PROCESS OPTIMISATION AND QUALITY EVALUATION OF COCOA BASED CHOCOLATES

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(2016-24-002)

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2019

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THESIS

Submitted in partial fulfilment of the requirements for the degree of

Doctor of philosophy in Community Science

(FOOD SCIENCE AND NUTRITION)

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF COMMUNITY SCIENCE

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

2019

DECLARATION

I, hereby declare that the thesis entitled "**PROCESS OPTIMISATION AND QUALITY EVALUATION OF COCOA BASED CHOCOLATES**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed during the basis for the award to me of any degree, diploma, associate ship, fellowship or other similar title, of any other University or Society.

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ACKNOWLEDGEMENT

It is with utmost respect and great devotion, I place on record my deep sense of gratitude and indebtedness to my major advisor ***Dr. Seeja Thomachan Panjikkaran****., Assistant Professor and Head, Dept. of Community Science, College of Horticulture, Kerala Agricultural University, Vellanikkara for constant inspiration and evaluation without which this endeavor would not have been fruitful. I also thank for her sustained and valuable guidance, constructive suggestion, consistent patience, friendly approach, constant support, encouragement and care to me during the conduct of this research work and critical scrutiny of the manuscript which helped a lot for the preparation and submission of the thesis in time. I consider myself being fortunate in having the privilege of being guided by her. I express my heartfelt and sincere thanks.*

I express my deep sense of gratitude, indebtedness and heartfelt thanks to my Advisory committee members ***Dr. Aneena E. R.*** *and* ***Dr. Sharon C. L.*** *Assistant Professors, Dept. of Community Science for their enthusiasm, the right support at the right time, and the constant inspiration throughout my doctoral research work. I take this opportunity to extend for the great support showered upon me by both of my teachers from the right and the left.*

I am very much obliged and grateful to ***Dr. B. Suma*** *Professor and Head, Cocoa Research Centre, Kerala Agricultural University and member of my advisory committee for untiring encouragement, advice and support for carrying out my field and laboratory works at the Cocoa Research Centre.*

I have no words to express my unreserved thanks to ***Dr. J. S. Minimol****, Associate Professor, Plant Breeding & Genetics, Cocoa Research Centre and member of my advisory committee for her guidance right from the planning and formulation of the research programme, constructive criticisms, timely interventions and corrections and constant encouragement throughout my study period.*

My special thanks to ***Dr. Lakshmy P.S.,*** *Assistant Professor, Dept. of Community Science, College of Horticulture, Kerala Agricultural University was a real support for me during the last phase of research work and I remember with gratitude for clearing doubts and sharing with me valuable opinion that helped in fine tuning some procedures and processes.*

I wish to express my sincere thanks to ***Sri. S. Krishnan,*** *Assistant Professor, Department of Agricultural Statistics, College of Horticulture for the unparallel and valuable advice extended for the statistical analysis and interpretation of my research data at various stages of observations.*

I convey my special appreciation and heartfelt gratitude to my batch mate and friend ***Remya, P. R.*** *and my beloved juniors****, Ajisha, Simla Thomas, Aathira, Remya Molu, Revathi, Amitha, Aiswariya Girijan, Sruthi, Lovely, Meera*** *and* ***Riya,*** *for their assistance throughout my field, laboratory and preparation of the manuscript. I also thank* ***Mrs. Kumari*** *of Department of Community Science, for the support and help rendered during my study.*

I would also like to express my sincere gratitude to ***Chithira, Athira, Simya, Jayashri, Priya, Sudha, Suma*** *and all other* ***staffs*** *of Cocoa Research Station.*

I express my sincere thanks to the ***Department of Post Harvest Technology****,* ***Radio Tracer Laboratory*** *and the* ***Dept. of Animal Nutrition (KVASU)*** *for all the facilities provided, for their cooperation and support during the conduct of the research. I convey my sincere gratitude to* ***Dr. A. T. Francis,*** *Librarian for the support and guidance in searching and collecting vast research publications relevant to my research work.*

My special thanks to ***Mr. Aravind*** *and* ***Ms. Rajitha*** *staff of Student's Computer Club. I convey my earnest thanks to my beloved batch mates and all the seniors, juniors and well-wishers, who directly or indirectly helped me, successfully complete this project.*

*I am thankful to **Kerala Agricultural University** for providing with technical, administrative, financial assistance and for providing **KAU Fellowship** in pursuit of my study and research work.*

I am very much grateful to express my gratitude to my most beloved parents- Razak master and Jameela, father and mother–in–laws, my spouse and our beloved kids Aman, Ameen and Aadil Mon; and my brothers and all the in laws in my family needs special mention. With their relentless back up and encouragement, I was able to do more justice to my doctoral research work and thesis preparation.

Above all, I bow my head to the Almighty for the blessings showered up on me throughout the life.

Shahanas E.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDIES

Sl. No.	TITLE
1.	Score card for organoleptic evaluation of alkalised chocolates made at 60^0C and 70^0 C
2.	Score card for organoleptic evaluation of non alkalised chocolates made at 60^0C and 70^0 C
3.	Score card for organoleptic evaluation for the standardisation of dehydrated mint powder in the chocolate
4.	Score card for organoleptic evaluation for the standardisation of white pepper powder in the chocolate
5.	Score card for organoleptic evaluation for the standardisation alkalised chocolateswith dried fruits, nuts, dehydrated mint leaves and white pepper powder
6.	Score card for organoleptic evaluation for the standardisation non alkalised chocolates with dried fruits, nuts, dehydrated mint leaves and white pepper powder
7.	Sensory evaluation of alkalised and non alkalised blended chocolates with jack fruit, almond and white pepper powder

ABBREVIATIONS

A. O. A. C	- Association of Official Analysis Chemists
g	- Gram
mg	- Milligram
ml	- Millilitre
^{o} brix	-Degree brix
%	- Per cent
TSS	-Total soluble solids
cfu	- colony form units

Introduction

INTRODUCTION

"Chocolate is a perfect food and a little chocolate now and then doesn't hurt, but keeps the doctor at a bay".

Marela Cavington

The cocoa tree (*Theobroma cacao* L.) is the source of most popular food product chocolates which have a rich history forms the basis for one of the world's most popular food products chocolate, which have a rich history of cultures and carrying important economic and social implications to millions of people worldwide (Krahmer *et al*., 2015). This tree was designated as *Theobroma cacao* by Linnaeus in 1735. *Theobroma* the genus names is from the Greek and translates into "Food of the Gods."

Cocoa is a supporting crop to farmers due to its remunerative income. The quality of final product depends upon the fermented dried beans. The economic value of the cocoa beans was reduced due to high free fatty acid (FFA) content. An increase in the per cent of FFA is one of the clear indications of deterioration in cocoa quality. FFA are carboxylic acids released from triglycerides (Selamat *et al*., 1996) through the effect of a lipase or oxidation.

Recently, the cocoa trade has assumed a more scientific position and a lot of emphasis are placed on the content of free fatty acid. A reduction in the free fatty acids level will definitely have a positive impact. The different types of free fatty acid present in cocoa beans are oleic acid, acetic acid and palmitic acid. It is expected that the free fatty acids content must be less than 1.75per cent in dry cocoa beans (Jonfia and Navarro, 2010). As such there is a need to carry more research to develop a technology for the standardisation of free fatty acid content in the processing stages of cocoa in both primary and secondary processing.

Cocoa processing or chocolate making consists of a multistep process mainly primary and secondary processing. In the primary processing, after harvesting the cocoa beans are subjected to fermentation. The cocoa fruit contains

about 30–40 seeds covered by a mucilaginous pulp removed by yeast and bacteria during fermentation, which is a key step for the development of chocolate flavour and it produces aroma precursors. After fermentation, a drying step is required to reduce the water content to 5–7per cent; this ensures product stability before further processing. Dried cocoa beans or nibs are then roasted to develop the chocolate flavour (Mattia and Sager, 2015).

Secondary processing involves the conversion of cured beans into different finished products. Secondary processing includes cleaning, alkalisation, roasting, kibbling, winnowing, blending, grinding, extraction of cocoa butter and production of chocolate (Fowler, 1999). For the production of milk chocolate, the basic ingredients are cocoa liquor, sugar, cocoa butter and milk powder. Milk and other ingredients may be added, mixed and then refined to reduce the particle sizes of solids. After refining, the conching, which consists of the agitation of the chocolate mass at high temperatures, and finally tempering, which consists of heating, cooling and mixing process, are required for the development of the final texture and flavour.

Commercial production of chocolates are dominated by multinational companies. Small scale production of chocolates are mainly done at household levels. Small scale producers fail to reach to the standard of commercial producers due to the inappropriate processing techniques. Chocolates when subjected to tempering and conching operations will definitely improve the texture of the products. Hence, in this study an attempt has been made to optimise the time and temperature of fabricated small scale tempering and conching machine suitable for small scale producers.

Chocolate, due to its unique structure and flavour, is a food usually consumed for pleasure that has been recently reconsidered as a source of healthy compounds. Chocolate is a processed food produced from the seed of the tropical tree *Theobroma cacao* L. Chocolates are increasingly being seen as capable of promoting good health.

Chocolate has been known for its good taste and proposed health effects for centuries. The chocolate is lauded for its tremendous antioxidant potential. The biologically active phenolic compound in cocoa has changed the perception of chocolates which was criticized for its high fat and sugar content. Several researches have shown beneficial effect of chocolate in reducing oxidative stress, blood pressure regulation and cardio vascular diseases (Buijsse *et al*., 2006).

Cocoa and cocoa derived products comprise one of the most popular super foods and it contain high density of essential nutrients like energy, carbohydrate, protein, fat, polyphenol and minerals. According to USDA (2016) the 100gram cocoa powder contain the nutrients like carbohydrate (54.3g), protein (19.6g), fat (12.7g), fibre (5.1g) and minerals like magnesium (590 mg), phosphorus (960 mg) and potassium (1700 mg).

Several types of chocolate are available in the market. In the present scenario, consumers give importance not only for health benefits and nutritional qualities but also for variety in taste. Cocoa beans are mostly processed into chocolate and also for production of cocoa based products such as cocoa liquor, cocoa butter, cocoa cake and raw cocoa powder (Afoakwa *et al*., 2007). Value addition in chocolates has an immense scope in the present scenario. Chocolate blends well with fruits, nuts and spices. Hence, in this study an attempt was also made to evaluate the organoleptic, physiological and shelf life properties of blended chocolates.

The present study entitled "Process optimisation and quality evaluation of cocoa based chocolates" was under taken with the following objectives

- To develop protocol for primary processing of cocoa beans based on free fatty acid content (<1.75 %).
- To standardise the time and temperature of chocolate making using machine
- To evaluate the quality attributes and shelf life of the products.

Review of Literature

REVIEW OF LITERATURE

Chocolate, is a food usually consumed for pleasure due to its good taste, flavour and proposed health benefits. The scientific name of cocoa tree, *Theobroma cacao*, derived from the Greek words *theo* means 'God' and *broma* means 'Food' (Dillinger, 2000). The Food of the Gods, provides the raw material for the chocolate production and it is a treasure of bioactive compounds polyphenols.

The literature pertaining to the study entitled "Process optimisation and quality evaluation of cocoa based chocolates" is presented under the following sub headings,

2.1. Cocoa- Scenario of cultivation

2.2. Processing of cocoa

2.2.1. Primary processing of cocoa

2.2.2. Secondary processing of cocoa

2.3. Chemical constituents of cocoa

2.3.1. Primary metabolites

2.3.2. Secondary metabolites

2.4. Value added products from cocoa

2.5. Health benefits of chocolates

2.6. Storage and keeping quality of chocolates

2.1. Cocoa- Scenario of cultivation

Cocoa plant (*Theobroma cacao* L.) is a small (4 to 8 m height) evergreen tree and is an international commodity which is important and even vital for both producer and industrial countries (Misnawi *et al*., 2003).

Theobroma cacao L is an important agricultural commodity and it is grown by about 6 million farmers globally. World Cocoa Foundation (2010) reported that about 5–6 million farmers in developing countries in Africa, Asia and Latin

America produced around 90 per cent of cocoa in worldwide. About forty million people depend on cocoa for their livelihoods (Beg *et al*., 2017).

Cocoa is a third important beverage crop next to coffee and tea and is the third highest traded commodity in the world after coffee and sugar. It is mainly grown in tropical countries of the world. When cultivation is confined to under developed countries, major production is consumed by the people in richest countries (Amma *et al*., 2009).

Cocoa is cultivated in areas with suitable environment, on lands covering over 70,000 km^2 worldwide between 20° north and south of the equator (Fowler, 1999). According to Dillinger *et al*. (2000), about 70 per cent of the world's cocoa production takes place in the equatorial region of West Africa and the rest in the equatorial regions of Central and South America, the West Indies and tropical areas of Asia. The Mayan people were first cultivated the cocoa plant in 400AD (Verna, 2013).

Cocoa is originated from the Amazonian basin (Wood and Lass, 1985 and Motamayor *et al.,* 2002), and today, it is cultivated in many regions of the humid tropics. ICCO (2015) reported that the annual global cocoa production was more than 4 million tons per in 2013/2014 crop season. Pipitone (2016), also reported that the estimation of International Cocoa Organization (ICCO) was more than 4.0 million metric tons of cocoa beans were produced worldwide and of this Africa contributed approximately 74 per cent (2.92 million tonnes) in the 2015-16 season.

Cocoa is a perennial tree crop in many tropical countries and West African countries of Ghana, Ivory Coast, Nigeria and Cameroon together accounted for 68 per cent of world production, followed by Indonesia and Brazil with 13 per cent and 5 per cent respectively (Misnawi *et al*., 2002).

The major cocoa producing countries are Cote d'Ivoire, Ghana, Indonesia, Brazil and Nigeria, their contribution being 84 per cent of the world total. The African countries produce 69 per cent and the central and South American countries

produces 13 per cent of the total. The Asian countries produce the remaining 18 per cent. The contribution of India is negligible (Amma *et al.*, 2009).

Verna (2013) also opined that the cocoa plant is grown in several countries, mainly, Ivory Coast, Ghana, Indonesia, Nigeria, Brazil, Cameroon, Ecuador, Dominica Republic and Papua New Guinea. The majority of world cocoa production (approximately 80%–90%) comes from small holder farmers (Khader, 2015).

The cocoa crop is originated from the Amazonian basin and it is cultivated in many regions of the humid tropics (Motamayor *et al*., 2002; Wood and Lass, 1985). The cocoa bean production contributes the economy of the growing regions and it also serves as a main source of income for millions of small holder farmers (Darkwah and Verter, 2014)

The production of cocoa begins in the tropical regions around the Equator, where the hot and humid climate is well suited for growing cocoa trees (Fowler, 1999). ICCO (2008) reported that the world production dropped by almost nine per cent to 3.4 million tonnes in 2006-2007, as a consequence of unfavourable weather conditions.

According to the International Cocoa Organization (2012), In 2009/2010 season, the cocoa production from Ghana accounted for about 17.5 per cent of global production, second to Cote D'Ivoire, which produced 34 per cent. Khader (2015) also stated that about 70 per cent of the world's cocoa beans come from four West African countries: Ivory Coast, Ghana, Nigeria and Cameroon. The Ivory Coast and Ghana are the two largest producers of cocoa, accounting for more than 50 per cent of the world´s cocoa. In 2016, the Ivory Coast alone produced approximately 1.6 million metric tons of cocoa beans and the production in 2016-2017 is 1.9 million metric tons. These countries collectively accounted for 68per cent of world production, followed by Indonesia and Brazil with 13 per cent and 5 per cent respectively. Statistics revealed that the production of cocoa beans, by the

six major cocoa producing countries, increased from 3.53 to 4.05 million metric tons of cocoa beans from the crop year 2012-2013 to 2017-2018 (WTO, 2018).

Grey (2000) reported that about 75 per cent of the world cocoa population come from Asia, Africa and Latin America. Cocoa plays an important role in most of these economies as a source of foreign exchange. It provides jobs for an estimated fourteen million people (FAO, 2005).

The developing countries are the large producer of cocoa but it is mostly consumed by industrialised countries. The processors and the chocolate manufactures are the purchasers of cocoa in the consuming countries. But now chocolates and other cocoa products are popular throughout the world (Amma *et al*., 2009).

Bhavani, (2017) reported that in India, it is mainly cultivated in Karnataka, Kerala and Tamil Nadu mainly as intercrop with Arecanut and Coconut. The commercial scale cultivation of cocoa started in 1960s. But the middle of 1970s, there was a boost in the acceptance of cocoa as a crop due to the impressive price of raw beans in Indian and international markets.

Amma *et al*. (2009) and Bhavani, (2017) reported that the area increased to 1927 hectares by1970-71 and further to 29000 hectares by 1980-81 and then there was a drastic decline due to the reduction in price. In 2008, India produced approximately 8500 metric tons of cocoa. In 2009, the area under the crop is 31885 hectares and the production is 10560 metric tons with a mean productivity of 535 kg dry beans per hectare.

The area expansion programme in Andhra Pradesh resulted in remarkable increase in area of cocoa by 39.98 per cent. This programme is implemented in Tamil Nadu to cover 50000 hectares in a span of five years (Amma *et al*., 2009).

Cocoa is an important plantation crop grown for chocolates around the world. In India, the states of Kerala, Karnataka, Andhra Pradesh and Tamil Nadu have major cocoa cultivation in an area of 78000 ha with total production of 16,050

MT. Tamil Nadu ranks first with an area of 26,969 ha whereas Andhra Pradesh ranks first in production. The highest productivity is in Kerala which is 785 kg/ha. The average productivity of cocoa in India is 475 Kg/ha (DCCD, 2019).

Kerala accounts for about 33.04 per cent of the area in the country with production share of 56.81 per cent. In Kerala, large area additionally brought under cultivation, mainly in Idukki district especially due to the attractive prices and remunerative returns. Statistics shows that the production of cocoa beans, by the four major cocoa producing states in India increased from 5.19 – 10.56 million metric tons of cocoa beans from the crop year 1998-99 to 2007-2008 (Amma *et al*., 2009). Kerala state have more cocoa production of 6.00 million metric tons of cocoa beans in the crop year 2007-2008 compared to other states Karnataka and Andhra Pradesh (2.76 and 1.58 million metric tons respectively) .

