agriculture.

4.7.Total Viable Count (TVC) or Total Bacterial Count

Significantly *($p \leq 0.05$)* high level of Total Viable Count (TVC) or Total Bacterial Count (966.67, 816.67, 180 per g) has found in the control of BANANA, PAPAYA and TOMATO respectively. The lowest amount of Total Viable Count (TVC) or Total Bacterial Countlevel (1.1, 3.1, 3.1333 per g) was observed with the sample of treatment-1 in BANANA, PAPAYA and TOMATO respectively. Fig. 4.7 shows that number of Total Viable Count (TVC) or Total Bacterial Countsignificantly *($p \leq 0.05$)* varied among the control, treatment-1 and treatment-2 in different fruits and vegetables. Level of Total Viable Count (TVC) or Total Bacterial Countamong different fruits and vegetables is in the order of,

For BANANA: Banana Control >Banana treatment-2>Banana treatment-1.
For PAPAYA: Papaya Control > Papaya treatment-1.
For TOMATO: Tomato Control > Tomato treatment-1.

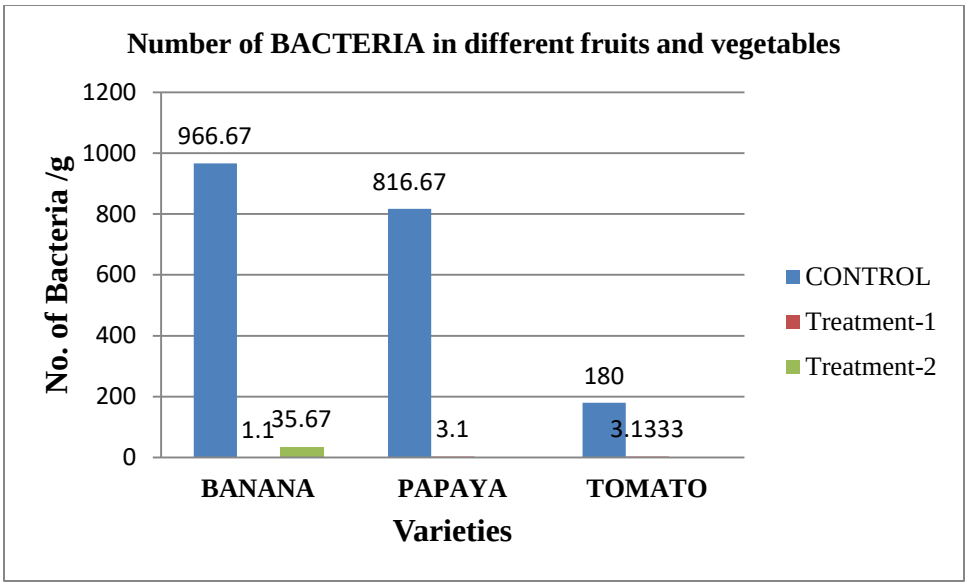


Figure4.7. Distribution of number of Bacteria in different fruits and vegetableswith three replications (Datawere recorded at days 0, 5 and 10).

From the result, it can be said that there is a significant difference ($p \leq 0.05$) among the varities in case of Total Viable Count (TVC) or Total Bacterial Count. The significant difference is higher in control of Banana, Tomato and Papaya than treatment-1 and treatment-2 of Banana, Tomato and Papaya. As, microbial growth is higher, the fruits and vegetables are rotten more fast and the market value will be lessered.In case of Total Viable Count (TVC) or Total Bacterial Count, if number of Total Viable Count (TVC) or Total Bacterial Countis low, it is beneficial. Fig. 4.7 shows that number of Total Viable Count (TVC) or Total Bacterial Count significantly ($p \leq 0.05$) varied among the control, treatment-1 and treatment-2 in different fruits and vegetables.From the result, it can be said that, CHITOSAN played an effective role as preservative. So, we can use CHITOSAN as natural preservative which can play a vital role in our agriculture.

4.8.Total Fungal Count (TFC) or Yeast & Mold Count (YMC)

Significantly ($p \leq 0.05$) high level of Total Fungal Count (TFC) or Yeast & Mold Count (YMC) (966.67, 816.67, 180 per g) has found in the control of BANANA, PAPAYA and TOMATO respectively. The lowest amount of Total Fungal Count (TFC) or Yeast & Mold Count (YMC) level (1.1, 3.1, 3.1333 per g) was observed with the sample of treatment-1 in BANANA, PAPAYA and TOMATO respectively. Fig. 4.8 shows that number of Total Fungal Count (TFC) or Yeast & Mold Count (YMC) significantly ($p \leq 0.05$) varied among the control, treatment-1 and treatment-2 in different fruits and vegetables. Level of Total Fungal Count (TFC) or Yeast & Mold Count (YMC) among different fruits and vegetables is in the order of,

For BANANA: Banana Control > Banana treatment-2 > Banana treatment-1.

For PAPAYA: Papaya Control > Papaya treatment-1.

For TOMATO: Tomato Control > Tomato treatment-1.