The cocoa belongs to the Malvaceae family and more than 20 species are known within the *Theobroma* genus (Wood and Lass, 1985). Among these, *Theobroma cacao* is the only species cultivated extensively. The species has three genetic groups based on morphological and anatomical characteristic *Criollo, Forastero* and *Trinitario* (Pridmore *et al*., 2000).

Traditionally, cocoa can be classified into three types, namely *Criollo, Forastero* and *Trinitario* based on genetics, morphology, geographical origin and flavour quality attributes (Cheesman, 1944). Three primary cocoa types: *Forastero* (bulk grade), *Criollo* (fine grade) and *Trinitario* (fine grade) showed wide variations in flavor quality (Awua, 2002; Amoye, 2006). According to Ferrazzano *et al.* (2009) based on botanical point of view three main varieties of cocoa trees are *Criollo* (fine grade), *Forastero* (bulk grade) and *Trinitario* (fine grade).

Criollo variety is good and high quality and requires good growing conditions. The cocoa beans *criollo* variety have a distinctive flavours and aromas (Rusconi and Conti, 2010). The tropical forests of Mexico, Central America, and northern part of South America are the growing region of *criolla* variety (Bertazzo *et al*., 2011).

Forastero cocoa variety growing in the Upper Amazon, Lower Amazon, the Orinoco, or the Guyanas (Motamayor *et al*., 2003). Most of the harvests come from Ghana, Ivory Coast, Sao Tome, Togo, Nigeria, Brazil, Indonesia, and Malaysia (Franzen and Mulder, 2007). *Forastero* variety of trees includes many subtypes, from which the strong and disease-resistant cocoa beans are obtained (Rusconi and Conti, 2010).

Most of the world's chocolate production (approximately 80%) comes from the *Forastero* type of cocoa; this variety is favoured over the *Criollo* for its disease resistant and high-yielding nature and beans from this variety are relatively cheaper than those from the Criollo type (Rusconi and Conti, 2010). The flavour and taste of these beans is much less aromatic than the fine varieties.According to Caligiani *et al*. (2010) the seeds of *Forastero* variety have excellent aroma and taste similar to *Criollo* and *Trinitario*.

Trinitario is a new hybrid variety with beneficial sensory traits and more resistant to adverse environmental conditions (Lachenaud *et al.*, 2007). This species is a hybrid of beans of *Criollo* from Trinidad and *Forastero* from the upper region of the Amazon basin. *Trinitario* trees were originally grown in Trinidad.

2.2. Processing of cocoa

Cocoa is an important crop around the world and is an important cash crop for growing countries, as well as an important branch of the food industry for processing and consuming countries. Cocoa (*Theobroma cacao* L.) is a cash crop of huge economic significance in the world and the key raw material for chocolate manufacturing (Krahmer *et al*., 2015).

The expectations of chocolate manufacturers directed on the qualitative parameters by country origin aim at having uniform and constant raw materials to produce chocolate. The quality and flavor of cocoa products strongly depend on the various stages of cocoa processing (Saltini *et al.*, 2013). These processes begin very

early with cocoa farming, storage, fermentation, drying and packing the cocoa beans and continue with the manufacturing of chocolate.

Harvest and post harvest operations affect the quality of cocoa for marketing and chocolate preparation. The pods mature in about 150 to 170 days from of pollination. The stage of maturity of pods is judged by change of colour of pods. Pods, which are green when immature turn yellow when mature and the reddish pods turn yellow or orange (Amma and Minimol, 2012).

Chocolate industry is a competitive market with different branded chocolates. So it is very important to produce the good quality cocoa beans (Arikiah, 2008). These processes include pulp pre-conditioning, fermentation and drying (Krahmer *et al*., 2015).

The pulp is the substrate metabolised by a sequence of microorganisms during fermentation. Pulp pre-conditioning involves changing the properties of the pulp prior to the development of microorganisms in fermentation (Afoakwa *et al*., 2010).

Pulp preconditioning can be done in three basic ways prior to fermentation and these are pod storage, depulping (mechanical or enzymatic depulping) and bean spreading (Schwan and Wheals, 2004). Nazaruddin *et al.* (2006) reported that prefermentation treatments have significant effects in changing the acidity and polyphenol content and flavour of the cocoa beans.

Nazaruddin *et al*. (2006) reported that pulp preconditioning of cocoa prior to fermentation was significant in affecting the changes in acidity, causing a significant reduction in the content of polyphenol compounds especially epicatechin and catechin during fermentation. Pulp pre conditioning reduce the astringency and bitterness in cocoa and cocoa products. The harvested pods can be stored for three to five days. According to Afoakwa *et al*. (2010) pod storage is storing harvested cocoa pods for a period of time before opening the pods and fermenting the cocoa beans

According to Amma *et al.* (2009) the post harvest storage of cocoa beans enhances the pre fermentation activity inside the pods and helps to facilitate rapid rise in temperature during fermentation, reduces acidity and imparts stronger chocolate flavour. Afoakwa *et al*. (2010) also observed that pod storage appears to have highly beneficial effect on the chemical composition of cocoa beans and subsequent development of chocolate flavour.

Krahmer *et al*. (2015) also reported that post harvest treatment of cocoa play an important role in the flavour profile of the cocoa beans. Post harvest treatment involves all the primary process, fermentation harvested of cocoa beans drying of fermented cocoa beans. The good quality cocoa can be produced if care is bestowed in cultivation, processing and drying techniques.

After post harvest treatment the cocoa pods are broken by hitting against a hard surface and beans are extracted and kept for fermentation. Amma *et al.* (2009) reported that the processing of cocoa involves manly two steps, primary processing and secondary processing.

2.2.1. Primary processing of cocoa

Primary processing denotes production of dry cured beans for the market. This involves fermentation and drying. The microbial fermentation and drying process are two major steps in the processing of cocoa beans and they are essential in the formation of flavour, which is an important parameter of quality in chocolate processing (Hii *et al*., 2009)

Fermentation is a metabolic process in which carbohydrates and related compounds are oxidized with the release of energy by the action of microorganisms (Rose, 1983). Raw cocoa beans are covered with sugary mucilaginous pulp and the beans with the pulp around are called ‘wet beans’. The kernel which is also called ‘nib’, is the economically important part. Fresh nib is bitter and is not suitable for manufacture of different products (Amma *et al.,* 2009).

Thompson *et al.* (2001) reported that to develop the typical flavour of cocoa, the cocoa beans were subjected to fermentation process during which highly bitter tannins present in them are oxidized, resulting in the formation of aromatic substances and the development of the typical brown to deep red-brown colour. Kadow *et al*. (2013) stated that fermentation of cocoa beans is very crucial as it promotes dramatic biochemical changes in the type and concentration of flavour precursors in cocoa beans. According to Wollgast and Anklam (2000) during fermentation the chemical and biochemical compounds in cocoa interact in cotyledons to give cocoa aromatic flavour.

Fermentation is the initial step needed in the development of various flavour precursors in the beans (Hii *et al*., 2009). Khader (2015) stated that all the standard method of fermentation involve keeping together a mass of reasonable quantity of wet beans for periods ranging from four to six days.

In most of the standard methods, mass of beans is mixed on alternate days as a result of fermentation most of the pulp around the beans were lost and a series of bio-chemical changes occur in the beans, which are necessary for imparting chocolate flavour (Aculey *et al*. 2010). Heat is produced by keeping fresh beans compactly and the heat must be conserved so that chemical changes inside the bean are completed (Amma and Minimol, 2012).

During fermentation, the temperature of the beans rise from ambient to about 50 to 55°C due to the exothermic oxidation reaction (Wood and Lass, 1985). During fermentation a series of biochemical reactions occurs in the beans. Chocolate flavour is developed during fermentation and roasting.

Amma and Minimol (2012) opined that the astringent and bitter raw beans attain chocolate flavor during fermentation and roasting. Fermentation of cocoa beans is very crucial as it promotes dramatic biochemical changes in the type and concentration of flavour precursors in cocoa beans (Kadow *et al*., 2013).

Wood and Lass, (1985) opined that the beans are put for fermentation immediately after taking out of the pods. Several methods are employed in the fermentation of cocoa beans, the choice being dependent on the quantity of beans available for fermentation and the circumstances prevailing (Schwan and Wheals, 2004). Camu *et al.* (2008) stated that different fermentation methods are used for fermenting cocoa beans which depends on farmers, area and countries. Guehi *et al.* (2010) also reported that fermentation methods depends on areas and countries.

Fermentation method consists of keeping a reasonable quantity of beans together for 6-7 days with sufficient insulation (to maintain the temperature developed), with sufficient aeration (to help aerobic fermentation) and mixing in alternate days (to ensure uniform fermentation) (Amma and Minimol, 2012). Hii, *et al*. (2009) reported that raw cocoa beans have an astringent and unpleasant taste and have to be fermented and dried to obtain the characteristic cocoa taste and flavour.

The duration and method of fermentation are crucial to the formation of flavour compounds and flavour precursors (Aculey *et al*. 2010). Longer fermentation periods induces the growth of unwanted moulds and consequently off-flavors (Guehi *et al.*, 2010).

Amma *et al*. (2009) standardised and recorded the different types of fermentation methods like plastic gunny bags, heaps, baskets and boxes, which are the mostly used fermentation methods in large scale and small scale methods. Guehi *et al.* (2010) reported that in contrast to the box method of fermentation, the temperature increases faster in the heap method at the beginning of the process. The fermentation in boxes shows a relatively low concentration of sugars, ethanol and acetic acid, as well as a high pH (Wallace and Giusti, 2011).

Shamsuddin and Dimmick (1986) reported that cut test is used as a criteria to know the index of fermentation. Based on the colour changes in cotyledons during fermentation the fermentation index was recorded. Misnawi *et al.* (2003)

also reported that cut test or fermentation index determine the degree of cocoa beans fermentation, along with the formation of brown colour.

Cocoa beans are dried after fermentation in order to reduce the moisture content from about 60 per cent to about 7-8 per cent (Afoakwa, 2010). The drying process of fermented cocoa beans initiates major polyphenol oxidizing reactions catalysed by polyphenol oxidase, giving rise to new flavour components and loss of membrane integrity, inducing brown colour formation (Afoakwa *et al*., 2010).

Drying helps to reduce bitterness and astringency and also the development of the chocolate brown colour of well fermented cocoa beans. Drying process prevent the mould infestation during storage and also allow some of the chemical changes which occurred during fermentation to continue and improve flavour development (Kyi *et al*., 2005).

Amma *et al*. (2009) stated that the moisture content has to be brought down to about 6per cent for safe storage and transportation. Drying should commence immediately on cessation of fermentation, unless the beans are skin dry within 24 hours after fermentation, moulds set in and damage the beans. The aim of cocoa drying is to remove water so as to reach moisture content below 7per cent and is usually carried out by sun heating in static conditions and heating dryers are also used (Carla *et al*., 2017).

Drying continues the process of oxidation that follows after the fermentation process and therefore plays an important role in the composition of polyphenols (Wollgast and Anklam, 2000). The drying process interferes with biochemical reactions initiated during fermentation, leading to a reduction in the bitterness, astringency and acidity of cocoa beans. Darkening of cotyledons contribute to the formation of precursors for desirable flavour in the final product (Beckett, 2009). Hii *et al.* (2009) also showed that the dried cocoa beans are less in bitterness and astringency, having the best flavour profile with a high level of cocoa flavour.

Payne *et al.* (2010) also reported that drying has an important role in the flavour development of chocolates. Drying rate during the drying process is have the importance for the final quality of the cocoa beans. Too slow drying rate would result in low acidity, poorer colour and high presence of moulds (Bharath and Connor, 2008). Amma *et al*. (2009) opined that the biochemical oxidation of acetic acid from the beans continues during drying, so very quick drying or heating of the beans will not be suitable. A too fast drying affects the excessive production of acids, including acetic acid, which is deleterious to the flavour, while too slow drying results in lower pH, the absence of the optimal colour of cocoa beans and increased growth of moulds (Zahouli *et al.*, 2010). Saltini *et al.* (2013) also suggested that the drying process must not be too rapid otherwise the beans tend to retain an excessive amount of acetic acid, and this is deleterious to flavour.

There are two methods for drying the beans, sun drying and artificial drying. Sun drying is the simplest and the most popular method and generally gives good quality beans. Depending up on the climatic conditions, the beans are exposed to sun with intermittent stirring for about 7-10 days (Amma *et al*., 2009). The quality of dried cocoa beans depends on the use of conventional hot air dryers and sun drying (Hii *et al*, 2009). Amma and Minimol, (2012) suggested when climatic conditions are not suitable for drying, artificial drying method becomes necessary.

Several types of artificial dryers are being used in drying of cocoa beans. Raw cocoa beans were artificially dried using an air ventilated oven at temperature of 60^0C until the moisture content of 7 per cent as reported (Hii *et al*., 2009). The major conditions recognised are temperature, rate of air flow, bean depth and extend of bean stirring (Amma and Minimol, 2012).

Amma *et al*. (2009) opined that the bean recovery of cured beans varies from 23-46 per cent depending up on the season and variety of cocoa. PHAMA, (2018) stated that the expected bean recovery rate is between the range of 30 – 40 per cent.

2.2.2. Secondary processing of cocoa

Secondary processing denotes the steps involved in conversion of cured beans in to different finished products. The development of flavour in cocoa and chocolates lies by roasting the beans followed by extraction of cocoa butter from nib to produce cocoa powder and addition of cocoa butter and sugar in chocolate making. The major steps involved in secondary processing are cleaning and sorting, alkalisation, roasting, extraction of butter and making of cocoa powder and production of chocolate (Amma *et al*., 2009).

The beans are cleaned to remove the flat and poor quality beans. It is then sorted to separate the small or broken beans. Cocoa nibs, cocoa liquor, and cocoa powder can be modified by treatment with alkali, also known as alkalisation (Giacometti *et al*., 2015).

Lacueva *et al.* (2008) reported that the alkalisation process modify the flavor and colour of cocoa powders. When beans are used for manufacture of cocoa powder, it is treated with alkali to improve the colour and to develop flavour. Beans is alkalised by soaking one kilograms of fermented dried beans for three hours in one litre water with ten gram of sodium bicarbonate then drained after three hours and dried. The protocol for alkalisation is standardised by Kerala Agricultural University (Amma *et al*., 2009).

Alkalisation caused a progressive reduction of polyphenols as well as their antioxidant activity (Miller *et al.,* 2008). The cocoa butter flavour will be altered, depending on alkalisation. Sulistyowati and Misnawi, (2008) describe that reduction of the polyphenol antioxidant activity was triggered by alkalisation.

Due to alkalisation, flavones are reduced, polyphenols such as anthocyanins and catechins are reduced into quinones which undergo polymerization to form darker chocolate (Miller *et al*., 2008). Polyphenols, which has beneficial health effects, are decreased during alkalisation. Hence, considering the health point of view there is a huge demand from the consumers for natural non alkalised

chocolates. A study by Payne *et al.* (2010) found that compared to natural cocoa powders, alkalisation caused a loss in both epicatechin (up to 98%) and catechin (up to 80%).

Roasting is one of the important steps which affects the quality characteristic of cocoa beans during industrial processing. Roasting of cocoa beans termed as treatment of cocoa beans in hot air, can be done using direct or indirect heating (Misnawi *et al.*, 2003). According to Oliviero *et al.* (2009) roasting of the fermented cocoa beans remove the undesired compounds with low boiling points, such as acetic acid, and also roasting caused the formation of the typical roasty, sweet odorants of cocoa.

Roasting determines the character of the chemical and physical processes that occur inside the beans, as well as the quality of the final products (Krysiak, 2006). According to Cooper *et al.* (2007) roasting modifies the precursor compounds of flavour and aromas of origin, those formed during fermentation and drying. Afoakwa *et al*. (2008) reported that during roasting, there is evaporation of volatile acids from the beans causing a reduction in acidity, hence, reducing sourness and bitterness of the cocoa beans. Roasting involves complex chemical transformations, attributed to Maillard reactions, caramelization of sugars, protein degradation, and synthesis of sulphur compounds (Sacchetti *et al*., 2016).

Several studies revealed that the temperature and duration of roasting substantially affect the chemical and physical changes occurring in cocoa beans (Oliviero *et al.*, 2009). Amma *et al*. (2009) also reported that the most favoured temperature for roasting of cocoa beans for chocolate making lies between 120°C and 125°C. Farah *et al*. (2012) also reported that the cocoa roasting process is carried out at high temperatures of 120–150°C from 15 to 45 minutes. Kothe *et al*. (2013) reported that time and temperature of the roasting process depend on several factors, such as cocoa material (beans, nibs or liquor roasting), final cocoa product (dark or milk chocolates) and type of cocoa (Criollo or Forastero).

Misnawi *et al.* (2002) studied sensory properties of cocoa liquor as affected by the polyphenol concentration and duration of roasting. They found that cocoa polyphenols had a negative effect on flavour properties. Roasting generally depletes the antioxidant activity of cocoa. Arlorio *et al*. (2008) reported that the antioxidant activity decreased between 37 and 48 per cent after pre-roasting at 100°C and also roasting at 130°C.

Cocoa butter is a valuable ingredient in food, pharmaceutical, cosmetic, health and other industries. It consists of three fatty acids: palmitic, stearic and oleic acid (Rahoma *et al.,* 2002). For the extraction of coca butter the roasted seeds are ground using a ball mill crusher or a grinding machine to obtain a cocoa mass or paste used to make cocoa butter or chocolate. The cotyledons (nibs) are ground to get 'mass'or 'liquor' (Amma *et al*., 2009).

About 50 per cent of cocoa seed is cocoa butter, which is extracted by solvent extraction and mechanical extraction (Estephen, 2005). According to Amma and Minimol, (2012) cocoa mass contains about 55-58 per cent fat, which is also called 'cocoa butter'. Cocoa butter has the characteristics of melting at body temperature. Adabe and Samnick, (2014) reported that during grinding of cocoa nibs heat is produced by friction, which melts the cocoa butter. Normal grinding is by means of either cylinder rollers or a ball mill.

The yield of extraction of butter increased with increasing the time and temperature of grinding of cocoa nibs (Asep *et al.,* 2008). Cocoa butter is extracted from mass or liquor with the help of a hydraulic press. According to Amma *et al*. (2009) the equipment could extract 44.8 per cent of the butter by applying pressure and the cake left behind at the bottom of the presses after the extraction of butter contains a further 12-20 per cent of butter. Adabe and Samnick, (2014) also reported that 100 g of cocoa paste produces on average 46g of butter and 54 g of cake. The butter obtained is filtered, centrifuged and deodorised by steam distillation. The cocoa butter is used to produce chocolate or cosmetics.