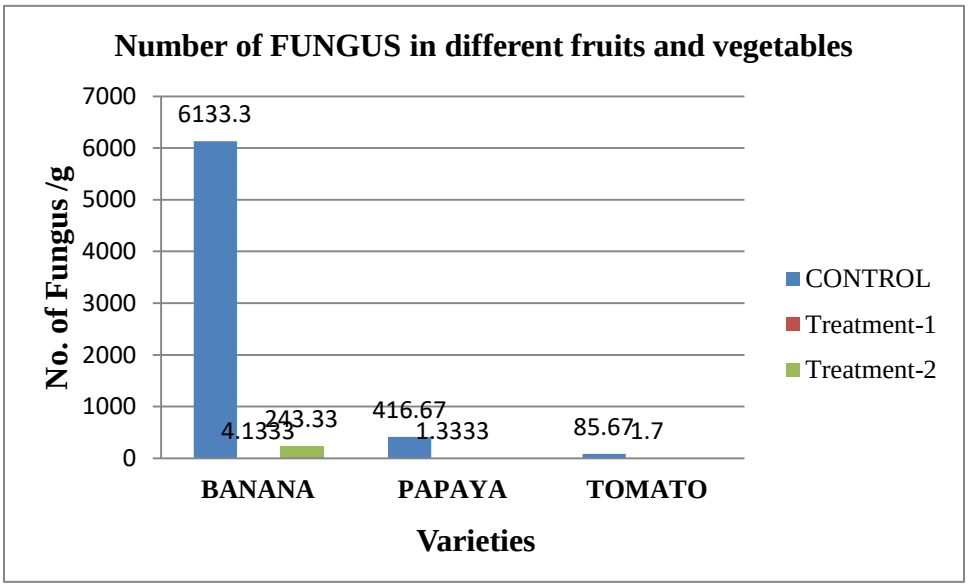


Figure4.8. Distribution of number of Fungus in different fruits and vegetableswith three replications (Datawere recorded at days 0, 5 and 10).

From the result, it can be said that there is a significant difference ($p \leq 0.05$) among the varities in case of Total Fungal Count (TFC) or Yeast & Mold Count (YMC). The significant difference is higher in control of Banana, Tomato and Papaya than treatment-1 and treatment-2 of Banana, Tomato and Papaya. As, microbial growth is higher, the fruits and vegetables are rotten more fast and the market value will be lessered.In case of Total Fungal Count (TFC) or Yeast & Mold Count (YMC), if number of Total Fungal Count (TFC) or Yeast & Mold Count (YMC)is low, it is beneficial. Fig. 4.8 shows that number of Total Fungal Count (TFC) or Yeast & Mold Count (YMC) significantly ($p \leq 0.05$) varied among the control, treatment-1 and treatment-2 in different fruits and vegetables.From the result, it can be said that, CHITOSAN played an effective role as preservative. So, we can use CHITOSAN as natural preservative which can play a vital role in our agriculture.

4.9. Total Nitrogen (N) percentage (%)

Nitrogen content is significantly *($p \leq 0.05$)* high (0.9439%, 1.215%, 3.38%) in the treatment-1 in BANANA, PAPAYA and TOMATO respectively. The lowest amount of nitrogen content (0.7593%, 1.02367%, 1.9233%) was associated with the sample of control of BANANA, PAPAYA and TOMATO respectively. Fig. 4.9 shows that percentages of nitrogen content significantly *($p \leq 0.05$)* varied among the control, treatment-1 and treatment-2 in different fruits and vegetables. The descending order of the amount of nitrogen percentages among different Rice field is as follows,

For BANANA: Banana treatment-1 > Banana treatment-2 > Banana Control.
For PAPAYA: Papaya treatment-1 > Papaya Control.
For TOMATO: Tomato treatment-1 > Tomato Control.

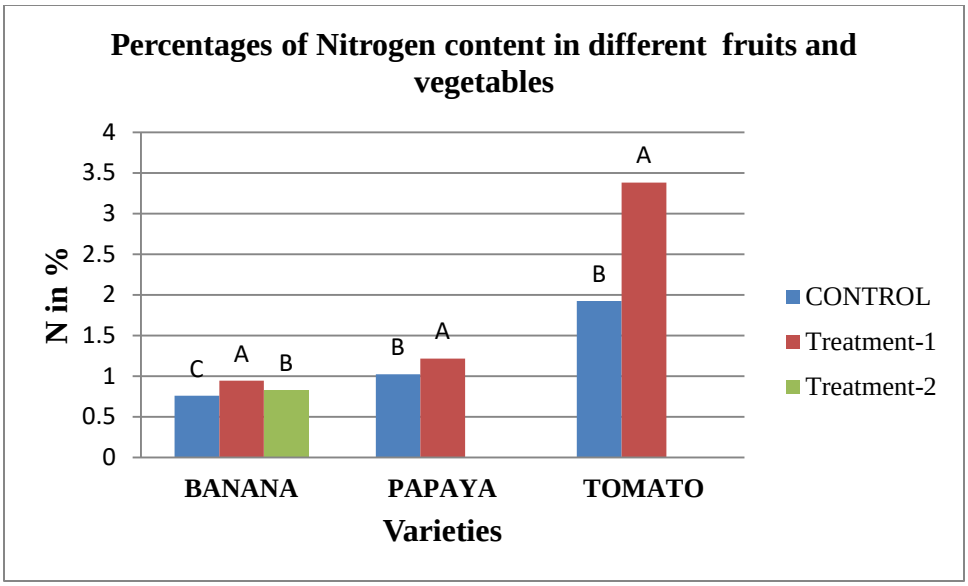


Figure4.9. Distribution of Nitrogen content in different fruits and vegetableswith three replications (Datawere recorded at days 0, 5 and 10).

Chowdhury*et al.* (2011) stated that optimum limit of percentage of total nitrogen (N) is four categories such as low (< 1.180%), medium (1.180-2.360%), high (2.361-4.450%) and very high. According to their standard, Nitrogen level is low in Banana , medium in Papaya and medium to high in Tomato of the experimental fruits and vegetables which might be attributed to limiting effects of all factors (Sumner, 2000). Rahman*et al.* (2014) observed low level of Nitrogen of 1.1-1.3% and stated that the low Nitrogen content may be attributed to low organic matter contents of most of the soils in our country. Portch and Islam (1984) also found that 100% of the fruits and vegetables studied in our country were medium to high in available nitrogen, which was similar to the present findings. The poor nitrogen status of fruits and vegetables is due to high cropping intensity, high rates of decomposition of organic matter and inadequate application of organic matter in terms of manure, compost, and high volatilization of ammonium nitrogen.In case of nitrogen content, if percentages of nitrogen contentis remaining similar or high, it is beneficial. Fig. 4.9 shows that percentages of nitrogen content significantly *($p \leq 0.05$)* varied among the control, treatment-1 and treatment-2 in different fruits and vegetables. From the

result, it can be said that, CHITOSAN played an effective role as preservative. So, we can use CHITOSAN as natural preservative which can play a vital role in our agriculture.