Cocoa mass is pressed to produce cocoa butter and cocoa cake or it is used as the base ingredient for chocolate manufacture. The pressed cocoa butter is also used as an ingredient for some formulations, cocoa powder or fat-reduced cocoa powder can be added in products (Yates, 2009). The quality, quantity and type of ingredients used in chocolate manufacture all affect the fundamental basic flavour of the end chocolate. The cocoa powder is used to produce chocolate, pastries, milk-based drinks or cosmetics (Adabe and Samnick, 2014).

According to Becket (2000), conching is the grinding of chocolate mass at high temperatures (above 50°C) in a machine for the development of proper viscosity, the attainment of final texture and flavour of chocolates. The perceived quality of milk chocolate is affected by conching. Conching is a mixing step that involves volatilization of fatty acids and aldehydes and development of smooth texture. Volatilization reduces the bitterness of the chocolate and develops the typical chocolate flavour (Prawira and Barringer, 2008). Yates, (2009) reported that the flavour can be fine-tuned during processing, through roasting of the cocoa beans and the conching and tempering conditions, particularly time and temperature.

To make chocolate bars, the chocolate liquor and cocoa butter are blended with sugar, milk and vanilla. The ingredients are conched, super refined for giving smooth texture. It is also stirred at very high temperatures. Conching is a mechanical treatment of the chocolate mass in large containers fitted with rollers, paddles, or a variety of other devices (Mentink and Serpelloni, 1994). Chemical and physical changes take place under the influence of air which is brought into the mass, at a temperature of about 60 °C, and of rubbing and shearing forces. The result is the formation or liberation of flavour components which give the delicate chocolate (Ziegleder, 2009).

During conching, solid particles are ground, disassociated by friction and become rounded, thus the particle size of the solid particles in the chocolate is steadily reduced (El-deep *et al.* 2000). Conching also contributes to the physical properties of the chocolate, as particle size is reduced, the texture of the chocolate

becomes smoother (Liang and Hartel, 2004). Januszewska, (2018) stated that during, conching the level of volatile acids decreases and a more balanced final aroma develops. If the conching process is too long or too high temperatures are applied, most of the positive, volatile compounds may be lost.

Dand, (2011) describe conching is the final stage of bulk chocolate production. While there are differently designed conching machines, their purpose is to add the final touches to the texture and flavour of the product. Winkler, (2014) opined that conching improve the flavour of chocolates. The cocoa mass is added into the conche, where mixing starts and after time the other ingredients like cocoa butter, emulsifiers, flavours, nut pastes were added to the conche. This process usually takes several hours, and is performed at a wide range of temperatures (50–90°C).

Tempering is a technique of controlled pre-crystallisation employed to induce the most stable solid form of cocoa butter, a polymorphic fat in finished chocolates (Herrera and Hartel, 2000). The process consists of shearing chocolate mass at controlled temperatures to promote crystallisation of triacylglycerol's (TAGs) in cocoa butter to effect good setting characteristics, foam stability, demoulding properties, product snap, contraction, gloss and shelf-life characteristics (Toro-Vazquez *et al*., 2004).

Bomba, (1993) opined that the production of top quality chocolate depends not only on raw materials and their preparation, but also on the final solidification of the molten chocolate of tempering. Tempering is an important process in creating the correct texture of cocoa butter in the chocolate suspension. Tempering means put through a slow decrease of temperature. The chocolate is cooled and then warmed, over and over until it gets to the correct temperature. This process is done so that there is even crystallization throughout the chocolate (Smith, 2009).

Depending on the cocoa butter content of the chocolate and addition of other ingredients, the tempering temperature of chocolate varies. According to Afoakwa

et al. (2008) attainment of optimal temper during tempering of chocolate is vital to the desired texture and appearance of products.

2.3. Chemical constituents of cocoa

The cocoa beans are treasure of chemical compounds and their physical qualities during pod storage and subsequent fermentation processes would have important commercial implications (Duncan, 1984). Cocoa bean quality is made up of several components such as flavour volatiles, nutritional composition, polyphenolic content and fermentative quality (Schwan and Wheals, 2004). The most important components are the flavour volatiles of the beans as these affect cocoa bean acceptability (Owusu, 2013).

Relative to other seeds, the chemical constituents and nutritive value of cocoa are excellent. Based on its overall composition, it serve as a relatively complete food. Due to the nutritional qualities of chocolate, people have called cocoa a complete food. The fresh cocoa bean has an approximate composition of 32–39per cent water, 30–32per cent fat, 10–15per cent proteins, 5–6per cent polyphenols, 4–6per cent starch, 4–6per cent pentosans, 2–3per cent cellulose, 2–3per cent sucrose, 1–2per cent theobromine, 1per cent acids and 1per cent caffeine (Bertazzo *et al*., 2011).

The characteristic flavours of cocoa beans are due to a very rich volatile fraction composed of a mixture of hundreds of compounds (Magi *et al*., 2012). According to Crafack *et al*. (2014) more than 600 flavour compounds have been identified from cocoa beans and cocoa products.

2.3.1. Primary metabolites in cocoa

Cocoa and cocoa-derived products comprise one of the most popular super foods and it contain high density of essential nutrients like carbohydrate, protein, fat, polyphenol and minerals. According to USDA (2016) the 100gram cocoa powder contain the nutrients like carbohydrate (54.3g), protein (19.6g), fat (12.7g),

fibre (5.1g) and minerals like magnesium (590mg), phosphourus (960mg) and potassium (1700mg).

Chocolate is a highly nutritious energy source, with a fast metabolism and good digestibility. The presence of cocoa, milk and sugar in its composition help for an appropriate ingestion of proteins, carbohydrates, fats, minerals and vitamins (Campos and Benedet, 1994).

A primary metabolite is a kind of metabolite that is directly involved in normal growth, development and reproduction. Primary metabolites do not show any pharmacological actions or effects. Lipids, proteins, nucleic acids, and carbohydrates, are called primary metabolites (Vince *et al*., 2011).

The fat in cocoa (cocoa butter) is neutral in flavour. The caloric content of the whole beans about 50 – 60 percent mainly comes from cocoa butter. The fatty acids in cocoa are oleic, palmitic and stearic acids in combination with several polyunsaturated fatty acids, mostly arachidonic and linoliec acids, that predominate the triacylglycerol fraction of cocoa butter (Porsgaard and Hoy, 2000). The lipids in cocoa butter not absorbed rapidly or efficiently because of the complex molecular configuration of fatty acids in cocoa triglycerides, which slows the time for overall metabolism and slows intestinal absorption (Sanders *et al*., 2003).

The predominant fatty acids in cocoa butter are saturated (stearic; 18:0, 35% and palmitic; 16:0, 25%) and monounsaturated (oleic; 18:1, 35%), with the remaining fat being primarily polyunsaturated linoleic (3%). Cocoa fat contains about 95% triacylglycerols, 2% diacylglycerols, 1% monoacylglycerols, 1% polar lipids, and 1% free fatty acids (as percentages of lipids) (Biehl and Vioght, 2003).

Denke (1994) reported that by consuming cocoa, plasma LDL cholesterol (bad cholesterol), and apo B (an important lipoprotein polypeptide critical to LDL function) are decreased, while plasma HDL cholesterol (good cholesterol) is increased. Mursu *et al*. (2004) observed that the consumption of cocoa butter and

its effects on serum lipids, slower the rate of absorption of the triacylglycerides in cocoa butter and the high polyphenolic content in cocoa have a beneficial effect.

The solubility and digestibility of the nitrogenous compounds in cocoa are important considerations in determination of the value of cocoa and chocolate as sources of protein in nutrition. The protein content in cocoa beans is typical of many seeds but, the protein quality based on the distribution of certain essential amino acids is relatively poor (USDA, 2016).

Zak and Keeney (2000) found four predominant fractions of protein in cocoa beans representing 95 per cent of total seed proteins, and these are albumins (water-soluble), globulins (salt-soluble), prolamins (alcohol-soluble) and glutelins (soluble in dilute acids and alkali). Albumin is a major polypeptide accounting for about 52 per cent of total bean protein and is not degraded during fermentation (Dodo *et al*., 1994).

Adeyeye *et al*. (2010) reported that there was a more positive build-up of protein in fermented cocoa nibs than in unfermented cocoa nibs. According to Afoakwa (2010) the cotyledon protein degradation into peptides and free amino acids performs vital to the flavour development in cocoa.

Kirchoff *et al.* (1989) reported that the degradation of seed proteins during fermentation resulted in increase in the free amino acids. Beihl *et al.* (1995) recorded a very high proteolytic activity in ripe ungerminated cocoa seeds, which digested the vascular storage protein during fermentation and responsible for the release of hydrophobic aminoacids and a large number of oligopeptieds which were the essential cocoa flavour precursors.

The biological value and chemical score of cocoa serves as a comparative baseline, the chemical score for cocoa protein is less than 25, due to the low amount of methionine (Young *et al*., 1999). Afoakwa *et al.* (2013) observed that the crude protein content was 16-22 per cent during fermentation and bean storage. The

protein content was varies and depends up on the type of products or chocolates, it varies from 3-26 per cent (USDA, 2016).

Chocolate contains a variety of ingredients which contain different nutrients and affect the body's functioning in different ways. Depending on the relative proportions of cocoa, milk and other ingredients, the carbohydrate content of chocolate varies considerably. Based on Nutrient Data Base (2018) the carbohydrate content vary from 24- 63 per cent from cocoa powder to different types of chocolates.

Freshly harvested cocoa beans contain about 12 – 14 per cent potentially digestible carbohydrate and considerable quantities of nondigestible carbohydrate or fibre. After fermentation, the digestible carbohydrate content and composition of the cocoa pod (pulp and beans) is altered significantly. During fermentation the pulp is digested and a number of complex products are produced, like various short chain sugars, ketoacids, acetic acid and alcohol (Dei, 2006). Afoakwa. (2010) reported that the predominant sugars in cocoa beans are sucrose, fructose and glucose with sucrose being the major component (about 90% of total sugars), followed by fructose and glucose (about 6%).

Bucheli *et al*. (2001) reported that the fermentation process reduces the carbohydrate content of beans to 5-6 per cent. Pod storage influenced the carbohydrate content. The carbohydrate content was higher in fermented samples than in unfermented samples with beans stored for 21 days prior to fermentation having the highest carbohydrate content (Afoakwa, 2010).

An apparent inverse relationship appears to exist between the levels of fat and total carbohydrate in fermenting cocoa. Afoakwa *et al*. (2013) reported that carbohydrate content was significantly higher in fermented samples than in unfermented samples.

Cocoa is an excellent source of most essential minerals, especially calcium, copper, iron, manganese, magnesium, phosphorus, potassium and zinc (Lefeber *et*

al. 2010). Dei *et al.* (2006) opined that when cocoa consumed with maize and condiments and spices, especially pigmented fruits and vegetables, provide vitamins whose amounts are missing in cocoa. Afoakwa *et al*. (2013) in their study found that the micro nutrients decreases with fermentation and increasing pod storage.

Magnesium is found at significant levels in cocoa (2–4 mg/g dry powder). Dark chocolate (70% cocoa) contain 40 mg of magnesium (Volp, 2012). Cocoa powder known as an effective source of potassium, sodium, magnesium and phosphorus (Pedrol *et al*., 2016).

2.3.2. Secondary metabolites in cocoa

Secondary metabolites are molecules that are not essential for the growth and development, but they are biosynthetically derived from primary metabolites. Eg: Alkaloids, glycosides, phenolics, terepenoids. These secondary metabolites are called bioactive compounds (Bernhoft, 2010).

Bioactives have been defined as inherent non-nutritive constituents in food plants with anticipated health promoting / beneficial effects, when ingested (Gry *et al*., 2007). According to Biesalski *et al*. (2009) the bioactive compounds are essential and non- essential compounds that occur in nature, are part of the food chain and have an effect on human health.

Nutritionally, cocoa contains biologically active substances that may affect human health: flavonoids (epicatechin and oligomeric procyanidins), theobromine and anthocyanin are the some of the bioactive compounds in cocoa (Wollgast and Anklam, 2000). Cocoa and its derived products (cocoa powder, cocoa liquor, and chocolate) are a very rich source of bioactive components such as polyphenols (Hi *et al*., 2009).

Bioactive compounds in cocoa are classified in to two groups, polyphenols and methylxanthines. Polyphenols are a large class of chemical compounds found in plants and are natural antioxidants. Polyphenols consists of flavanoid and non

flavanoid. The primary flavanoids in cocoa are the monomeric epicatechin, catechins and polymeric procyanidins (Kim *et al*., 2006).

Wollgast and Anklam (2000) reported that three groups of polyphenols can be distinguished in cocoa beans these are catechins or flavan-3-ols (37%), anthocyanins (4%) and proanthocyanidins (58%). The polymers of epicatechin and catechin are procyanidins, also known as condensed tannins, which through formation of complexes with salivary proteins, are responsible for the astringency of cocoa (Manach *et al*., 2004).

According to Manach *et al*. (2004) procyanidins are the most abundant polyphenol in cocoa and chocolate products with reported levels ranging from 1.08 to 85.36 mg/g and epicatechins and catechins are the next most abundant in cocoa and cocoa products. Other polyphenols found in cocoa beans are the flavonol glycosides such as quercetin (Bordiga *et al*., 2015).

Polyphenols play an important role in shaping the sensory properties of cocoa beans and products derived as a result of processing. This is due to a complex formation of polyphenols with polysaccharides and proteins (Ferrazzano *et al*., 2009).

Bonvehi (2005) reported that phenolic compounds are responsible for pungent and bitter taste of raw beans, affect the stability and digestibility of cocoa beans and they are biosynthesised from proteins and fatty acids.

The flavonoids constitute the largest and are the main sub groups of poly phenols in cocoa beans. The main constituents of flavanoids are flavan-3-ols are monomeric epicatechin and catechin, together with proanthocyanidins, formed from monomeric flavanols (Ellam and Williamson, 2013).

Flavanoids in cocoa contains large concentrations of flavonoids, epicatechin, catechin, and procyanidins. Cocoa has the maximum levels of flavonoids, greater than even tea and wine. Dark chocolate contains considerably

higher amounts of flavonoids than milk chocolate. The biological effects of flavonoids greater in dark chocolate and less in milk chocolates. The milk in milk chocolateslow down the intestinal absorption of flavonoids (Lee *et al*., 2003).

Flavan-3-ols are lost during fermentation, treatment with alkali and roasting (Kim and Keeney, 1984). Miller *et al.* (2006) reported that the total flavan-3-ol content of commercial cocoa varies by greater than 10 fold. Niemanak *et al.* (2006) conducted a study in cocoa beans and analysed the flavanoid contents in them and found that proanthocyanidin content was more in cocoa beans (58-65%) compared to catechin (29-38%) and anthocyanin (4%). Processing can result in some epimerization of epicatechin to form catechin (Cooper *et al*., 2007). The total flavanoids content in dark chocolate is 28.30 mg in 100g of chocolates (Miller *et al.*, 2009).

Methylxanthines are group of phytochemicals derived from the purine base xanthine. Caffeine, theophylline and theobromine are the three naturally occurring methylxanthines. Theobromine and caffeine are the major methylxanthines present in cocoa and cocoa products (Kim *et al.*, 2006).

Dark chocolate and milk chocolate contain 240-520 mg and 65-160 mg of theobromine per 50 g portion, respectively (Smith, 2009). Meng *et al*. (2009) conducted a study on the theobromine content in different types of chocolates, they noticed that dark chocolate contain more theobromine (88.11 mg/g) followed by semisweet (48.12mg/g) and milk chocolate (12.15mg/g). Theobromine is the primary alkaloid found in cocoa and chocolate and is a 3, 7-dimethylated xanthine alkaloid, formed during caffeine metabolism (Franco *et al.*, 2013).

The high levels of theobromine content in cocoa, is about 2.5% of dry weight, whereas caffeine is 0.24per cent. Theobromine and epicatechin are absorbed efficiently in the small intestine and procyanidins are poorly absorbed in the small intestine (Cooper *et al*., 2008). According to Risner. (2008) theobromine is not degraded during cocoa processing Franco *et al.* (2013) found out the

methylxanthine content in cocoa beans as 2-3 per cent of theobromine, 0-2 per cent of caffeine and trace amount of theophylline.

The amounts of polyphenols vary due to the type of cultivar and the country of origin, as well as production processes of cocoa bean and chocolate manufacturing processes (Cooper *et al.*, 2007).

According to Counet *et al*. (2004) *Criolla*, cultivar have high level of procyanidins, reducing sugar and free aminoacids. Forastero roasted cocoa beans have three times higher amount of pyrazines than Criollo (Elwers *et al.*, 2009). Wallace and Giusti. (2011) reported that fermentation in boxes shows a relatively low concentration of sugars, ethanol and acetic acid, as well as a high pH.

Misnawi *et al.* (2003) observed that sensory properties of cocoa liquor is affected by the polyphenol concentration and duration of roasting polyphenols had a negative effect on flavor properties. Payne *et al.* (2010) found that when cacao beans were roasted to 120°C, the catechin level in beans increased compared to the same unroasted beans. These results suggest that roasting in excess of 70°C generates significant amounts of catechin.

Cocoa polyphenols are thermally labile to some degree, the exact stability of epicatechin in chocolate will depend on stabilizing interactions with other components of the cocoa nib. The enzymatic and nonenzymatic oxidations occur during manufacturing processes of cocoa (Tomas-Barberán *et al.,* 2007). Hurst *et al.* (2011) also reported that the enzymatic and nonenzymatic oxidations reduce the amount of polyphenols available in cocoa beans due to high processing temperatures and longer processing times.

Alkalisation process is applied to modify the flavor and colour of cocoa powders and it decrease in epicatechin and catechin of 67per cent and 35per cent (Andres-Lacueva *et al.*, 2008). According to Miller *et al.* (2008) alkalisation caused a progressive reduction of polyphenols as well as their antioxidant activity. Reduction of the polyphenol antioxidant activity to be triggered by heat and alkali

synergistically (Sulistyowati and Misnawi, 2008). Payne *et al.* (2010) found that compared to natural cocoa powders, alkalization caused a loss in both epicatechin (up to 98%) and catechin (up to 80%).