4.10.Phosphorus (P) Content (ppm)

Phosphorus (P) content is insignificantly *(at 5% level)* high (6.7266, 20.163, 42.81 ppm) in the treatment-2 and treatment-1 in BANANA, PAPAYA and TOMATO respectively. The lowest amount of Phosphorus (P) content (5.9576, 18.572, 41.35 ppm) was associated with the sample of control of BANANA, PAPAYA and TOMATO respectively. Fig. 4.10 shows that Phosphorus (P) contentinsignificantly *(at 5% level)* varied among the control, treatment-1 and treatment-2 in different fruits and vegetables. The descending order of the amount of Phosphorus (P) among different Rice field is as follows,

For BANANA: Banana treatment-2>Banana treatment-1> Banana Control . For PAPAYA: Papaya treatment-1 > Papaya Control.

For TOMATO: Tomato treatment-1 > Tomato Control.

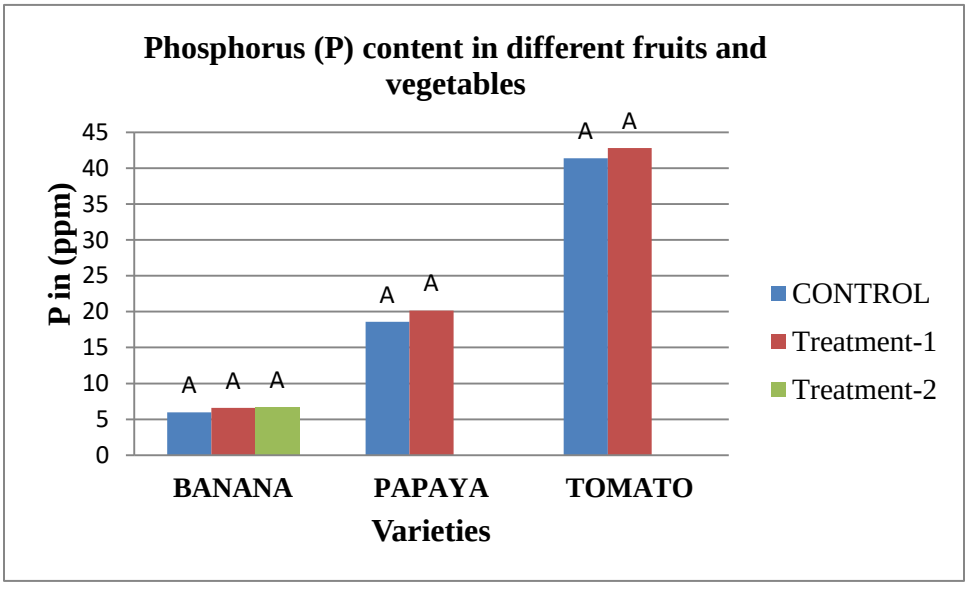


Figure4.10. Distribution of Phosphorus (P) content in different fruits and vegetableswith three replications (Datawere recorded at days 0, 5 and 10).

Four categories for optimum limit of total Phosphorus percentage is such as low (< 12 ppm), medium (12.1-24.00 ppm), high (24.0-30.00 ppm) and very high (> 30. 0 ppm). Thus, Content of total Phosphorus is very low in the sample of all Banana fruits (Fig.: 4.10). Chowdhuryet al. (2011) registered that 41% of the fruits and vegetables of Bangladesh contained Phosphorus below the critical level and 35% of the fruits and vegetables contained Phosphorus above the critical level but below the optimum level. Low range of fruits and vegetables total Phosphorus under study area might be due to the effect of past fertilization, pH, organic matter content, texture and various soil management and agronomic practices (Vermaet al., 2005).In case of Phosphorus (P) content, if Phosphorus (P) contentis remaining similar, it is beneficial. Fig. 4.10 shows that Phosphorus (P) contentinsignificantly (at 5% level) varied among the control, treatment-1 and treatment-2 in different fruits and vegetables. From the result, it can be said that, CHITOSAN played an effective role as preservative. So, we can use CHITOSAN as natural preservative which can play a vital role in our agriculture.

4.11. Available Potassium (K) Content (ppm)

Available Potassium (K) content is significantly ($p \leq 0.05$) high (7.4667, 7.5, 18.1 ppm) in the treatment-1 in BANANA, PAPAYA and TOMATO respectively. The lowest amount of Available Potassium (K) content (6.2, 6.3333, 14.2 ppm) was associated with the sample of treatment-2 and control of BANANA, PAPAYA and TOMATO respectively. Fig. 4.11 shows that Available Potassium (K) content significantly ($p \leq 0.05$) varied among the control, treatment-1 and treatment-2 in different fruits and vegetables. The descending order of the amount of Available Potassium (K) among different Rice field is as follows,

For BANANA: Banana treatment-1 > Banana control> Banana treatment-2 .

For PAPAYA: Papaya treatment-1 > Papaya Control.

For TOMATO: Tomato treatment-1 > Tomato Control.

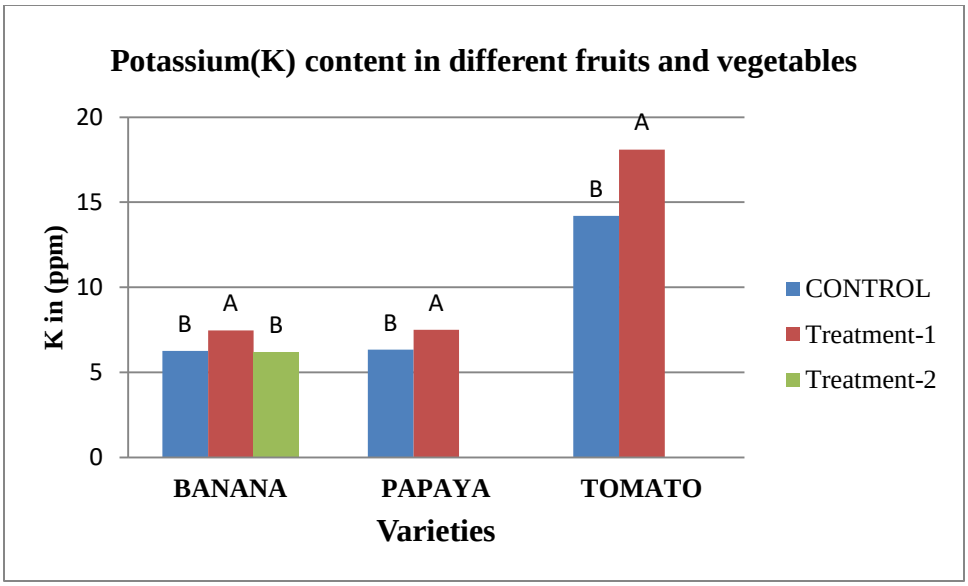


Figure4.11. Distribution of Potassium (K) content in different fruits and vegetableswith three replications (Datawere recorded at days 0, 5 and 10).