2.4. Value added products from cocoa

Cocoa is a supporting crop to farmers due to its remunerative income. The chocolate industry is frequently undergoing dynamic change and it depends on the nature of the demand for chocolate (Frost *et al*., 2011). Value added products can be made from cocoa and cocoa by products, both during the primary processing stage as well as with secondary processing. There are several value added products and by products from cocoa (Rossini *et al*. 2011).

Cocoa beans are mostly processed into chocolate and cocoa products using a wide range of intermediate products such as cocoa liquor, cocoa butter, cocoa cake and raw cocoa powder (Afoakwa *et al*., 2007). Cocoa powder is essentially used in flavouring biscuits, ice cream and other dairy products, drinks and cakes and in the manufacture of coatings for confectioneriess and frozen desserts (Pandey and Singh, 2011).

Cocoa powder can be used as an ingredient in almost all confectioneries. It is used in chocolate flavoured drinks, chocolate flavoured desserts such as ice cream, chocolates, spreads, sauces, cakes and biscuits (Yates, 2009). Amma *et al.* (2009) also reported that cocoa powder can be made by pressing the cocoa mass and produce cocoa butter and cocoa cake (which is kibbled and then further processed into cocoa powder), or it is used as the base ingredient for chocolate manufacture.

The dried, fermented cocoa bean is the main ingredient used in the manufacture of chocolate. The processing of dried fermented cocoa is largely limited to the production of the local chocolate, commonly called ‘Creole Chocolate’. This is basically a crude form of the pure unsweetened (bitter) chocolate, which is used to make a beverage (Sukha, 2003).

Unsweetened chocolate contains 100 per cent cocoa and zero per cent sugar. It's held together by cocoa butter and too bitter to eat as is, but it is often used in baking (Sukha and Mujaffar, 2002).

Dark chocolate contains over 70 per cent cocoa and not all 70 per cent chocolates have the same flavours or bitterness since the provenance of the cocoa bean can radically alter the flavour, but all will contain the same amount of cocoa to sugar ratio (Sukha and Mujaffar, 2002). Miller *et al*. (2009) opined that the dark chocolate contain 86-100 per cent of cocoa, very little amount of sugar (0.2%) and milk is not added in dark chocolate. The bitter sweet chocolate contain 61-85 per cent of cocoa, 13-45 per cent of sugar and 10-20 per cent of milk (Miller *et al*., 2009).

Bitter sweet chocolate that contains around 70 per cent cocoa and 30nper cent sugar. This chocolate is more used in baking than the traditional unsweetened chocolate. In Europe, bittersweet chocolate is simply known as dark chocolate (Sukha and Mujaffar, 2002).

Semi-sweet Chocolate contains around 60 per cent cacao and 40 per cent sugar. This chocolate can be eaten, used in baking, or even melted for decorating pastries (Sukha and Mujaffar, 2002). Semisweet chocolates contain 35-60 per cent of cocoa, 45-65 per cent of sugar and 15-25 per cent of milk (Miller *et al*., 2009).

Milk chocolate that contains only 10-40per cent cocoa, mixed with sugar and milk solids. Occasionally vanilla is added for extra flavour and lecithin for smoothness (Sukha and Mujaffar, 2002). In milk chocolate, milk content is more compared to other chocolates, it is 30-35 per cent milk, 10-20 per cent of cocoa and sugar content was 50 per cent).

White chocolate not contained cocoa and is simply made up of cocoa butter and sugar and a little vanilla for flavour. Chocolate bars are nice and versatile. They melt well and if broken up into chunks work beautifully as chocolate chips. In fact,

in countries where it might be hard to find chocolate chips, most bakers simply use chopped chocolate bars (Sukha and Mujaffar, 2002).

Chocolate chips are often treated with stabilizers to help them retain their shape when baking. Higher quality chocolate chips contain fewer stabilizers and can be used for melted chocolate. Chocolate wafers look like large chocolate chips and are specially formulated for easy melting. These are ideal for covering fruit or anything else ((Miller *et al*., 2009).

Cocoa butter is used in the manufacture of chocolate. It is also widely used in cosmetic products such as moisturising creams and soaps (Amma *et al.,* 2009).

Pectin for jam and marmalade is extracted from the sweatings by precipitation with alcohol, followed by distillation and recycling of the alcohol in further extractions (Sukha, 2003). Pectin is present in cocoa pulp or mucilage and the pulp is extracted by pressing the beans just before they are fermented to produce a range of jams and jellies.

The cocoa pulp and juice is also fermented to give a good quality wine and liqueur (Freire *et al.*, 1996). Cocoa sweatings have also been used to provide alcohol, vinegar and other products. The products like ice cream, yogurt (pulp made into a nectar and stirred into yougurt), pancake syrup, juices and shakes, wine, vinegar and nata - a processed agar-like product packed in syrup and consumed as a desert in Asia (Sukha, 2003) .

The pulp and juice is also fermented to give a good quality wine and liqueur. Cocoa sweatings have also been used to provide alcohol, vinegar and other products (Freire *et al.,* 1996). Amma *et al.* (2009) reported that the concentration of alcohol in the sweatings is about 2-3per cnt acetic acid 2.5per cent, citric acid (0.77-1.52%), pectins (0.90-1.10%) and salts (K, Na, Ca, Mg) with pH of 3.2-3.5. The pectin from mucilage show slow setting characteristic.

Cocoa liquor is used, with other ingredients, to produce chocolate. Chocolate is used as a product on its own or combined with other ingredients to form confectionery products (Amma *et al.*, 2009).

Once the beans have been fermented and dried, they can be processed to produce a variety of products. In the preparation of soft drinks, fresh cocoa pulp juice (sweatings) is collected, sterilised and bottled. For the production of alcoholic drinks, such as brandy, the fresh juice is boiled, cooled and fermented with yeast. After 4 days of fermentation the alcohol is distilled (Sukha, 2003).

Adomako, (1995) also reported that many different types of cocoa products can be made from cocoa, viz. Animal feed from cocoa husk, production of soft drinks and alcohol, jam and marmalade, cocoa butter, cocoa powder and cocoa liquor. Ntiamoah and Afrane (2008) reported that cocoa beans are also used in the beverage industry, for example in the preparation of chocolate milk. Cocoa butter is used in the manufacture of chocolate confectionery, soap and cosmetics (Schumacher *et al*., 2010). Other by-products such as cocoa pulp juice is also fermented to produce industrial alcohol and alcoholic beverages such as brandy and wine (Jayathilakan *et al*., 2011).

About 70-75% of the pod is constituted by pod husk and it generally discarded after collection of beans (Freire *et al.*, 1996). Fresh or dried pod husks are sliced and mixed with other feeds or used in the manufacture of animal feed pellets (Sukha, 2003). According to Ntiamoah and Afrane. (2008) the pod husks and shells are used for the preparation of animal feed and fertilizer.

According to USDA (2016) the pod husk contains crude protein (5.69-9.69%), fatty substances (0/03-0.15%), glucose (1.16-3.92%), sucrose (0.02-0.16%), pectin (5.30-7.06%), crude fibre (33.19-39.45%), theobromine (0.20-0.21%) and ash (8.83-10.18%). Incorporation of a 20% pod husk in cattle feed has shown beneficial effect. The dry pod husk contains 5.3-7.08 per cent pectin.

Cocoa pod husk ash is used mainly for soft soap manufacture. It may also be used as fertiliser for cocoa, vegetables, and food crops. To prepare the ash, fresh husks are spread out in the open to dry for one to two weeks. The dried husks are then incinerated in an ashing kiln (Sukha, 2003).

Cocoa bean shells can be used an organic mulch and soil conditioner for the garden (Sukha, 2003). It contains 2.8 per cent starch, 6.5per cent pectin, 18.6 per cent fibre, Theobromine 1.3 per cent, fat 3.4 per cent, tannins 1.3 per cent and vitamin D 300IU etc. As a fertilizer, shells act as a humus forming base. The do not decompose easily. This can be overcome by heaping for one season. Theobromine is extracted commercially and methylated to form caffeine, which have a greater demand than theobromine. Shell is a very good mulch and manure and it is used in anyhurium, orchid and foliage plants (Amma *et al*., 2009).

2.5. Health benefits of chocolates

Nutritionally, cocoa contains biologically active substances that may affect human health (Dillinger *et al*., 2000). The cocoa, as part of the wonderful nature, provides the mankind a wide variety of valuable food products and health benefits. The cocoa or dark chocolate may possess certain beneficial effects on cocoa (Amma *et al*., 2009).

Dark chocolate is a rich source of the flavanoids, epicatechin and gallic acid which possess a significant antioxidant action, protecting against LDL oxidation than other polyphenol antioxidant rich foods and beverages (Djousse *et al*., 2011). There are several proven health benefits by the intake of cocoa and cocoa based products for the cardiovascular system, cancer, neurological/nervous system, endocrine system, diabetes and dermatological system (Baba *et al*., 2007).

There are several proven beneficial effects of cocoa in cardiovascular diseases (CVD). Cocoa has aspirin-like effects on platelet function and the joint effects of the cocoa and aspirin are additive in nature, suggesting improved clot

prevention afforded by cocoa (Pearson *et al*., 2002). The flavanol rich cocoa due to its chemical structure improve vasodilatation of artery by producing nitric oxide.

Theobromine inhibits platelet aggregation in the blood vessels. Nitric oxide has an inflammatory effect and counteracting platelet aggregation at the site of inflammation (Hu *et al*., 2001). Murphy *et al*. (2003) have observed that the cocoa flavanol drink have reduced the platelet count and aggregation in cardiovascular patients. Chocolate has a dual effect on platelets it not only decreases platelet aggregation but also reduces platelet adhesion (Pearson *et al.,* 2005). Zomer *et al.* (2012) have reported that the daily consumption of dark chocolate could be an effective cardiovascular preventive strategy in patients with metabolic disease.

The bioactive compounds in cocoa decrease LDL cholesterol and increase HDL cholesterol. The function of flavanoids in cocoa will scavenge the free radicals (reactive nitrogen species) nitrogen dioxide and reduce lipid peroxidation of LDL (Cooper *et al*., 2008). Baba *et al. (*2007) reported that after twelve weeks of cocoa powder consumption LDL cholesterol was decreased and HDL cholesterol was increased in cocoa group compared to control group.

Bioactive compounds in cocoa reduce the blood pressure. Under pathological conditions reactive oxygen species are produced in artery wall, this will cause hyper tension. The polyphenol in cocoa increased nitric oxide bioavailability and improve arterial stiffness and endothelial function (Buijse *et al*., 2006). Law *et al*. (2009) reported that there is favourable decrease in diastolic blood pressure in a population of 1 mm Hg is enough to reduce the incidence of coronary heart disease events by 5 per cent and of stroke by 7 per cent in person aged 50–69 years with a systolic (high) blood pressure of 150 mm Hg and a diastolic blood pressure of 90 mm Hg.

Shiina *et al*. (2009) also found that the consumption of dark chocolates improve the coronary circulation, platelet activation and reduce the cardiovascular diseases. The effects are likely due mostly to the actions of flavanols, minerals

(potassium, magnesium, and calcium) in cocoa or stearic acid present in in chocolate (Pearson *et al*., 2005).

Cancer is a disorder which is characterized by abnormal growth of cells. This abnormal growth and multiplication caused by free radicals. Chronic inflammation and oxidative stress are significant contributing factors to carcinogenesis. Reactive oxygen (RO) and nitrogen intermediates (RNI) can damage DNA or interfere with DNA repair, leading to mutations in cells to avoid controls on growth and replication (Carnesecchi *et al*., 2002). Inflammation increases the production of ROI and RNI stimulated by cytokines and chemokines, and angiogenesis (Federico *et al*., 2007).

Free radicals damage the components of a cell including the DNA and lead to the development of cancer. Cocoa contains a high polyphenol content, which have antioxidant activity. The antioxidants combine with free radicals and prevents cancer. John (2011) reported that cocoa and dark chocolate contain more polyphenol having more antioxidant index compared to other products like milk and white chocolates. Spadafranca *et al.* (2010) observed that dark chocolate consumption significantly improved DNA resistance to oxidative stress in the short-term.

Bioactive compounds in cocoa help to control the abnormal cell growth by interacting with the mechanism of a cell. The bioactive compounds such as procyanidins and catechins, influence the immune responses by modulating the activation of the transcription factor NF-kB, involved in inflammatory responses, cellular proliferation, cell adhesion, and regulating cytokine production (Mackenzie *et al*., 2004). Martin *et al*. (2009) conducted a study on cancer preventive effects of cocoa polyphenols. At the end of the study they found that there is decrease in cell growth and TNF induced oxidant formation in cancer suffering persons.

Diabetes mellitus (DM) is a group of metabolic disorders in which the blood sugar level is high for a prolonged period. According to Konopatskaya *et al*. (2003) diabetes is due to either the beta cells of pancreas not producing enough insulin or

the cells of the body not responding properly to the insulin produced. The flavanols in cocoa may improve insulin resistance by reducing oxidative stress, improving endothelial function and altering glucose metabolism. The oxidative stress is the underlying mechanism for both insulin resistance and cardiovascular disease (Ceriello and Motz, 2004).

The bioactive compounds in cocoa will reduce the postprandial hyperglycaemia, plasma free fatty acids and a biomarker of oxidative stress 8-isoprostane (Jalil *et al*., 2008). The function of bioactive compound in cocoa, especially epicatechin will increase insulin production and control blood sugar levels and help beta cells to work better and become stronger (Olasope *et al.*, 2016).

The bioactive compounds in cocoa improve the endothelial function and effect on insulin sensitivity. According to Kim *et al*. (2006) increased insulin sensitivity improves endothelial function; conversely, improvement in endothelial function can increase insulin sensitivity. Balzer *et al.* (2008) reported that flow-mediated dilatation improved by the consumption of flavanol rich cocoa daily for 30 days in medicated.

Cocoa flavanols was attributed to enhance blood flow, promote brain perfusion and protect from neuro degradation and neuronal injury through interaction with a number of signaling pathways (Larsson *et al*., 2012). The bioactive compounds improve the brain functioning by maintaining the cerebral blood flow (CBF), supporting constant oxygen and glucose supply to neurons and waste excretion (Walters and Williamson, 2012).

Hunot and Hirsch (2003) opined that the pathogenesis of Parkinson's disease, Alzheimer's disease, and neuronal injury associated with stroke will involve neuro inflammation. Spencer (2009) reported that the flavonoids in cocoa modulate the chronic responses and the flavonoids interact with a variety of neuronal protein kinase and lipid kinase signaling cascades and can prevent excitotoxic death in neurons. Epicatechin in cocoa induces the enzymes extracellular signal regulated kinase and cyclic AMP response element binding

protein (CREB) activation in cortical neurons increasing CREB regulated gene expression involved in the formation of long-term memory (Schroeter *et al*.,2007).

The consumption of polyphenol enriched cocoa enhance the cerebral blood flow (CBF). Spencer (2009) opined that increased blood flow to the cerebral gray matter induces angiogenesis and new nerve cell growth in the hippocampus. Sokolov *et al*.(2013) reported that those who are drinking high flavanol drink have increased blood flow up to140 ml/100g/min compared to low flavanol drinking group.

Brain perfusion is the amount of blood taken up by certain areas of brain. Cocoa flavanol intake will increase perfusion in Anterior Cingulate Cortex (ACC) and parietal cortex (Francis *et al*., 2006). According to Patel *et al*. (2008) blood perfusion in ACC and parietal cortex increase attention, learning capacity and taste monitoring ability. The antioxidant properties of catechin and epicatechin derivatives can protect from neuronal injury and neuroinflammation. According to Nurk *et al*. (2008) cocoa can also increase cerebral blood flow, which can be neuroprotective and epicatechin is involved in the processes of formation of long-term memory.

Skin is prone to the development of several diseases, and the mechanisms in the pathogenesis of aged skin are still poorly understood. The bioactive compounds of cocoa have a positive impact on skin health. Cocoa butter is a common ingredient in skin moisturizers, but cocoa's beneficial effects on skin extended beyond its use as a topical agent. Boelsma *et al*. (2003) reported that the vasodilatory effect of cocoa flavanol in nutrient delivery and thermoregulation is dependent on cutaneous microcirculation. The antioxidant actions of cocoa flavanols are one possible mechanism by which skin protection could be conferred.

The antioxidants in cocoa scavenge the free radicles and reduce the skin problem and increase skin health (Heinrich *et al*., 2006). Neukam *et al*. (2007) also reported that the consumption of high flavanol cocoa for 12 weeks increased dermal blood flow and oxygen saturation by 70 per cent and 80 per cent respectively. When

we are exposed to ultra violet light it will cause to over production of reactive oxygen species (ROS) (Williams *et al*., 2009). This will cause activate inflammation, accelerate physiological ageing and epidermal degeneration.

Obesity is one of the major risk factors in the development of cardiovascular diseases. Cocoa consumption led to a significant decrease in total body weight, mesenteric white adipose tissue weight and serum triglycerides (Balzer *et al*., 2008). When DNA analysis was carried out on liver and mesenteric fat tissue samples, the results showed a reduction in expression of various genes associated with fatty acid transport and synthesis in liver and mesenteric fat and increased expression of genes associated with thermogenesis (Matsui *et al*., 2005).

Nitric oxide (NO) has been shown to increase uptake of glucose, increase oxidation of fatty acids and glucose, inhibit fat synthesis, and enhance lipolysis in adipose tissue (Johnston *et al*., 2005). Cocoa has been found to decrease visceral adipose by changing the expression of genes for enzymes and transport molecules involved in fatty acid synthesis and thermogenesis in liver and white adipose tissue (Jobgen *et al*., 2006)

Chocolate consumption can improve a negative mood state and the sensory experience of eating chocolate improved mood, rather than neurochemical effects (Zellner *et al*., 2004). Macht and Mueller (2007) found that a chocolate bar elevated mood and elicited joy to a greater extent than an apple, but these effects were most pronounced at 5 and 30 min after consumption. Martin *et al*., (2009) reported that the effects cocoa and dark chocolate consumption reduced urine cortisol and catecholamines and partially normalised other metabolic parameters associated with the high-anxiety trait.

Cocoa have the psychoactive effect due to flavanols or methylxanthine compounds in them. Scholey *et al*., (2010) conducted a study and they observed that the flavanol-rich preparations significantly increased cognitive performance and reduced mental fatigue relative to the control beverages.