Hossainet al. (2015) stated that fruits and vegetables of Bangladesh having Potassium level of (5 to 50 ppm) representsoptimum condition. Thus treatment-1 in different fruits and vegetables as it has a Potassium concentration. Adequate Potassium in different fruits and vegetables may be due to the application of different types of organic and inorganic fertilizers, especially potash fertilizer and the decomposition of the minerals containing potassium (Islam et al., 1985). Potassium is rarely a limiting factor in different fruits and vegetables. Thus it can be stated that different fruits and vegetables cultivation may not be inhibited by lower Potassium content in the study area.In case of Potassium (K) content, if Potassium (K) contentis remaining similar or high, it is beneficial. Fig. 4.11 shows that Available Potassium (K) content significantly ($p \leq 0.05$) varied among the control, treatment-1 and treatment-2 in different fruits and vegetables. From the result, it can be said that, CHITOSAN played an effective role as preservative. So, we can use CHITOSAN as natural preservative which can play a vital role in our agriculture.

CHAPTER 5

SUMMARY AND CONCLUSION

Bio-based polymers are closer to the reality of replacing conventional polymers than ever before. Nowadays, bio-based polymers are commonly found in many applications from commodity to hi-tech applications due to advancement in biotechnologies and public awareness. However, despite these advancements, there are still some drawbacks which prevent the wider commercialization of bio-based polymers in many applications. This is mainly due to performance and price when compared with their conventional counterparts, which remains a significant challenge for bio-based polymers.It is important that the concentration of chitosan be standardized tosuit the produce and its storage conditions. This study points out that 500 ppm chitosan is sufficient for storage of tomatoes, papayas and bananas at low temperature and high humidity. This study shown that coating with chitosan and a chitosan derivative can be employed to extend the shelf life and to improve quality of fruits and vegetables by delaying ripening, reducing weight loss and reducing microbial growth in fruits and vegetables. From the experiment, it can be concluded that 500ppm dose of chitosan is more effective compared to that of 1000ppm dose of chitosan in Banana fruits. The application of chitosan was observed more tolerant in the treatment fruits and vegetables compared to the control fruits and vegetables. The microbial growth were shown more effective result by the application of 500 ppm chitosan compared to the 1000 ppm chitosan. From the result, in case of banana, after 5 days the application of chitosan was observed more tolerant in the treatment fruits compared to the control fruits. After 15 days initially fungus growth was observed in the papaya fruits in control compared to the treatment fruits. It is concluded that, the application of preservatives is more effective to the fungul attack area rather than the whole fruits. In case of tomato initially it was unspoiled about 8 days. After that it got rottend compared to the treatment fruits. Future studies are recommended to assess the effect of coatings on the internal atmosphere of fruit; internal gas must be analyzed for $CO_2$and O_2 levels. One can conclude that the effect of a chitosan coating appears to be

comparable to the control which is without coating in improving postharvest preservation of fruits and vegetables. Due to its lower cost, chitosan isprobably the most attractive and effective biopolymer for achieving conservation of different fruits and vegetables in tropical countries.Chitosan is a strong oxidizing agent that hasgreat potential as a decontamination agent for minimally processed fruits and vegetables. It has high effectiveness against a wide variety of microorganisms. Microbial cellmembrane has been identified as the primary target ofchitosan on microorganisms. It is less effective when microorganismsare hidden in produce wounds. It is useful to prolongthe shelf-life of fruits and vegetables. It does not produce significant amounts of toxic by-products. More research is needed about its effect on the fruit and vegetable physiology and on toxicology issues and nutrients stability. My results suggest that biochemical treatment appears to be a promising new technique for improving the postharvest storage life of tomato, papaya, banana and possibly other fruits and vegetables.

RECOMMENDATION

From the conclusions, the following recommendations can be made:

- Farmers could be adopt the use of chitosan (naturally occurring polymers – as starch, collagen, gelatin, alginate, cellulose chitin etc.) to inhibit the activity of enzymes involved in loss of food quality and used as a food preservative method due to its ability to sterilize microorganisms.
- Studies should be needed to find out the suitable doses of chitosan preservatives to reduce the use of chemical preservatives such as plastic.
- Future studies should be recommended to assess the effect of coatings on the internal atmosphere of fruit; internal gas must be analyzed for CO2 and O2 levels due to its lower cost, chitosan is probably the most attractive and effective biopolymer for achieving conservation of fruits and vegetables in tropical countries.
- A recent survey and market study of the possible future applications of new process technologies (like microwave, ultrasound) in the food industry should be revealed that many companies are reluctant to apply these new technologies.

CHAPTER 6

REFERENCES

Abrol, I.P.; Yadav, J.S.P and Massud, F. 1998.Salt-affected soils and their management. In*:* FAO Soil Bulletin 39, Rome.

Alexnader D.A., Kong H.J. and Mooney D.J.: Alginate hydrogels as biomaterials. *Macromolecular Biosciences* 2006, **6**: 623–633.

Austin J.W., Dodds K.L., Blanchfield B., Farber J.M. 1998. Growth and toxin production by Clostridium botulinum on inoculated fresh cut packaged vegetables. J Food Prot **61**(3):324-8.

Bae K.P, Moo-Moo K: Applications of chitin and its derivatives in biological medicine. *Int J Mol Sci* 2010, **11**: 5152–5164.

Bangladesh Agricultural Research Council (BARC). 2005. *Fertilizer recommendation guide*.

Bansal V, Pramod K.S, Nitin S, Omprakask P, Malviya R: Applications of chitosan and chitosan derivatives for drug delivery. *Adva Biol Res* 2011, **5**: 28–37.

Barkalow D.G, Chapedelaine AH, Dzija MJ: Improved pullulan free edible film compositions and methods of making same. *PCT International Application WO 02/43657, US 01/43397, 21 Nov* 2002.

Bauer R.: Physiology of Dematium pullulans de Bary. *Zentralbl Bacteriol Parasitenkd Infektionskr Hyg Abt2* 1938, **98**: 133–167.

Ben-Yehoshua, S. 1987. Transpiration, water stress, and gas exchange. Pages 113-170. In: Post harvest Physiology of Vegetables. J. Weichmann (Ed.), Marcel Dekker, New York.

Bernier B.: The production of polysaccharides by fungi active in the decomposition of wood and forest litter. *Can J Microbiol* 1958, **4**: 195–204.

Bisanda E.T.N., Ansell M.P.: Properties of sisal-CNSL composites. *J Mater Sci* 1992, **27**: 1690–1700.

Black W.A.P., Woodward F.N.: Alginates from common British brown marine algae. In Natural plant hydrocolloids. *Adv.Chem.Ser Am Chem Soc*1954, **11**: 83–91.

Catley B.J.: Utilization of carbon sources by Pullulariapullulans for the elaboration of extracellular polysaccharides. *Appl.Microbiol* 1971, **22**:641–649.

Chen G.Q., Martin K.P.: Plastics derived from biological sources: present and future: a technical and environmental review. *Chem Rev* 2012, **112**: 2082–2099.

Cheng K.C., Demirci A., Catchmark J.M.: Pullulan: biosynthesis, production, and applications. *Appl Microbiol Biotechnol* 2011, **92**: 29–44.

Chowdhury, M. A.; Khairun, Y.; Salequzzaman, M. and Rahman, M. M. 2011.Effect of combined shrimp and rice farming on water and soil quality in Bangladesh.*Aquaculture International*, **19**(6): 1193-1206.

Conca K.R., Yang T.C.S.: Edible food barrier coatings. In *Biodegradable polymers and packaging*. Edited by: Ching C, Kaplan DL, Thomas EL. Technomic, Lancaster; 1993:357–369.

de Guzman D.: *Bioplastic development increases with new applications*. 2010. . Accessed October 2010

Doug S.: Bioplastics: technologies and global markets. *BCC research reports PLS050A* 2010.

Draget K.I., Skjåk-Braek G., Smidsrød O.: Alginate based new materials. *Int J BiolMacromol* 1997, **21**: 47–55.

Eichhorn S.J., Baillie C.A., Zaferiropouls N., Mwaikambo L.Y., Ansell M.P., Dufresne A., Entwistle K.M., Herrera-Franco P.J., Escamilla G.C., Groom L., Hughes M., Hill C., Rials T.G., Wild P.M.: Review: current international research into cellulosic fibres and composites. *J Material Sc* 2001, **36**:2107–2131.

Elazar, R. 2004. Postharvest Physiology, Pathology and Handling of Fresh Commodities. Lecture Notes. Department of Market Research.Ministry of Agriculture and Rural Development, Israel.

Erich Lück and Gert-Wolfhard von Rymon Lipinski "Foods, 3.Food Additives" in *Ullmann's Encyclopedia of Industrial Chemistry*, 2002, Wiley-VCH, Weinheim.

Erwin T.H., David A.G., Jeffrey J.K., Robert J.W., Ryan P.O.: The eco-profiles for current and near-future NatureWorks polylactide (PLA) production. *Industrial Biotechnology* 2007, **3**: 58–81.

EurActiv.com: *EU calls time on first-generation biofuels*. 2012. . Accessed Oct 2012

FAO 1989 Document Repository, Prevention of post-harvest food losses: Fruits, vegetables and root crops: Atraining manual, Forest Finance Working Paper - 17-2

Georg J.M., Kristensen M., Astrup A.: Effect of alginate supplementation on weight loss in obese subjects completing a 12-week energy restricted diet: a randomized controlled trail. *Am J ClinNutr* 2012, **96**: 5–13.

Goh G.H., Heng P.W.S., Chan L.W.: Alginates as a useful natural polymer for microencapsulation and therapeutic applications. *Carbohydr Polym*2012, **88**: 1–12.

Gomez, K.A., Gomez, A.A., 1984. Statistical Procedures for Agricultural Research. 2nd ed. International Rice Research Institute, Los Banos, Philippines, p. 627.

Gomez-Guille M.C., Gimenez B., Lopez C.M.E., Montero M.P.: Functional bioactive properties of collagen and gelatin from alternative sources: a review. *Food Hydrocolloids* 2011, **25**: 1813–1827.

Guangwen C., Shulian L., Fengjun J., Quan Y.: Catalytic dehydration of bioethanol to ethylene over TiO_2/γ-Al_2O_3 catalyst in microchannel reactors. *Catal today* 2007, **125**: 111–119.

Hafdani F.N., Sadeghinia N.: A review on applications of chitosan as a natural antimicrobial. *World Academy of Sci Engg Technol* 2011, **50**: 252–256.

Hanggi J.U.: Requirements on bacterial polyesters as future substitute for conventional plastics for consumer goods. *FEMS Microbioly Rev* 1995, **16**: 213–220.

Haung Y.M., Li H., Huang X.J., Hu Y.C., Hu Y.: Advances of bio-ethylene. *Chin J Bioprocess Eng* 2008, **6**: 1–6.

Helga E., Svein V.: Biosynthesis and applications of alginates. *Polym Degradation and Stability* 1998, **59**: 85–91.