2.6. Storage and keeping quality of chocolates

Chocolate is one of the most popular foods and common confectionery material in the world and the people enjoyed for its wonderful taste. Fowler (2009) opined that chocolate is a complex emulsion based on cocoa, the consumption of cocoa activates pleasure centres of the human brain through its flavour.Chocolate is a product of cocoa, made by mixing cocoa mass, cocoa butter and sugar (sucrose) using special machinery (Afoakwa, 2007).

Chocolate is a highly nutritious energy source, with a fast metabolism and good digestibility. The presence of cocoa, milk and sugar in its composition can be the warrant for an appropriate ingestion of proteins, carbohydrates, fats, minerals and vitamins (Campos and Benedet, 1994). The taste of chocolate is partially determined by the chemistry of the product; which is the typical formulations of ingredients used manufacturing of chocolate (Kulozik *et al*., 2003).

Lees (1990) reported that major causes of deterioration of chocolate and confectionery products are fermentation, rancidity and moulds. A good chocolate is shiny brown, breaks cleanly, and is free of lumps, tiny burst bubbles and white specks. It melts on the tongue like butter, has a true aroma of chocolate rather than of cocoa powder, and is neither greasy nor sticky (Afoakwa, 2007). The shelf life of chocolate depends on several parameters like storage temperature and humidity, availability of oxygen in the environment, packaging material used and as well as the addition of other ingredients such as fats, nuts (Nattress *et al*., 2004).

Nevzat (2013) reported that the chocolate loses its taste and flavour and became rancid during storage. The composition of the chocolate can play an important role on its shelf life (Bernard, 1989). Ali *et al*. (2001) reported that colour and texture of filled dark chocolate stored at 30°C were significantly less preferred than the control and chocolate stored at 18°C as a result of fat migration which adversely affected product texture appearance. Chocolate without milk can be stored for several months (or even years) if it is protected from damp and stored at 20°C temperature. Chocolate is very sensitive to temperature and humidity.

The quality of chocolate was determined in terms of moisture content, free fatty acids, peroxide value (Pandey and Singh, 2011). Yadav *et al*. (2011) found that during storage of chocolates the moisture content was increased. The heat sealed laminated chocolates absorb less per cent of moisture as compared to butter paper packaging whereas the unpacked chocolate is very sensitive to absorb moisture at lower relative humidity.

In some cases moisture content decreased throughout the storage period. One of the possible reasons for increasing hardness during storage of confections is decrease in moisture content (Fox and Sweeney 1998). Ali *et al*. (2001) also reported post hardening in chocolates during storage at 18°C. An increase in titratable acidity as an indicator of glycolytic changes in milk based confectionery during storage was also reported by Sarkar *et al*. (2002).

The free fatty acid (FFA) is the primary quality attribute for edible grade oil / fat. The Food and Adulteration Act specify a maximum acceptable limit of FFA as 3 per cent (Ranganna, 2005). The unpleasant odour and taste which develops spontaneously in fats, known as rancidity. Yadav *et al*. (2011) observed the free fatty acid content in unpacked chocolate was higher than packed (butter paper and heat-sealed laminated) chocolates during storage. The similar observation have recorded by Ali *et al*. (2001).

Lipid oxidation is the main cause of spoilage and off flavour formation in chocolate. Antonelli (2002) reported that peroxide value is one major factor of lipid oxidation. Lipid degradation is related as one of the main deteriorative problems and one of the first mechanisms of quality loss of food products and chocolates. The peroxide values were increased with increasing temperature, Rh and storage duration of chocolates (Yadav *et al*.,2011).

During storage, chocolate surfaces turn greyish (“fat bloom”), inducing considerable colour changes, i.e. of lightness and saturation of colour. The turning grey of chocolate, principally, appears as the result of errors during defined phases of the technological production processes, such as tempering, forming, cooling, or

as a consequence of extremely long storage (Bricknell *et al*., 1998). Lacueva *et al*. (2008) reported that colour and appearance decreased during storage of chocolate at temperatures (11, 20 and 25°C). Color and appearance score decreased in the chocolate samples stored at temperature 25°C (Mishra *et al.*, 2016).

Mexis *et al*. (2010) also reported that there was slight change in taste in chocolate stored at 4°C with respect to chocolate stored at 20°C. Overall acceptability of guava milk chocolate decreased during storage period and degradation in overall acceptability was observed very fast at 25°C and no change in overall acceptability was observed in chocolate at temperature 2°C up to 56 days storage (Mishra *et al.*, 2016).

Fadel *et al*. (2006) carried out sensory evaluation of a cocoa substitute sample (mixture of chicory roots and carob bean) in comparison to that of a cocoa sample. The cocoa substitute showed a decrease in sensory attributes while for chocolate-like attribute reported an opposite trend. Jonfia *et al*. (2010) reported that dietary chocolates, which consisted of fructose, cocoa butter, whole milk powder, cocoa mass, skimmed milk powder, hazelnut paste, and lecithin as emulsifier, along with added aromas, was evaluated sensory as excellent during storage up to one year.

Ziegleder (1997) reported that typical chocolate deterioration effects, frequently encountered in fat migration are softening and blooming of the surface layer and unacceptable texture. Jonfia *et al*. (2010) observed that dietary chocolate had relatively good sensory quality during storage and after production it was already evaluated with a very good mean score.

Ali *et al*. (2001) reported that colour and texture of filled dark chocolate stored at 30°C were significantly less preferred than the control and chocolate stored at 18°C as a result of fat migration which adversely affected product texture and appearance. Nattress *et al*. (2004) found that bitterness decreased during storage which is in agreement with the postulation that dark chocolate tends to “mellow” over time.

Microbiological evaluation is an indicator for quality evaluation of chocolates during storage. Microorganisms that cause spoilage in butter are involved with spoil of chocolates. But vegetables oils are usually more resistant to lipolytic breakdown than milk fat (Vernam and Sutherland, 1994). The microbiological investigations suggest that chocolate coating led to a marked reduction in the population of spoilage microorganisms during storage and the product was of satisfactory bacteriological quality (Mehta, 2017)).

Swanson and Anderson (2000) observed that the bacteriological counts of the chocolate flavoured milks were much higher than those of the non flavoured milk samples. Baylis *et al*. (2004) in his study observed that the microbiological changes in chocolate samples during storage were showed that optimized chocolate have lesser standard plate count as compared to control chocolate throughout the storage period.

Materials and Methods

MATERIALS AND METHODS

The study entitled “Process optimisation and quality evaluation of cocoa based chocolates” was carried out with the objectives to develop a protocol for primary processing of cocoa beans based on free fatty acid content, to standardise the time and temperature of chocolate making using machine and to evaluate the quality attributes and shelf life of the products. The methods followed and materials used in the study are given under the following headings.

3.1. Collection of raw materials

3.2. Development of protocol for primary processing of cocoa

3.2.1. Standardisation of fermentation methods based on free fatty acid content

3.2.1.1. Basket method

3.2.1.2. Heap method

3.2.1.3. Sack method

3.2.2. Physico-chemical analysis of fermented beans

3.2.2.1. Bean recovery

3.2.2.2. Cut test

3.2.2.3. Moisture

3.2.2.4. pH.

3.2.2.5. Peroxide value

3.2.2.6. Lipase activity

3.2.2.7. Total fat

3.2.2.8. Free fatty acid

3.2.2.9. Temperature

3.2.3. Standardisation of drying methods based on free fatty acid

3.2.3.1. Sun drying

3.2.3.2. Oven drying

3.2.4. Physico-chemical analysis of dried beans

3.2.4.1. Bean recovery

3.2.4.2. Moisture

3.2.4.3. pH

3.2.4.4. Peroxide value

3.2.4.5.Total fat

3.2.4.6. Lipase activity

3.2.4.7. Free fatty acid

3.2.5. Standardisation of method of storage based on free fatty acid content

3.2.6. Physico-chemical analysis of stored beans

3.2.7. Microbial enumeration

3.3. Alkalisation of stored beans

3.4. Standardisation of time and temperature for chocolate making using machine

3.4.1. Physico-chemical analysis of chocolates

3.4.2. Organoleptic evaluation of chocolates

3.4.3. Enumeration of total micro flora

3.5. Blending of chocolates with other ingredients

3.5.1. Quality evaluation of selected blended chocolates

3.5.1.1. Physico- chemical analysis

3.5.1.2. Organoleptic evaluation

3.5.1.3. Enumeration of total micro flora

3.6. Cost of production of the selected chocolates

3.7. Statistical analysis of the data

3.1. Collection of raw materials

Cocoa beans required for the research purpose were procured from Cocoa Research Centre of Kerala Agricultural University.

Ingredients like dried fruits and dried nuts were purchased from the local market. Pepper and mint were collected from households and processed to white pepper powder and mint powder.

3.2. Development of protocol for primary processing of cocoa

3.2.1. Standardisation of fermentation methods based on free fatty acid content

Cocoa beans were harvested at the right stage of maturity. Cocoa pods mature in about 150 to 170 days from the day of pollination. The harvested pods were stored for two to five days for enhancing prefermentation activity inside the pods. The harvested pods were broken by hitting against a hard surface and beans were extracted and any black or diseased beans and germinated beans were removed. The wet cocoa beans were grouped in to nine lots. The beans were subjected to three different types of fermentation. The three fermentation methods selected were basket method, heap method and sack method. All the fermentation methods were subjected to three different periods of fermentation. The periods selected for the study was 5^{th}, 6^{th} and 7^{th} day of fermentation.

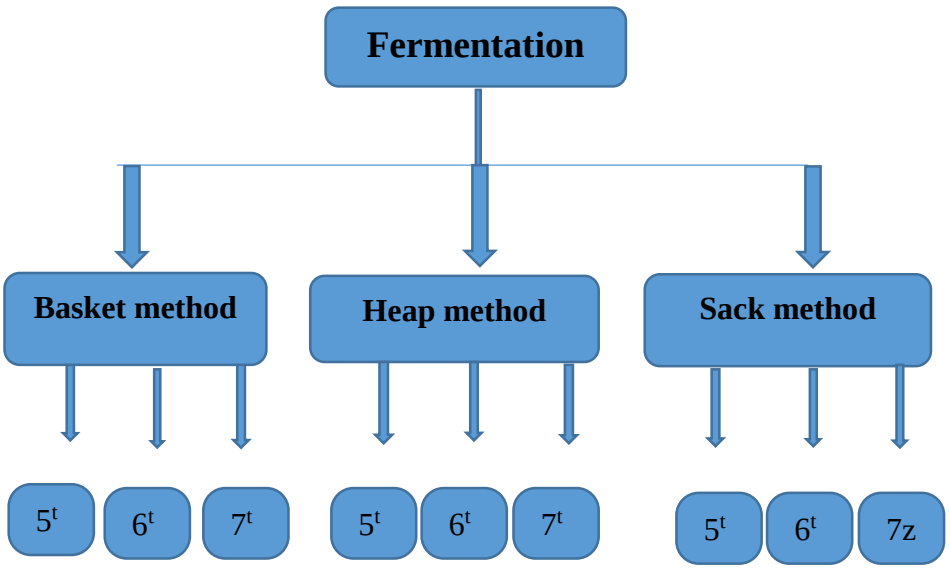


Fig.1. Schematic representation of fermentation methods and periods

3.2.1.1. Basket method

In this method bamboo baskets were used. The baskets were lined with one or two layers of torn banana leaves to facilitate drainage of sweatings. Two kilogram wet beans were filled, compacted and covered with banana leaves. The baskets were placed on a raised platform to allow the flow of drippings and after 24 hours covered with gunny sack. The beans were mixed on the third and fifth day of fermentation. The beans were taken out for drying on the fifth, sixth and seventh days of fermentation (Amma and Minimol, 2012).

3.2.1.2. Heap method

In this method, a mass of fifty kilogram wet cocoa beans was heaped over a layer of banana leaves on a sloppy floor. Banana leaves were folded and kept over the beans. As soon as the beans were heaped, flow of sweatings will be initated and it will continue for the first two days. The beans were mixed on the third and fifth day of fermentation. The beans were taken out for drying on the fifth, sixth and seventh day of fermentation (Amma and Minimol, 2012).

3.2.1.3. Sack method

In sack method, three kilogram wet cocoa beans were filled in plastic gunny bags and tied loosely. The sacks were shaken for mixing the beans thoroughly without opening the sack on the third and fifth day of fermentation. The beans were taken out for drying on the fifth, sixth and seventh day of fermentation (Amma and Minimol, 2012).

3.2.2. Physico-chemical analysis of fermented beans

The physico- chemical qualities of beans fermented using basket, heap and sack methods were carried out initially and on fifth, sixth and seventh day of

fermentation using standard procedures. The analysis was carried out with three replication for each treatment.

3.2.2.1. Bean recovery

Ripened pods were harvested, split opened and beans were extracted. Two kg of beans for basket method, fifty kg of beans for heap method and three kg of beans for sack method were collected. Before fermentation fresh weight of beans was recorded. After fermentation the weight of beans was recorded. Then the bean recovery or fermentation recovery was estimated and expressed in per cent.

$$\text{Bean recovery (Fermentation Recovery)} = \frac{\text{Weight after fermentation (g)}}{\text{Fresh weight before fermentation (g)}} \text{ x } 100$$

3.2.2.2. Cut test

Cut test or fermentation index was used to determine the extent of fermentation of fermented cocoa beans. It is one of the cocoa grading schemes based on visual assessment of cocoa quality, which relies on changes in colour of the beans test (Amma *et al*., 2009). Hundred seeds from the fermented lot of each method was taken and cut longitudinally with a sharp knife and observed the cotyledon colour by placing on a white back ground. Based on the colour, beans were characterized into fully fermented, partially fermented, not fermented, slaty and mouldy. White colour at center or full dark brown colour indicated as fully fermented, partly pink colour or brown colour across and along margin indicated as partially fermented beans, fully purple colour indicated as not fermented, dark black colour indicated as slaty beans. The value is expressed in per cent based on the number of beans recorded under each category.

3.2.2.3. Moisture

Moisture content of the fermented beans were estimated by the method of A.O.A.C (1980). To determine the moisture content, ten grams of the sample was taken in a Petridish and dried at 60 to 70^0C in a hot air oven, cooled in a desiccator and weighed. The process of heating and cooling was repeated till a constant weight

was obtained. The moisture content of the sample was calculated from the loss in weight during drying.

$$\text{Moisture content (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

3.2.2.4. pH estimation

pH of cocoa beans after different methods and periods of fermentation were recorded. Five gram samples of cocoa beans was homogenized for 30 seconds in 100 ml of hot distilled water and vacuum filtered through Whatman filter paper. A 25 ml aliquot was pipetted into a beaker and the pH was measured using a pH meter (AOAC, 1980).

3.2.2.5. Peroxide value

Peroxide value of fermented beans were estimated to find the rate of rancidity during fermentation. It was estimated by the method given by Sadasivam and Manickam (1997). One gram of extracted oil sample was taken in boiling tube and to that one gram of potassium iodide and 20ml of solvent mixture (glacial acetic acid and chloroform) were added. The tube was placed in boiling water for 30 seconds and the contents were transferred to a conical flask containing 20ml of 5 per cent potassium iodide solution. The tubes were washed twice with 25 ml water and collected in a conical flask. This was titrated against N/ 500 sodium thiosulphate solution until yellow colour disappears. Later 0.5ml of starch solution was added and titrated till the appearance of blue colour. A blank solution was also prepared and peroxide value was calculated and expressed in milliequivalent per kg of the sample.

3.2.2.6. Lipase activity

Lipase activity was estimated to find out the production of lipase enzyme that is responsible for the production of free fatty acid. It was estimated by the method given by Sadasivam and Manickam (1997). 20 ml of substrate (Emulsion of 2ml cocoa butter, 25 ml water, 100 mg bile salt and 2gm gum arabic) was taken in a 500 ml beaker and add 5ml of phosphate buffer. The beaker was set on the top

of a magnetic stirrer cum hot plate and was maintained the temperature at 35^0C. Noted the pH of reaction mixture and adjusted it to 7.0. Added enzyme extract (0.5ml), record the pH and set the timer on and let it be pH on zero time. At frequent intervals (10min) the pH drops by about 0.2 unit added 0.1 N NaOH to bring the pH to initial value. Continue the titration for 30-60 minutes. Noteed the volume of alkali consumed.

$$\text{Activity meq/min/g sample} = \frac{\text{Volume of alkali consumedx Strength of alkali}}{\text{Wt of sample(g)xTime in min}}$$

3.2.2.7. Total fat

Fermented cocoa nibs were defatted to extract the fat with petroleum ether (40-60℃) in a Soxhlet apparatus (Sadasivam and Manickam, 1997). Ten grams of cocoa bean powder was wrapped in a blotting paper and tied with twine. The sample was placed in the extraction tube of Soxhlet apparatus. The fat present in the cocoa powder was extracted through siphoning of petroleum ether through the apparatus and fat will get settled at the bottom of the flask along with a little amount of petroleum ether. This was transferred to a pre-weighed beaker and kept open for the petroleum ether to evaporate. The cream coloured substances left behind after the evaporation of solvent was the fat and it was weighed and expressed as percentage.

3.2.2.8. Free fatty acid analysis

One to ten gram of cocoa butter extracted from fermented cocoa beans was weighed into 250 ml conical flask to which added 50 ml of a mixture of equal volumes of alcohol and diethyl ether previously neutralised, after the addition of 1ml of phenolphthalein indicator. The solution was titrated with N/10 KOH with constant shaking until pink colour persists for 15 seconds. The titre value in ml (V) was noted (Sadasivam and Manickam, 1997).

$$\text{Acid value} = \frac{\text{Titre value X Normality of KOH X 56.1}}{\text{Weight of sample(gm)}}$$

After statistical analysis the best treatment based on free fatty acid content (<1.75%) were selected for further studies.

3.2.2.9. Temperature of fermented beans

Temperature was measured by inserting an ordinary thermometer (0-50^{0}C range) into the fermenting mass at three different positions of basket, heap and sack. From each position observation was taken thrice and the average was calculated.

3.2.3. Standardisation of drying methods based on free fatty acid content

The selected fermented beans (Heap method subjected to 7 days of fermentation) were subjected to sun drying and oven drying (65^{0}C) with twenty replications until the moisture content reached 7 per cent.

3.2.3.1. Sun drying

After seventh day of fermentation the cocoa beans were exposed to sun for drying with intermittent stirring for two or three times in a day for 7 days.