Helgerud T., Gaserød O., Fjreide T., Andresen P.O., Larsen C.K.: Alginates. In *Food stabilisers, thickeners and gelling agents*. Edited by: Imeson A. Wiley Blackwell, Oxford; 2009:50–72.

Iain A.B., Seal C.J., Wilcox M., Dettmar P.W., Pearson P.J.: Applications of alginates in food. In *Alginates: biology and applications. Microbiology monographs 13*. Edited by: Brend HAR. Springer, Hiedelberg; 2009:211–228.

Irtwange S. V. 2006 Application of Modified Atmosphere Packaging and Related Technology in Postharvest Handling of Fresh Fruits and Vegetables. Agricultural Engineering International: The CIGR Ejournal. Invited Overview No. 4. Vol. VIII.

Islam, M. S., Altamash, S., Sarker, N. I., and Hossain, K. M. 1985.Potassium responses in greenhouse and field studies in Bangladesh.*Proceedings of the International Symposium on Potassium in Agricultural Soils* (pp. 70-89). Soil Science Society of Bangladesh and Bangladesh Agricultural Research Council.

Jackson, M.L. 1962. *Soil Chemical Analysis*. Prentice hall, In: Englewood cliffs, New Jersey, USA.

Jackson, M.L. 1973. *Soil chemical analysis*.Prentice Hall of India Pvt. Ltd. New Delhi. *pp. 495-498.*

Jane J: Starch properties, modifications and applications. *J Macromolecular Sci* 1995, 32: 751–757.

Johnston-Banks F.A.: Gelatin. In *Food gels*. Edited by: Harris P. Elsevier, London; 1990:233–289.

Jones, J. 1999. Tomato plant culture: in the field, greenhouse, and home garden. CRC Press. Boca Raton, FL 1-30.

Jong E.D., Higson A., Walsh P., Maria W.: Bio-based chemicals: value added products from biorefineries. *IEA Bioenergy Task 42 Biorefinery* 2010, 1–34. . Accessed 15 Feb 2012

Kader, A. A. 1992 Postharvest Technology of Horticultural Crops (2nd Edition). UC Publication 3311. University of California, Division of Agriculture and Natural Resources, Oakland, California 94608

Kader, A.A, 1993. Postharvest Handling. In: Preece, J.E. and Read, P.E., The Biology of Horticulture- An Introductory Textbook. New York: John Wiley & Sons. p. 353

Kathiraser Y., Aroua M.K., Ramachandran K.B., Tan I.K.P.: Chemical characterization of medium-chain-length polyhydroxyalkanoates (PHAs) recovered by enzymatic treatment and ultrafiltration. *J Chem Tech Biotech* 2007, **82**: 847–855.

Kitinoja, L. and Kader A.A., 1995 Postharvest Horticulture series no. 8 - March, Small-scale postharvest handling practices - A manual for horticultural crops - 3rd edition Department of Pomology university of California - Davis, CA

Krochta, J.M. and De Mulder-Johnston, C.L.C. 1997 Edible and biodegradable polymer films: Challenges and opportunities. Food Technol. **51**: 60 – 74.

Kupferman, E.M. 1990. Life after benlate: an update on the alternatives. Washington State University Tree Fruit Postharvest Journal **1**(1): 13-15.

Leathers T.D.: Biotechnological production and applications of pullulan. *Appl Microbiol Biotechnol* 2003, **62**: 468–473.

Lee H.C., Anuj S., Lee Y.: Biomedical applications of collagen. *International J of Pharmaceutics* 2001, **221**: 1–22.

Lee L., Arul J., Lencki R., Castaigne F. 1996. A review on modified atmosphere packaging and preservation of fresh fruits and vegetables: physiological basis and practical aspects - part 2.

Lemoigne M.: Production d'acide β-oxybutyrique par certainesbact'eries du groupe du Bacillus subtilis. *CR. Hebd. Seances Acad. Sci* 1923, **176**: 1761.

Li S., Juliane H., Martin K.P.: Product overview and market projection of emerging biobased products. *PRo-BIP* 2009, **1**: 1–245.

Liu M., Zhang Y., Wu C., Xiong S., Zhou C.: Chitosan/halloysite nanotubes bionanocomposites: structure, mechanical properties and biocompatibility. *Int J Biological Macromol* 2012, **51**: 566–575.

Luc A., Eric P.: Biodegradable polymers. In *Environmental silicate nano-biocomposites.Green energy and technology*. Springer, Hiedelberg; 2012:13–39.

Luiz A., De Castro R., Morschbacker : *A method for the production of one or more olefins, an olefin, and a polymer*. 2010. US 2010/0069691A1, 18 Mar 2010 US 2010/0069691A1, 18 Mar 2010

Maeda M., Tani S., Sano A., Fujioka K.: Microstructure and release characteristics of the minipellet, a collagen based drug delivery system for controlled release of protein drugs. *J Controlled Rel* 1999, **62**: 313–324.

Mandel K.G., Daggy B.P., Brodie D.A., Jacoby H.I.: Review article: alginate-raft formulations in the treatment of heartburn and acid reflux.*AlimentPharmacolTher* 2000, **14**: 669–690.

Marguerite R.: Chitin and chitosan: properties and applications. *Progress in PolymSci* 2006, **31**: 603–632.

Maurizio A., Jan J.D.V., Maria E.E., Sabine F., Paolo V., Maria G.V.: Biodegradable starch/clay nanocomposite films for food packaging applications.*FoodChem* 2005,**93**(3):467–474.

Michael C., Dirk C., Harald K., Jan R., Joachim V.: Policy paper on bio-based economy in the EU: level playing field for bio-based chemistry and materials. 2011. Accessed December 2012

Moleyar V., Narasimham P. 1994. Modified atmosphere packaging of vegetables: an appraisal. J Food Sci. Technol. **31**(4):267-78.

Nasiruddin, K.M. and Rahaman, L. 1989. *Effect of Salinity on seed germination and seeding establishment of Rapeseed and Mustard. Bangladesh Journal of Agricultural Science*, **17**(1): 147-152.

Onsoyen E.: Commercial applications of alginates. *Carbohydrates in Europe* 1996, **14**: 26–31.Packaging Technol Sci., **9**:1-17.