3.2.3.2. Artificial drying

For artificial drying the fermented cocoa beans were placed in hot air oven and dried at 65^{0}C until the moisture content reached to 7 per cent. Stirring of the beans were found necessary for both uniformity in drying and its efficiency.

3.2.4. Physico-chemical analysis of dried beans

The following physico-chemical analysis were done in sun dried and oven dried samples.

3.2.4.1. Bean recovery

After fermentation the beans weight was recorded. The fermented beans were dried and dry weight also recorded. Then fermentation recovery was estimated and expressed in per cent.

$$\text{Bean recovery} = \frac{\text{Dry weight after fermentation (g)}}{\text{Fresh weight after fermentation (g)}} \times 100$$

3.2.4.2. Moisture

Moisture content of the dried beans were estimated by the method of A.O.A.C (1980). To determine the moisture content, ten grams of the sample was taken in a Petridish and dried at 60 to 70^0C in a hot air oven, cooled in a desiccator and weighed. The process of heating and cooling was repeated till constant weight was obtained. The moisture content of the sample was calculated from the loss in weight during drying.

$$\text{Moisture content (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \text{ x } 100$$

3.2.4.3. pH estimation

pH of cocoa beans after different types of drying was recorded. Five gram samples of beans was homogenized for 30 seconds in 100 ml of hot distilled water and vacuum filtered through Whatman filter paper. A 25 ml aliquot was pipetted into a beaker and the pH was measured using a pH meter (AOAC, 1980).

3.2.4.4. Peroxide value

Peroxide value of fermented dried beans were estimated to find the rate of rancidity during fermentation. It was estimated by the method given by Sadasivam and Manickam (1997). One gram of extracted oil sample was taken in boiling tube and to that, one gram of potassium iodide and 20ml of solvent mixture (glacial acetic acid and chloroform) were added. The tube was placed in boiling water for 30 seconds and the contents were transferred to a conical flask containing 20ml of 5 per cent potassium iodide solution. The tubes were washed twice with 25 ml water and collected in a conical flask. This was titrated against N/ 500 sodium thiosulphate solution until yellow colour disappears. Later 0.5ml of starch solution was added and titrated till the appearance of blue colour. A blank solution was also prepared and peroxide value was calculated and expressed in milliequivalent per kg of the sample.

3.2.4.5. Total fat

Fermented dried cocoa nibs were defatted to extract the fat with petroleum ether (40-60℃) in a Soxhlet apparatus (Sadasivam and Manickam, 1997). Ten grams of cocoa bean powder was wrapped in a blotting paper and tied with twine. The sample was placed in the extraction tube of Soxhlet apparatus. The fat present in the cocoa powder was extracted through siphoning of petroleum ether through the apparatus and fat will get settled at the bottom of the flask along with a little amount of petroleum ether. This was transferred to a pre-weighed beaker and kept open for the petroleum ether to evaporate. The cream coloured substances left behind after the evaporation of solvent was the fat and it was weighed and expressed as percentage.

3.2.4.6. Lipase activity

Lipase activity was estimated to find out the production of lipase enzyme that is responsible for the production of free fatty acid. It was estimated by the method given by Sadasivam and Manickam (1997). 20 ml of substrate (Emulsion of 2ml cocoa butter, 25 ml water, 100 mg bile salt and 2gm gum arabic) was taken in a 500ml beaker. Added 5ml of phosphate buffer. The beaker was set on the top of a magnetic stirrer cum hot plate, and the temperature was maintained at 35^0C. Noted the pH of reaction mixture and adjust it to 7.0. Add enzyme extract (0.5ml), recorded the pH and set the timer on and let it be pH on zero time. At frequent intervals (10min), the pH drops by about 0.2 unit add 0.1 N NaOH to bring the pH to initial value. Continued the titration for 30-60 min period and noted the volume of alkali consumed.

$$\text{Activity meq/min/g sample} = \frac{\text{Volume of alkali consumed x Strength of alkali}}{\text{Wt of sample(g) x Time in min}}$$

3.2.4.7. Free fatty acid analysis

One to ten grams of cocoa butter extracted from fermented dried cocoa beans was weighed into 250 ml conical flask to which added 50 ml of a mixture of equal volumes of alcohol and diethyl ether previously neutralised, after the addition of 1ml of phenolphthalein indicator. The solution was titrated with N/10 KOH with constant shaking until pink colour persists for 15 seconds. The titre value in ml (V) was noted (Sadasivam and Manickam, 1997).

$$\text{FFA value} = \frac{\text{Titre value X Normality of KOH X 56.1}}{\text{Weight of sample(gm)}}$$

After statistical analysis the best method of drying based on free fatty acid content (<1.75%) were selected for further studies. Thus, sun drying were selected for further studies.

3.2.5. Standardisation of method of storage based on free fatty acid content

The selected dried beans (sun dried) were stored in polythene covers and plastic containers (PET bottles) under ambient condition for a period of six months with five replications. The control were stored in gunny bags.

3.2.6. Physico-chemical analysis of stored beans

The following physico-chemical qualities of cocoa beans stored in gunny bags, polythene covers and plastic container (PET bottles) were done initially and at an interval of one month for a duration of six months using standard procedures detailed in 3.2.4. The analysis was carried out in triplicate samples.

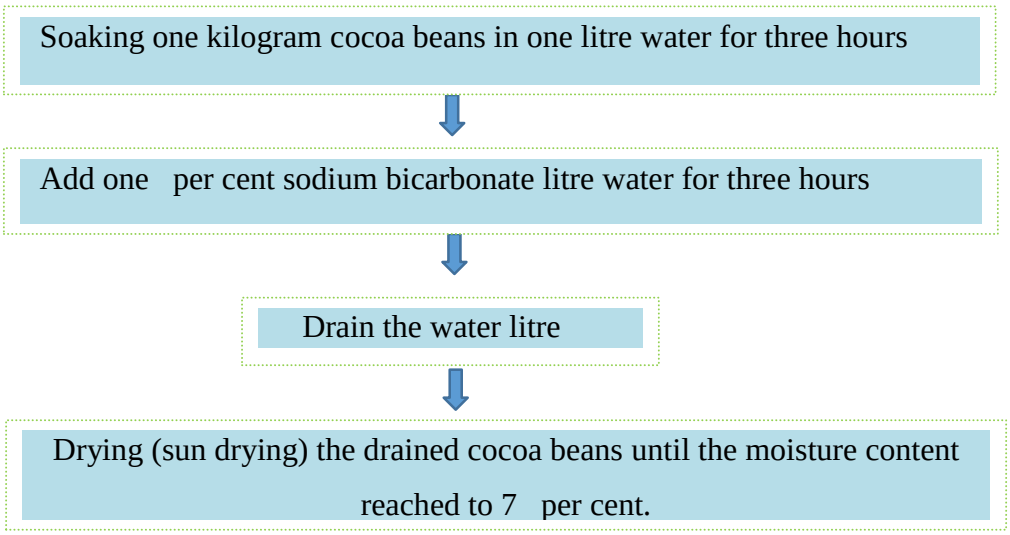
3.2.6.7. Microbial load

Microbial population of dried stored beans was determined by observing the total counts of bacteria and fungi through serial dilution and pour plate technique (Ranganna, 1986). The media used were Nutrient Agar for bacteria, Rose Bengal

Agar for fungus and Sabourouds Dextrose medium for yeast. The observations were expressed as number of colony forming units (cfu) per gram.

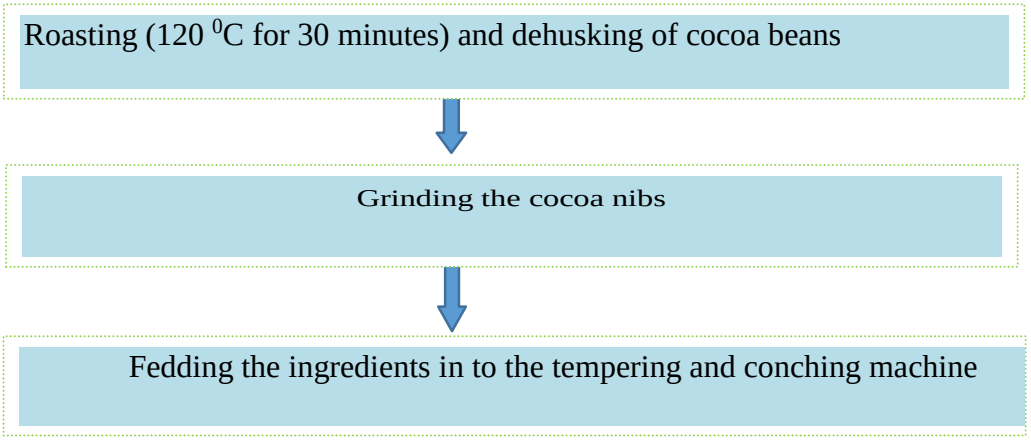
The stored cocoa beans in plastic container were divided into two lots. One lot was used as such and the second lot was subjected to alkalisation.

3.3. Alkalisation of stored cocoa beans



3.4. Standardisation of time and temperature for chocolate making using machine

The chocolate was prepared with alkalised and nonalkalised beans at different time and temperature by the following procedure standardised by Cocoa Research Centre, Kerala Agricultural University, Vellanikkara and is detailed below.



All the ingredients needed for chocolate making was fed in to machine

(Table.1.). Twelve sets of chocolates were prepared at different time periods 7, 9 and 11 hours at a temperature of 60^0C and 70^0C respectively in tempering and conching machine (Table.2.).

Table.1.Ingredients for chocolate making

Ingredients	Quantity
Cocoa nibs	800gm
Cocoa butter	1 kg
Sugar	2 kg
Milk powder	1 kg
Vanilla powder	25gm

Table.2.Details of time and temperature of chocolate making

Treatments	Combinations
AT1	60^0C for 7 hrs
AT_2	60^0C for 9 hrs
AT_3	60^0C for 11 hrs
AT_4	70^0C for 7 hrs
AT_5	70^0C for 9 hrs
AT_6	70^0C for 11 hrs
NAT_7	60^0C for 7 hrs
NAT_8	60^0C for 9 hrs
NAT_9	60^0C for 11 hrs
$NAT1_0$	70^0C for 7 hrs
$NAT1_1$	70^0C for 9 hrs
$NAT1_2$	70^0C for 11 hrs

A- Alkalised NA- Non alkalised

The chocolates prepared in tempering and conching machine were subjected to physico-chemical analysis, organoleptic evaluation and microbial enumeration.

3.4.1. Physico-chemical analysis of chocolates

The following physico- chemical qualities of chocolates were carried out using standard procedures. The analysis was carried out in triplicate samples.

3.4.1. 1. Texture Analysis

Texture is an important quality parameter which affects the consumer acceptability of chocolates and was determined using Texture Analyser (Stable Micro Systems, UK). The instrument had a microprocessor regulated texture analysis system interfaced to a personal computer. The instrument consists of two separate modules; the test bed and the control console (keyboard). Both are linked by a cable which route low voltage signal and power through it. The texture analyser measures force, distance and time and hence provide a three-dimensional product analysis. Forces may be measured to achieve set distances and distances may be measured to achieve set forces.

The sample was kept on the flat platform of the instrument and was subjected to double compression by a cylindrical probe with 5mm diameter. The test was conducted at a speed of 10 mm/s using 50 N load cell. The sample was allowed for double compression of 40 per cent with trigger force of 0.5 kg during various textural parameters were determined from the force deformation curve, the gel strength, rupture strength, brittleness and adhesiveness were determined.

3.4.1. 2. Moisture

Moisture content of chocolates were estimated by the method of A.O.A.C (1980). To determine moisture content of the sample, ten grams of the sample was taken in a petridish and dried in a hot air oven at 60^0C to 70^0C, cooled in a desicator and weighed. The process of heating and cooling was repeated till constant weight was achieved. The moisture content of the sample was calculated from the loss in weight during drying.

3.4.3. Energy

Energy content of selected chocolates were calculated according to Gopalan *et al*. (1989) and expressed as kilocalories (Kcal). The energy present in sample was calculated as per the formula given below.

Energy = (4 x Protein) + (4 x Total carbohydrates) + (9 x Fat)

3.4.1. 4. Total Soluble Solids (TSS)

The total soluble solids (TSS) of the chocolates was measured using refractrometer (Alvarez *et al*., 2003). To measure the TSS, the daylight plate was lifted up and the mucilage content was placed on top of the prism assembly. Then the daylight plate is closed so that the mucilage content spreads across the entire surface of the prism without any air bubbles or dry spots. The refractrometer was held in the direction of natural light source and when looked through the eyepiece, a circular field with markings and a partition with blue colour in the top and white below was found. The partition line indicates the TSS value and expressed it as degree brix.

3.4.1. 5 .Total sugar

The total sugar was determined using the method given by Ranganna (1986). From the clarified solution used for the estimation of reducing sugar, 50 ml was taken and boiled gently after adding citric acid and water. It was then neutralised with sodium hydroxide and the volume was made up to 250 ml. An aliquot of this solution was titrated against Fehling's solution A and B. The total sugar content was expressed as percentage.

$$\text{Total sugars (\%)} = \frac{\text{Fehling's factor x 250 x dilution x 100}}{\text{Titre value x 50 x weight of the sample}}$$

3.4.1. 6. Reducing sugar

Twenty five gram of chocolates was ground with 100 ml of distilled water and transferred to a conical flask. It was neutralised with 1N sodium hydroxide in the presence of phenolphthalein. For the clarification of the neutralised mixture, 2 ml of lead acetate was added followed by addition of 2 ml of potassium oxalate to neutralise the excess amount of lead acetate. It was then allowed to stand for 10 minutes for the settlement of the precipitate. Filtered the solution through Whatman's No.1 filter paper which was made upto 250 ml. Aliquot of the solution was titrated against a boiling mixture of fehlings solution A and B using methylene blue as indicator until the appears of brick red colour indicator (Ranganna, 1986). The reducing sugars present in chocolates were computed using the formula as follows.

$$\text{Reducing sugar (\%)} = \frac{\text{Fehling's factor x dilution x 100}}{\text{Titre value x weight of the sample}}$$

3.4.1. 7. Protein

The protein content of chocolates were estimated using Lowry's method given by Sadasivam and Manickam (1997). A sample of 500 mg was extracted using 5 to 10 ml of buffer (Tris buffer GR – tris hydroxymethyl amino methane) and centrifuged. An aliquot 0.1 ml from the supernatant was taken in a test tube, 5 ml alkaline copper solution was mixed well and allowed to stand for 10 minutes. Folin-Ciocalteau reagent of 0.5 ml was added and incubated at room temperature in the dark for 30 minutes and the developed blue colour was read at 660nm (OD). A standard graph was prepared using alkaline copper solution and Folin-Ciocalteau reagent by applying serial dilutions. From the standard graph, the amount of total protein present in sample was estimated and expressed in gram per 100 g of sample.

3.4.1. 8. Total fat

Chocolates were defatted to extract the fat with petroleum ether (40-60℃) in a Soxhlet apparatus (Sadasivam and Manickam, 1997). Ten grams of chocolates was wrapped in a blotting paper and tied with twine. The sample is placed in the extraction tube of Soxhlet apparatus. The fat present in the chocolates was extracted through siphoning of petroleum ether through the apparatus and fat will get settled at the bottom of the flask along with a little amount of petroleum ether. This was transferred to a pre-weighed beaker and kept open for the petroleum ether to evaporate. The cream coloured substances left behind after the evaporation of solvent was the fat and it was weighed and expressed as percentage.

3.4.1. 9. Polyphenols

Method: Folin- Ciocalteau (FC) reagent method

Powdered sample- 500 mg

Ethanol (80 percent)

Na_2CO_3 (20 percent)

FC reagent

Catechol – 100 mg

The powdered and defatted chocolates was used for the estimation of total polyphenols. The defatted samples were extracted exhaustively with ethanol. The total phenols in the extract then estimated by Folin- Ciocalteau reagent method developed by Malick and Singh (1980). The procedure followed is detailed below.

Exactly 500 mg of powdered defatted sample were taken and ground it with 80 percent ethanol using mortar and pestle and centrifuged at 10,000 rpm for 20 minutes. The supernatant was collected in a beaker and the remaining residue settled down was re extracted with five times the volume of 80 per cent ethanol. Again centrifuged and the supernatant was collected and pooled in the beaker. Then supernatant was allowed to evaporate. Five milli litre water was poured to the

residue to dissolve the phenols in it. Pippetted out 0.2 ml of the solution into a test tube and then make up the volume to 3 ml using distilled water followed by the addition of 0.5 ml of Folin-Ciocalteau reagent. Kept it for three minutes and add 2 ml of 20 per cent Na_2CO_3 solution and mixed well. The test tubes were kept in a boiling water bath exactly for one minute and after that cooled it to room temperature and incubated at room temperature for 60 minutes for colour development. A blue coloured complex, molybdenum blue was formed as the phenol undergoes a complex redox reaction with phosphomolibdic acid present in Folin-Ciocalteau reagent in alkaline medium. Absorbance was read at 650 nm.

The detector was caliberated for quantification of total phenols using following procedure. The total phenols in the extracts were assayed in terms of catechin taken as the reference. 100 mg of catechol dissolved in 100 ml of distilled water was taken as stock solution. Working standards were prepared from this. Pippette out 1 ml aliquot from the stock solution into a 10 ml standard flask and made up the volume. For the measurement of absorbance value, pipette out 0.2 ml from this to a test tube and made up the volume to 3 ml with distilled water followed by the addition of 0.5 ml of Folin-Ciocalteau reagent. Kept it for three minutes and 2 ml of 20 percent Na_2CO_3 solution was added and mixed thoroughly. The absorbance was read at 650 nm.

Concentration of phenols present in the extract was worked out by substituting the absorbance value, thus obtained in the calibration equation. The total phenol content was calculated as mg catechol equivalent of phenol per gram sample and expressed it as percent.

$$\text{Total phenol} = \frac{\text{OD sample}}{\text{OD standard}} \text{ x } \frac{\text{Conc.of standard}}{\text{Vol.of sample}} \text{ x } 100$$

Where, OD sample = absorbance value of sample

OD standard = absorbance value of standard

3.4.1.10. Total ash

The ash content of the chocolates were estimated using the method given by ISI (1980). Five gram of sample was taken in a crucible and then was ignited at 550- 600°C in a muffle furnace for 5-6 hours. Cooled in a desiccator at room temperature and weighed. The ash content of sample was expressed in percentage.

3.4.1.11. Calcium

The calcium content of the chocolates were estimated by atomic absorption spectrophotometric method using the diacid extract prepared from the sample (Perkin-Elmer, 1982). A sample of 0.20 g was predigested with 10 ml of9:4 mixture of nitric acid and perchloric acid and made up the volume to 50 ml and used directly in atomic absorption spectrophotometer for the estimation of calcium and expressed in mg 100 g^{-1} of sample.

3.4.1.12. Phosphorus

The phosphorous content was analysed colorimetrically (Jackson, 1973), which gives yellow colour with nitric acid vanadate molybdate reagent. To five ml of pre-digested aliquot, five ml of nitric acid vandate molybdate reagent was added and made up to 50 ml with distilled water. After 10 minutes, the OD was read at 420 nm.

3.4.1.13. Iron

Iron content present in chocolates were determined using method suggested by Perkin – Elmer (1982). One gram of chocolate was pre-digested using 10 ml of 9:4 ratio of nitric and percholoric acid. The prepared diacid extract of the chocolate sample was used for estimation of iron in Atomic Absorption Spectrophotometer. Iron content present in the sample was expressed as in mg100 g^{-1} of sample.

3.4.1.14. Lipase activity

Lipase activity was estimated to find out the production of lipase enzyme that is responsible for the production of free fatty acid. It was estimated by the method given by Sadasivam and Manickam (1997). 20 ml of substrate (Emulsion of 2ml cocoa butter, 25 ml water, 100 mg bile salt and 2gm gum arabic) was taken in a 500ml beaker. Added 5ml of phosphate buffer. The beaker was set on the top of a magnetic stirrer cum hot plate and maintained the temperature at 35^0C. Noted the pH of reaction mixture and adjust it to 7.0. Added enzyme extract (0.5ml), recorded the pH and set the timer on and let it be pH on zero time. At frequent intervals (10min), the pH drops by about 0.2 unit add 0.1 N NaOH to bring the pH to initial value. Continued the titration for 30-60 min period and noted the volume of alkali consumed.

$$\text{Activity meq/min/g sample} = \frac{\text{Volume of alkali consumed x Strength of alkali}}{\text{Wt of sample(g) x Time in min}}$$

3.4.1.15. Free fatty acid analysis

One to ten grams of cocoa butter extracted from chocolates was weighed into 250 ml conical flask to which added 50 ml of a mixture of equal volumes of alcohol and diethyl ether previously neutralised, after the addition of 1ml of phenolphthalein indicator. The solution was titrated with N/10 KOH with constant shaking until pink colour persists for 15 seconds. The titre value in ml (V) was noted (Sadasivam and Manickam, 1997).

$$\text{Acid value} = \frac{\text{Titre value X Normality of KOH X 56.1}}{\text{Weight of sample(gm)}}$$

3.4.1.16. Peroxide value

Peroxide value of chocolates were estimated to find the rate of rancidity during fermentation. It was estimated by the method given by Sadasivam and Manickam (1997). One gram of extracted oil sample was taken in boiling tube and to that one gram of potassium iodide and 20ml of solvent mixture (glacial acetic acid and chloroform) were added. The tube was placed in boiling water for 30

seconds and the contents were transferred to a conical flask containing 20ml of 5 per cent potassium iodide solution. The tubes were washed twice with 25 ml water and collected in a conical flask. This was titrated against N/ 500 sodium thiosulphate solution until yellow colour disappears. Later 0.5ml of starch solution was added and titrated till the appearance of blue colour. A blank solution was also prepared and peroxide value was calculated and expressed in milliequivalent per kg of the sample.

3.4.2. Organoleptic evaluation of chocolates

3.4.2.1. Organoleptic evaluation

Organoleptic evaluation of the standardised chocolates were conducted using score card by a panel of 15 judges.

3.4.2.2. Selection of judges

A series of organoleptic evaluation were done by using simple triangle test at laboratory level to select a panel of 15 judges between the age group of 18-35 years as suggested by Jellinek (1985).

3.4.2.3. Preparation of score card

Score card containing six quality attributes such as appearance, colour, flavour, texture, taste and overall acceptability was used for the evaluation of the developed chocolates. Each of the above mentioned qualities were assessed by a nine point hedonic scale. The score cards used for the evaluation of chocolates are given in Appendix I.

3.4.3. Enumeration of total micro flora

The microbial population present in the chocolates were estimated using serial dilution plate count method as suggested by Agarwal and Hasija (1986). The microbial analysis was carried out in chocolates.

The sample was prepared by mixing 90 ml of distilled water with 10 g of chocolate and shaken well using a shaker to obtain suspension. The serial dilutions were carried out in the prepared water blank. To 9 ml of water blank transfer one ml of the prepared suspension with a dilution of 10^{-2}. This is then diluted to 10^{-3} followed by 10^{-4}, 10^{-5} and 10^{-6} using serial dilution techniques. Bacteria, fungi and yeast count were assessed using Nutrient Agar (NA) for bacteria, Potato Dextrose Agar (PDA) for fungi and Sabouraud's Dextrose Agar (SDA) media for yeast respectively and results are given as cfu/g.

3.4.3.1. Enumeration of bacterial colony

Total number of bacterial colony was enumerated in 10^{-4} dilution in nutrient agar medium. In a sterile petriplate, pour one ml of 10^{-4}dilution using a micropipette. To petriplate pour about 20 ml of the nutrient agar medium which is uniformly spread in petriplate by rotating in clockwise and anticlockwise directions. For bacterial colony the enumerated petriplates were incubated for 48 hrs at room temperature. The total number of bacterial colonies were counted and expressed as cfu/g.

3.4.3.2. Enumeration of fungal colony

Total number of fungal colony was enumerated in 10^{-3} dilution in Martin Rose agar medium. In a sterile petriplate, pour one ml of 10^{-3} dilution using a micropipette. To petriplate pour about 20 ml of the Potato Dextrose Agar medium is uniformly spread. For fungal colony enumeration the petriplates were incubated for 4 to 5 days at room temperature. The total number of fungal colonies were counted and expressed as cfu/g.

3.4.3.3. Enumeration of yeast colony

Total number of yeast colony was enumerated in 10^{-3} dilution in Sabouraud's Dextrose Agar medium. In a sterile petriplate, pour one ml of 10^{-3} dilution using a micropipette. To petriplate pour about 20 ml of the Sabouraud's Dextrose Agar medium which is uniformly spread in the petriplate by rotating. For enumeration of yeast population, the petriplates were incubated for 4 to 5 days in room temperature. The total number of yeast colonies were counted and expressed as cfu/g.

The scores obtained for the 12 treatments with different time and temperature were analysed by Kendall Wallace Analysis of variance to select the most acceptable chocolates. Kendall's coefficient of concordance (W) which expresses the degree of association among the ten judges was carried out for each treatment. Based on sensory evaluation and free fatty acid content the best treatments were selected for further studies

After statistical analysis the most acceptable chocolates, one each from alkalised and one each from the non alkalised chocolates were selected for further studies. Thus the treatments (one each from alkalised and non alkalised) T_6 (70^0C for 11 hrs.) and $T1_2$ (70^0C for 11 hrs.) were selected for further studies.

3.5. Blending of chocolates with other ingredients

Selected chocolates were prepared by blending with dried fruits, nuts, dehydrated mint leaves and white pepper at various proportions as per treatment specifications as detailed in Table 5. The per cent of dehydrated mint and white pepper added in the chocolates were also standardized based on sensory evaluation. Standardisation of per cent of dehydrated mint leaves and white pepper powder to be added to chocolate detailed in Table 3 and 4. All the treatments mentioned in Table 5 were done with alkalized and non alkalised beans. The experiment was conducted using CRD with eighteen treatments and three replications.

Table 3. Standardisation of the quantity of dehydrated mint leaves powder in chocolates

Treatments	Combinations
T_1	99% C+1% DMP
T_2	98% C+2% DMP
T_3	97% C+3% DMP
T_4	96% C+4% DMP
T_5	95% C+5% DMP

C-chocolate DMP– Dehydrated Mint Powder

Table 4. Standardisation of the quantity of white pepper powder in chocolates

Treatments	Combinations
T_1	99% C+1% WPP
T_2	98% C+2% WPP
T_3	97% C+3% WPP
T_4	96% C+4% WPP
T_5	95% C+5% WPP

C-chocolate WPP – White Pepper Powder

Table 5. Details of combinations of chocolates with dried fruits, nuts, dehydrated mint leaves and white pepper

Treatments	Combinations
AT_0 (Control)	100 % C (alkalised beans)
AT1	95% C+5% DG
AT_2	95 % C+5% DD
AT_3	95 % C+5% ODJ
AT_4	95%C+5% ODP
AT_5	95 % C+5% CN
AT_6	95 % C+5% A
AT_7	95 %C + 3%DMP
AT_8	95 %C + 5%WPP
NAT_0 (Control)	100 % C(Non alkalised beans)
NAT1	95% C+5% DG
NAT_2	95 % C+5% DD
NAT_3	95 % C+5% ODJ
NAT_4	95%C+5% ODP
NAT_5	95 % C+5% CN
NAT_6	95 % C+5% A
NAT_7	97 %C + 3%DMP
NAT_8	95 %C + 5%WPP

A-Alkalised, DG- Dehydrated Grapes, ODJ- Osmodehydrated Jackfruit, NA-Non alkalised. DD-Dehydrated Dates, ODP- Osmodehydrated Pineapple, C- chocolate, DMP – Dehydrated Mint Powder, CN-Cashew Nut, A-Almond, WPP-White Pepper

The chocolates were prepared as per the procedure detailed in 3.2.1 with alkalised and non alkalised beans. The chocolate was taken out from the machine and blended with dried fruits, nuts, dehydrated mint leaves and white pepper at various proportions as detailed in table.3. It was then molded into desired shape and subjected to organoleptic evaluation by the selected panel of judges.

3.5.1. Selection of blended chocholates

The scores obtained for the 18 treatments with alkalised and non alkalised chocolates were analysed by Kendall Wallace Analysis of variance to select the most acceptable chocolates. Kendall's coefficient of concordance (W) which expresses the degree of association among the ten judges was carried out for each treatment.

After statistical analysis the most acceptable chocolates, three from alkalized chocolates and three from the non alkalised chocolates were selected for storage study. Thus, a total of eight treatments including controls (alkalized and non alkalised chocolates) were selected for further studies.

Based on organoleptic evaluation as per the procedure detailed in 3.2.3, the best six treatments (three each from alkalised and non alkalised) along with control (alkalised and non alkalised) were selected for further studies.

3.5.2. Quality evaluation of selected blended chocolates

The selected chocolates and control were subjected to quality evaluation studies. The chocolates were wrapped in aluminium foil and stored for six months in refrigerated conditions for further studies.

3.5.2. 1. Physico-chemical analysis of blended chocolates

The following physico- chemical qualities of blended chocolates were carried out at monthly intervals using standard procedures as detailed in 3.4.1. The analysis was carried out in triplicate samples.

3.5.2.2. Organoleptic evaluation of blended chocolates during storage

Organoleptic qualities of the blended chocolates as per the procedure detailed in 3.4.2 were conducted initially and at an interval of fifteen days for six months.

3.5.2.3. Enumeration of total micro flora

The presence of bacteria, fungi and yeast were evaluated at monthly intervals as per the procedure detailed in 3.4.3.

3.6. Cost of production of the selected chocolates

The cost of production of 40 g of the selected chocolates were computed using the market price of raw materials incurred for the product preparation along with labour charge, fuel charge, electricity charge and packaging cost.

3.7. Statistical analysis of the data

The data were recorded and analysed as completely randomised design (CRD). The best method of fermentation was differentiated using two factor analysis. The best method of drying was differentiated using two sample case t test. The physico- chemical qualities and shelf life qualities of the each treatment were compared using one way ANOVA and t test. Based on organoleptic evaluation, the best treatment was identified using Kendall's Coefficient of Concordance (W).

Results

RESULTS

The results pertaining to the study entitled 'Process optimisation and quality evaluation of cocoa based chocolates' are presented in this chapter under the following headings.

4.1. Development of protocol for primary processing of cocoa

4.1.1. Standardisation of fermentation methods based on free fatty acid content

4.1.2. Physico-chemical analysis of fermented cocoa beans

4.1.3. Standardisation of drying methods based on free fatty acid

4.1.4. Physico-chemical analysis of dried cocoa beans

4.1.5. Standardisation of method of storage based on free fatty acid content

4.1.6. Physico-chemical analysis of stored cocoa beans

4.1.7. Microbial load on cocoa beans during storage

4.2. Alkalisation of stored cocoa beans

4.3. Standardisation of time and temperature for chocolate making using machine

4.3.1. Physico-chemical analysis of chocolates

4.3.2. Organoleptic evaluation of chocolates

4.3.3. Enumeration of total micro flora

4.4. Blending of chocolates with other ingredients

4.4.1. Quality evaluation of selected blended chocolates

4.4.1.1. Physico- chemical analysis

4.4.1.2. Organoleptic evaluation

4.4.1.3. Enumeration of total micro flora

4.5. Cost of production of the selected chocolates

4.6. Statistical analysis of the data

4.1. Development of protocol for primary processing of cocoa

In the present study, a protocol was developed for primary processing of cocoa beans based on free fatty acid content of <1.75 % (WHO, 2001).

4.1.1. Standardisation of fermentation methods based on free fatty acid content

The cocoa beans were subjected to three different types of fermentation like basket method, heap method and sack method for three different periods of fermentation (Plate 1,2 and 3). The periods selected for the study was 5^{th}, 6^{th} and 7^{th} day of fermentation. Nine different treatments [(T_1 - 5^{th} day-Basket), (T_2- 6^{th} day-Basket), (T_3- 7^{th} day-Basket), (T_4- 5^{th} day-Heap), (T_5- 6^{th} day-Heap), (T_6 - 7^{th} day-Heap), (T_7- 5^{th} day-Sack), (T_8 - 6^{th} day-Sack), (T_9 - 7^{th} day-Sack)] of fermentation were carried out.

4.1.2. Physico-chemical analysis of fermented beans

The physico-chemical qualities of the fermented cocoa beans were carried out using standard procedures. The analysis was carried out in triplicate samples. The physico-chemical qualities of fermented cocoa beans such as fermentation bean recovery, fermentation index/cut test, moisture, pH, peroxide value, lipase activity, total fat and free fatty acid content were evaluated.

4.1.2.1. Cut test/ Fermentation index of cocoa beans during fermentation periods

One of the indices used to determine the final price of cocoa is cut test. Cut test / fermentation index was found out for the cocoa beans using different fermentation methods during different periods of fermentation and is presented in table 6. The beans after fermentation were scored based on the colour into fully fermented, partially fermented and unfermented (Plate 4,5 and 6).

Fermentation index or cut test score was observed highest in heap method at seventh day of fermentation (T_6) with 84.99per cent of fully fermented beans, 10.66% of partially fermented beans and 4.33per cent of unfermented beans. The basket method (T_3) exhibited 72.22per cent of fully fermented beans and 19.88per

Plate 1. Basket method of fermentation

Plate 2. Heap method of fermentation

Plate 3. Sack method of fermentation

Plate 4. Fully fermented beans

Plate 5. Partially fermented beans

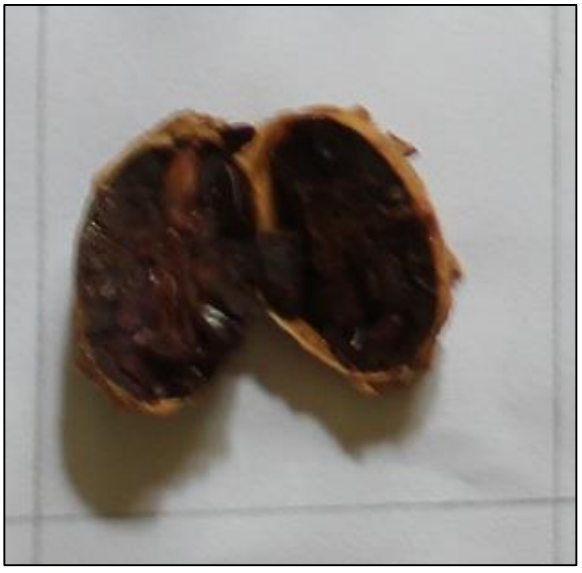


Plate 6. Not fermented beans

cent of partially fermented beans and 07.88per cent of unfermented beans. The sack method (T_9) was observed with least fully fermented beans at seventh day of fermentation (69.10%) and highest per cent in non-fermented beans among all the treatments.

The treatments T_3, T_5 and T_6 were observed with greater than 70 per cent fully fermented beans at seventh day of fermentation. The treatment T_1 was observed with least fully fermented beans of 28.99 per cent followed by T_7 with 29.88per cent at fifth day of fermentation.

Partially fermented beans were observed more in treatment T_1, T_4 and T_7 with the score of 35. 99 per cent, 35.77per cent and 34.88per cent at fifth day of fermentation in basket, heap and sack method respectively. Least score of partially fermented beans below 25 per cent were observed in treatment T_3, T_5, T_6 and T_9 with 19.88 per cent, 16.88 per cent, 10.66per cent and 24.44per cent respectively.

The treatments T_1, T_4 and T_7 were observed with highest unfermented beans of 34.99per cent, 21.77per cent and 35.21 per cent respectively at fifth day of fermentation. The lowest not fermented beans were observed in T_3, T_6 and T_9 respectively at seventh day of fermentation.

From the figure.2. It is clear that the fermentation index or cut test score was highest in heap method followed by basket and heap method at seventh day of fermentation respectively.

4.1.2.2. Fermentation bean recovery

Bean recovery of cocoa beans was worked out using different fermentation methods during different periods of fermentation. The data was interpreted using two factor analysis and is presented in table 7. Based on two way ANOVA, no statistically significant difference was observed between all the three periods (5^{th}, 6^{th} and 7^{th} day) of fermentation. However with regards to types of fermentation,

significant changes were observable for all the three types of fermentation namely basket, heap and sack method fermentation.

The highest fermentation bean recovery of 84.33per cent was noticed in treatment T_4 in heap method at fifth day of fermentation, followed by 82 per cent of bean recovery were noticed in treatment T_5 at sixth day of fermentation in heap method and 78.33 per cent of bean recovery were in treatment T_7 at fifth day of fermentation in sack method.

The lowest fermentation bean recovery of 52 per cent was observed in treatment T_3 at seventh day of fermentation and 57.33per cent in treatment T_2 at sixth day of fermentation in basket method. There was a reduction in fermentation bean recovery during fermentation periods in all three types of fermentation methods.