Peter W.D., Vicki S., Richardson J.C.: The key role alginates play in health. *Food Hydrocolloids* 2011, **25**: 263–266.

Phillips C.A., 1996. Review: modified atmosphere packaging and its effects on the microbiological quality and safety of produce. Intl J. Food Sci. Technol. **31**:463-79.

Picker K.M., Hoag S.W.: Characterization of the thermal properties of microcrystalline cellulose by modulated temperature differential scanning calorimetry. *J Pharmaceutical Sci* 2002, **91**: 342–349.

Portch, S. and M. S. Islam. 1984. Nutrient status of some important agricultural soils of Bangladesh. pp. 97-106. *In:* "Proceedings of the International Symposium on *Soil Test Crop Response Correlation Studies*", Bangladesh Agricultural Research Council and Soil Science Society of Bangladesh, Dhaka (Bangladesh).

Qin Y., Cai L., Feng D., Shi B., Liu J., Zhang W., Shen Y.: Combined use of chitosan and alginate in the treatment of waste water. *J ApplPolym Sci*2007, **104**: 3181–3587.

Rahman, M.A.; Hassan, K.M.; Alam, M. Akid, A.S.M. and Riyad.A.S.M. 2014.Effects of salinity on land fertility in coastal areas of Bangladesh.*International Journal of*

Renewable Energy and Environmental Engineering ISSN 2348-0157, Vol. 02, No. 03, July 2014.

Ramya R., Venkatesan ,Jayachanndran Kim S., Sudha P.N.: Biomedical applications of chitosan: an overview. *J Biomaterial Tissue Engg* 2012, **2**:100–111.

Rao, A. and Agarwal, S. 1999 Role of lycopene as antioxidant carotenoid in the prevention of chronic diseases: a review. Nutr. Res. **19**(2): 305-323.

Ravi Kumar M.N.V.: A review of chitin and chitosan applications. *Reactive Functional Polym* 2000, **46**: 1–27.

Ray S.S., Bousmina M.: Biodegradable polymers and their layered silicate nanocomposites: in greening the 21st century materials world.*Progress Material Sci* 2005, **50**: 962–1079.

Reis K.C., Pereira J., Smith A.C., Carvalho C.W.P., Wellner N., Yakimets I.: Characterization of polyhydroxybutyrate-hydroxyvalerate (PHB-HV)/maize starch blend films. *J Food Engg* 2008, **89**: 361–369.

Rekha M.R., Chrndra P.S.: Pullulan as a promising biomaterial for biomedical applications: a perspective. *Trends in Biomaterials and Artificial Organs* 2007, **20**: 21–45.

Roberts G.A.F.: Chitosan production routes and their role in determining the structure and properties of the product. In *Advances in Chitin Science, vol. 2, National Taiwan Ocean University, Taiwan*. Edited by: Domard M, Roberts AF, Vårum KM. Jacques Andre, Lyon; 1997:22–31.

Rubin A.L., Stenzel K.H., Miyata T., White M.J., Dune M.: Collagen as a vehicle for drug delivery: preliminary report. *J of Clinical Pharmacology*1973, **13**: 309–312.

Savenkova L., Gercberga Z., Nikolaeva V., Dzene A., Bibers I., Kalina M.: Mechanical properties and biodegradation characteristics of PHB-based films. *Process Biochem* 2000, **35**: 537–579.

Sena R.F., Costelli M.C., Gibson L.H., Coughlin R.W.: Enhanced production of pullulan by two strains of A. pullulans with different concentrations of soybean oil in sucrose solution in batch fermentations. *Brazilian J ChemEng* 2006, **2**: 507–515.

Sharma, P.; Vyas, S.; Mahure, N.V.; Kachhal, P.I.; Sivakumar, N. and yatnam, M. 2011."Effect of acid rain on geotechnical properties of soils of high and low plasticity. *International Journal of Earth Sciences and Engineering*, **4**: 158-160.

Shewfelt, R.L. 1986. Postharvest treatment for extending shelf life of fruits and vegetables. Food Technology **40**(5):7078, 89

Shewfelt, R.L. 1990. Quality of Fruits and Vegetables.A Scientific Status Summary by the Institute of Food Technologists' Expert Panel on Food Safety and Nutrition. Institute of FoodTechnologists, Illinois, USA

Siddhesh N.P., Edgar K.J.: Alginate derivatization: a review chemistry, properties and applications. *Biomaterials* 2012, **33**: 3279–3305.

Singh R.S., Saini G.K., Kennedy J.F.: Pullulan: microbial sources, production and applications. *CarbohydrPolym* 2008, **73**: 515–531.

Steinbüchel A., Valentin H.E.: Diversity of bacterial polyhydroxyalkanoic acids. *FEMS MicrobiolLett* 1995, **128**: 219–228.

Sumner, M. 2000. *Handbook of soil science*.pp. 2148. CRC Press, Boca Raton.

Talukder, S., Khalequzzaman, K.M., Khuda, S.M.K.E., Islam, M. S. and Chowdhury, M.N.A. 2003 Prepackaging, storage losses and physiological changes of fresh tomato as influenced by post harvest treatments, Pakistan J. Biol. Sci. **6** (14): 1205-1207

Teli M.D., Chiplunkar V.: Role of thickeners in final performance of reactive prints. *Textile Dyer Printer* 1986, **19**: 13–19.

Uddin, M. M. M., and Islam, M. S. 1998. Current status, problems and management of the coastal soils of Bangladesh.*Proceedings of the national Seminar on Coastal Environment and Energy Resources in Bangladesh*. Khulna, Bangladesh.

Umesha, T.S., Dinesh, S. V., and Sivapullaiah, P. V. 2012, "Effects of acids on geotechnical propertiesof black cotton soil.*International Journal of Geology,* 6: 69-76.

Valerie D., Vinod D.V.: Pharmaceutical applications of chitosan. *Pharmaceutical SciTechnol Today* 1998, **1**: 246–253.

Verma, V. K., Patel, L. B., Toor, G. S., and Sharma, P. K. 2005. Spatial distribution of macronutrients in soils of arid tract of Punjab, India.*Indian Journal of Agriculture and Biology,* **7**(2): 370-372.

Virginia E., Marie G., Eric P., Luc A.: Structure and properties of glycerol plasticized chitosan obtained by mechanical kneading. *Carbohydrate Polym* 2011, **83**: 947–952.

Walker, D.J. (Ed.) 1992. World Food Programme, Food Storage Manual. Chatham, UK: Natural Resources Institute, p53

Whitaker, J.R. 1996. Enzymes: In Food Chemistry. (3rd ed.) O.R. Fennema (Ed.), Marcel Dekker, New York, p 431-530.

Wills, R. 1981. Postharvest: an introduction to the physiology and handling of fruit and vegetables. McGlasson, Graham, and Joyce (ed). Avi Publishing Co., Connecticut, p 120.

Win N.N., Stevens W.F.: Shrimp chitin as substrate for fungal chitin deacetylase. *Appl Microbiol Biotechnol* 2001, **57**: 334–341.

Xie Z.P., Huang Y., Chen Y.L., Jia Y.: A new gel casting of ceramics by reaction of sodium alginate and calcium iodate at increased temperature. *J Mat SciLett* 2001, **20**: 1255–1257.

Yan Y.F., Krishnaiah D., Rajin M., Bono A.: Cellulose extraction from palm kernel cake using liquid phase oxidation. *J EnggSci Tech* 2009, **4**: 57–68.

Zagory D. 1995.Principles and practice of modified atmosphere packaging of horticultural commodities. In: Farber JM, Dodds KL, editors. Principles of

modified-atmosphere and sous-vide product packaging. Lancaster, PA: Technomic Publishing Co Inc. p 175-204.

Zajic J.E., LeDuy A.: Flocculant and chemical properties of a polysaccharide from Pullulariapullulans. *ApplMicrobiol* 1973, **25**: 628–635.

Zaman, T.M. and Bakri, D.A. 2003.*Dry land salinity and rising water table in the Mulyan Creek Catchment, Australia*. The University of Sydney Orange Leeds Parade, Orange, NSW 2800, Australia. pp. 23-27.

APPENDICES

Appendix I. The preservative effect of chitosan on some selected and available fruits and vegetables in Bangladesh

Controls & Treatments	Color Index	Weight Loss	Electrical Conductivity	pH	Total Suspended Solids (TSS)	Total Dissolved Solids (TDS)
BC	45.83b	16.5667a	0.00021a	4.8533a	3.543a	1.0553a
BT_1	50a	15.81c	0.00021a	4.6667a	3.5557a	1.042a
BT_2	50a	16.03a	0.00021a	4.877a	3.564a	1.03867a
PC	22.22a	12.9033a	0.00088a	5.5533a	4.7987a	1.17967a
PT_1	0b	7.3967b	0.00087a	5.35a	4.8123a	1.1953a
TC	16.66b	12.7867a	0.00023a	4.377a	2.7963a	1.01267a
TT_1	50a	9.49b	0.00023a	4.593a	2.6387a	1.011a
Significant level	*	*	ns	ns	ns	ns

*=Significant at 5% level, ns= Non-significant at 5% level

BC= Banana Control, BT_1= Banana Treatment 1, BT_2= Banana Treatment 2, PC= Papaya Control, PT_1= Papaya Treatment 1, TC= Tomato Control and TT_1= Tomato Treatment 1; T_1= 500 ppm Chitosan and T_2= 1000 ppm Chitosan

Appendix II. The preservative effect of chitosan on the major nutrient contents on some selected and available fruits and vegetables in Bangladesh

Controls & Treatments	Nitrogen (%)	Phosphorus (ppm)	Potassium(ppm)
BC	0.7593c	5.9576a	6.2667b
BT_1	0.9439a	6.5852a	7.4667a
BT_2	0.8277b	6.7266a	6.2b
PC	1.02367b	18.572a	6.3333b
PT_1	1.215a	20.163a	7.5a
TC	1.9233b	41.35a	14.2b
TT_1	3.38a	42.81a	18.1a
Significant level	*	ns	*

*=Significant at 5% level, ns= Non-significant at 5% level

BC= Banana Control, BT_1= Banana Treatment 1, BT_2= Banana Treatment 2, PC= Papaya Control, PT_1= Papaya Treatment 1, TC= Tomato Control and TT_1= Tomato Treatment 1; T_1= 500 ppm Chitosan and T_2= 1000 ppm Chitosan

Appendix III. The preservative effect of chitosan on the microbial effects on some selected and available fruits and vegetables in Bangladesh

Controls & Treatments	Number of Bacteria/g	Number of Fungal/g
BC	966.67a	6133.3a
BT_1	1.1c	4.1333c
BT_2	35.67b	243.33b
PC	816.67a	416.67a
PT_1	3.1b	1.3333b
TC	180a	85.67a
TT_1	3.1333b	1.7b
Significant level	*	*

*=Significant at 5% level

BC= Banana Control, BT_1= Banana Treatment 1, BT_2= Banana Treatment 2, PC= Papaya Control, PT_1= Papaya Treatment 1, TC= Tomato Control and TT_1= Tomato Treatment 1; T_1= 500 ppm Chitosan and T_2= 1000 ppm Chitosan

Plagiarism Detection Center

Original Document Information

Title	ASSESSMENT OF CHITOSAN AS PRESERVATIVE ON SELF LIFE AND MAJOR NUTRIENT CONTENTS ON FRUITS AND VEGETABLES
Author	MUST. ALIMA RAHMAN
Department	Agricultural Chemistry
University	Sher-e-Bangla Agricultural University
Year	2017

Plagiarism Detection Information

Plagiarism of this thesis below the accepting boundary.

This thesis can be forwarded for evaluation and defense.

N.B. This Report is auto generated and it requires no signature.

** This report is generated by the project of Controlling Plagiarism by using Digital Agricultural Theses Archive of Bangladesh (CP:3655)