Bean recovery of fermented beans were decreased by increasing fermentation periods. However the highest bean recovery at the end of fermentation day that is at seventh day of fermentation was recorded in heap method (76%) and is depicted in figure.3.

4.1.2.3. Moisture content

Moisture content in cocoa beans using different methods and periods of fermentation were interpreted using two factor analysis and are presented in table 8. Statistically no significant difference was noticed in moisture content between the different types and periods of fermentation.

The initial moisture content of 50.40 per cent, 53.67 per cent and 54.40 per centwas observed in sack method, heap method and in basket method respectively. Moisture content during fermentation periods was observed as 44.40 per cent in heap method, 47.40per cdent in basket method and 44.83per cent in sack method at fifth day of fermentation but it reduces into 37.83per dent, 39.43per cent and 39.80 per cent respectively at seventh day of fermentation. The lowest moisture content

of 37.83 per cent was recorded in heap method at seventh day of fermentation. A decrease in moisture content of fermented beans was observed with increasing duration of fermentation periods in all methods of fermentation (Figure 4).

Table.6. Fermentation index of cocoa beans during fermentation periods

Fermentation methods	**Fermentation index of fermented cocoa beans**			
	Treatments	**Fully fermented score (%)**	**Partially fermented score (%)**	**Unfermented score (%)**
Basket	T_1	28.99	35.99	34.99
	T_2	59.77	25.33	14.86
	T_3	72.22	19.88	07.88
Heap	T_4	43.55	35.77	21.77
	T_5	72.22	16.88	10.88
	T_6	84.99	10.66	04.33
Sack	T_7	29.88	34.88	35.21
	T_8	54.66	31.8	13.22
	T_9	69.10	24.44	07.32

T_1- 5 th day-Basket T_4- 5 th day-Heap T_7- 5 th day-Sack
T_2- 6th day-Basket T_5- 6 th day-Heap T_8- 6 th day-Sack
T_3- 7 th day-Basket T_6- 7 th day-Heap T_9- 7 th day-Sack

Table.7. per cent of fermented bean recovery of cocoa beans with different fermentation methods and fermentation periods

Fermentation methods	**Periods of fermentation**		
	5th day	**6th day**	**7th day**
Basket	T_1 -77.33 (20.22)	T_2- 57.33 (18.33)	T_3- 52.00 (19.25)
Heap	T_4-84.33 (7.70)	T_5- 82.00 (5.42)	T_6-76.00 (9.05)
Sack	T_7-78.33 (15.52)	T_8-67.00 (13.07)	T_9-61.66 (16.87)

Figures in parenthesis indicates the percentage deviation LSD (5% level): Fermentation methods (8.139); Fermentation period (NS); Fermentation methods × Fermentation period (NS)

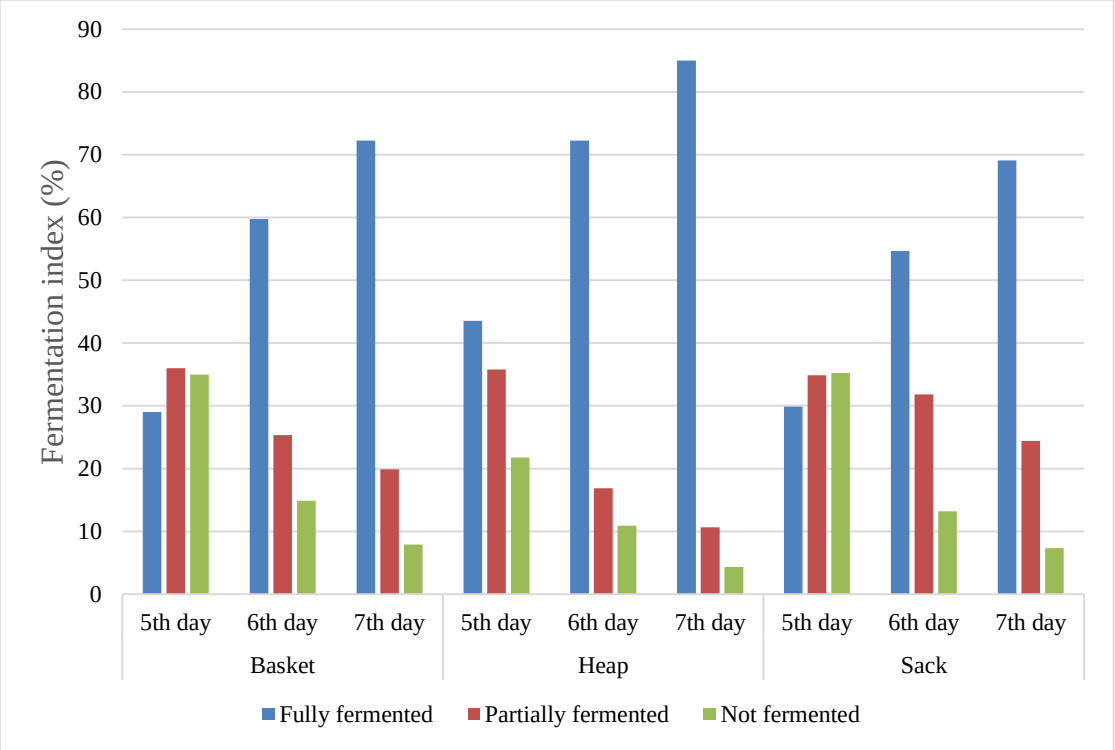


Fig. 2. Fermentation index of fermented cocoa beans

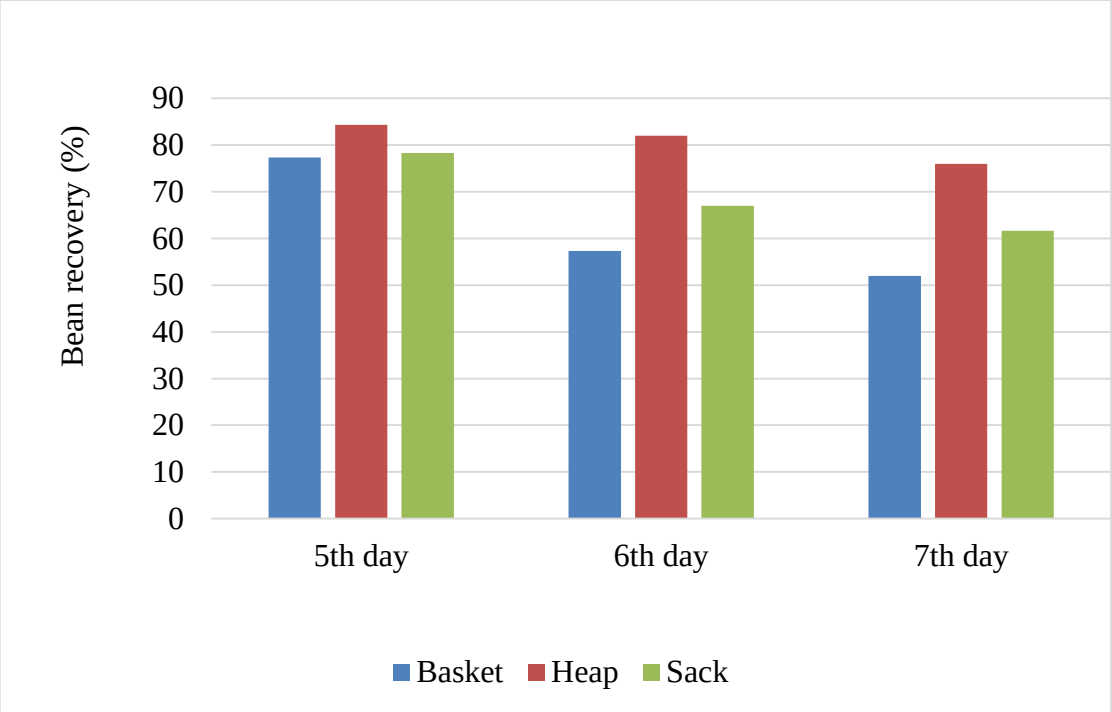


Fig. 3. Bean recovery of fermented cocoa beans

4.1.2.4. pH

Initially the pH value was 5.93, 6.11 and 5.82 in basket, heap and sack method respectively. The changes in pH of different treatments during fermentation was interpreted using two factor analysis (Table 9). Based on the percentage deviation, the pH did not have any significant difference with regard to fermentation methods namely basket, heap and sack method. However with days of fermentation significant changes were observable in all fermentation methods from fifth to sixth day of fermentation at LSD of 5% level. No significant difference was observed with 6^{th} and 7^{th} day of fermentation. The initial pH value of 6.11 was found to be maximum in heap method followed by 5.93 and 5.82 in basket and sack method respectively.

Figure 5 shows that the pH of fermented beans decreased during fermentation periods and there was a drastic decrease in pH from initial to fifth day of fermentation. A steady decrease in pH was observed among the three fermentation methods from fifth to seventh day of fermentation. It is evident from the figure that a decrease of 4.52 in pH from initial was noticed in T_4 during fifth day of fermentation for heap method. During seventh day of fermentation maximum pH of 4.38 was observed in T_6 in heap method of fermentation.

4.1.2.5. Total fat

The fat content of cocoa beans using different methods and periods of fermentation is represented in table 11. The initial fat content of 42.66 per cent was highest in heap method followed by 41.00 per cent and 40.44 per cent in sack and basket methods respectively Generally, the fat content decreased with the increase in the fermentation periods. No significant difference was observed with respect to the fermentation methods and period of fermentation. However the fat content was slightly more in cocoa beans fermented in heap method with 40.11 per cent, 37.00 per cent and 32.89 per cent at fifth, sixth and seventh day of fermentation respectively. There was no significant difference between fermentation methods and fermentation periods.

Table 8. Moisture content of cocoa beans with different fermentation methods and fermentation periods

Fermentation methods	Moisture content (%) for different fermentation periods			
	Initial	**5th day**	**6th day**	**7th day**
Basket	54.40	T_1-47.40 (11.24)	T_2-42.37 (8.28)	T_3-39.43 (9.67)
Heap	53.67	T_4- 44.40 (9.09)	T_5- 40.83 (11.58)	T_6- 37.83 (11.80)
Sack	50.40	T_7- 44.83 (4.13)	T_8- 42.03 (7.41)	T_9- 39.80 (10.70)

Figures in parenthesis indicates the percentage deviation
LSD (5% level): Fermentation methods (NS); Fermentation period (NS); Fermentation methods × Fermentation period (NS).

Table 9. pH of cocoa beans during fermentation

Fermentation methods	pH of different fermentation periods			
	Initial	**5th day**	**6th day**	**7th day**
Basket	5.93	T_1 4.61 (22.06)	T_2 4.43 (3.63)	T_3 4.28 (3.32)
Heap	6.11	T_4 4.52 (26.23)	T_5 4.45 (1.09)	T_6 4.38 (1.60)
Sack	5.82	T_7 4.53 (22.08)	T_8 4.39 (3.20)	T_9 4.26 (2.74)

Figures in parenthesis indicates the percentage deviation
LSD (5% level): Fermentation methods (NS); Fermentation period (4.340); Fermentation methods × Fermentation period (NS)

A mean fat content of 40.44 per cent recorded on the first day of fermentation was reduced into 31.00 per cent in basket method. Similarly the fat content of 42.66 per cent recorded on the first day in heap method was reduced to 32.89 per cent on completion of fermentation at seventh day. The initial fat content of 41.00 per cent in sack method was reduced in to 32. 55 percent at the end of fermentation during seventh day.

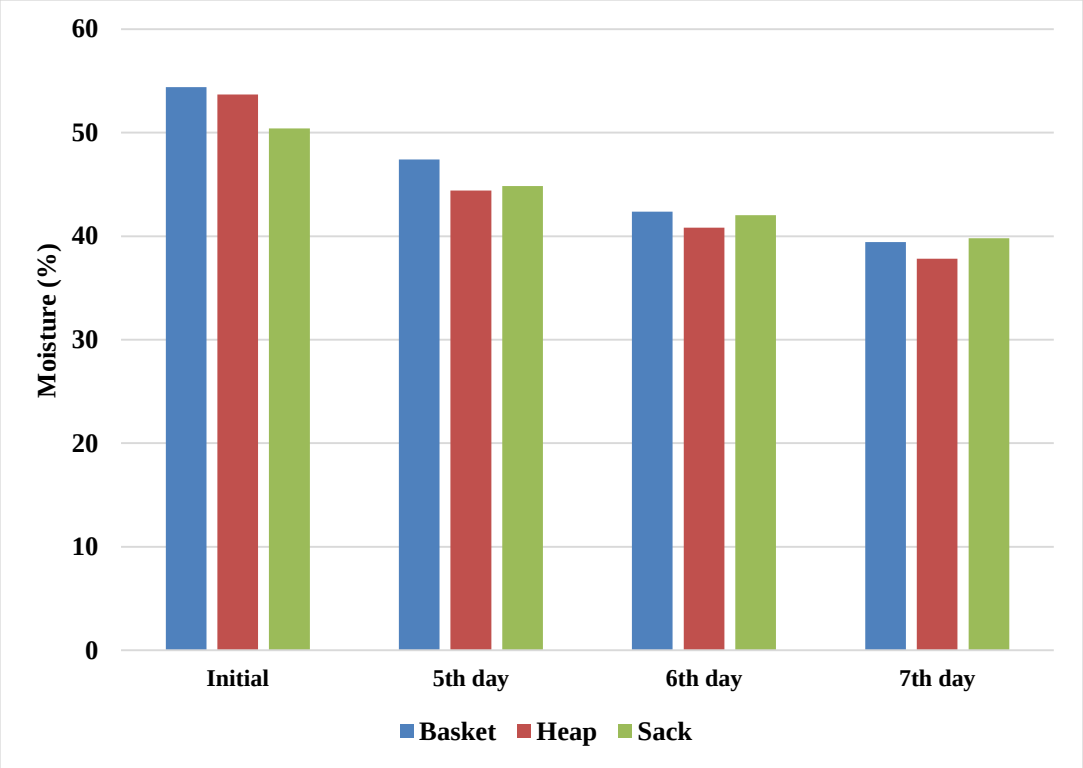


Fig.4. Moisture content of fermented cocoa beans

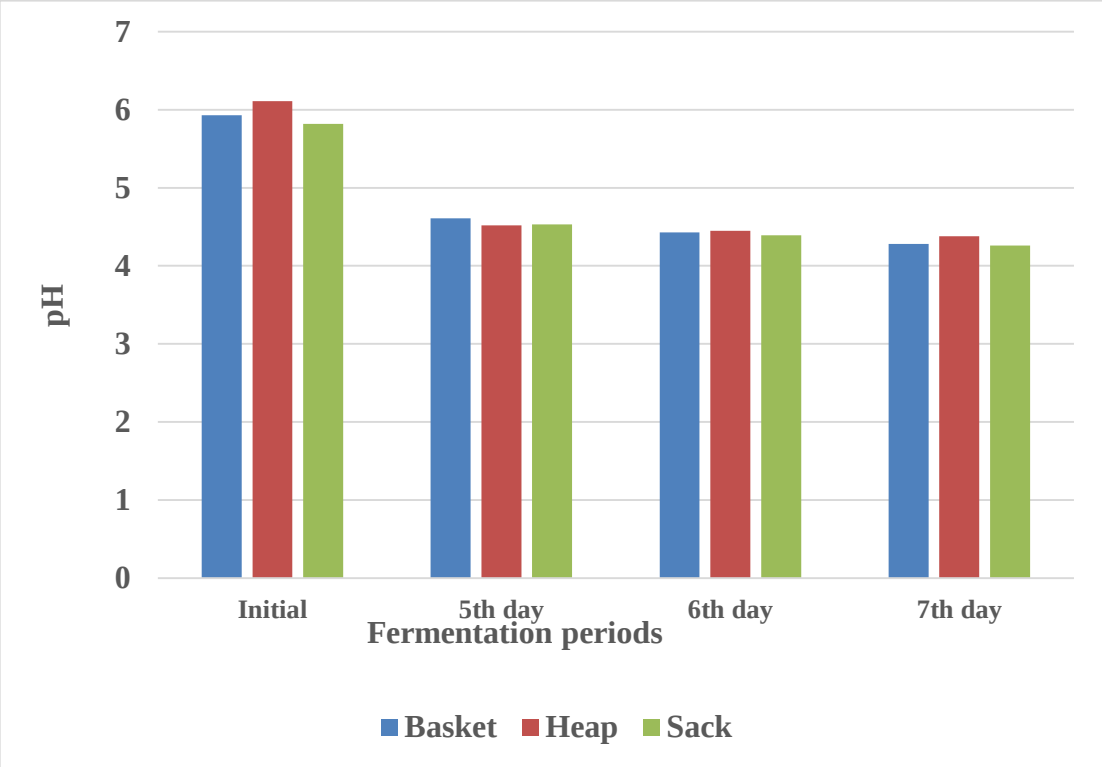


Fig.5. pH of fermented cocoa beans

From the figure 6 it is clear that there was a slight decrease in fat content during fermentation but it was not significant.

4.1.2.6. Peroxide value

The peroxide value of fermented cocoa beans were not detected throughout the fermentation periods in all nine treatments.

4.1.2.7. Lipase activity

The lipase activity of cocoa beans using different methods and periods of fermentation is presented in table 11. Based on the percentage deviation, the lipase activity did not have a significant response for fermentation methods. The interaction effects of fermentation methods and fermentation periods were not significant.

However in the traditional heap method, the lipase activity was observed with a decrease in per cent deviation at 25.46, 25.30 and 22.19% in 5^{th}, 6^{th} and 7^{th} days respectively. Lipase activity was decreased with fermentation periods in all three fermentation methods. In basket method the activity varied from initial of 0.0027 to 0.0011 at the end of fermentation, in heap method it varied from 0.0022 to 0.0005 and for sack it decreased from 0.0026 to 0.0012 respectively.

A decrease in lipase activity with advancement of fermentation periods was observed in all three methods. It is evident from the bar graph (Fig. 7) that, the lowest lipase activity of 0.0005 was recorded for the treatment T_6 for the heap method at seventh day of fermentation.

Table 10. Per cent of fat content of cocoa beans during fermentation

Fermentation methods	Per cent fat content for different fermentation periods			
	Initial	5th day	6th day	7th day
Basket	40.44	T_1 36.33 (9.36)	T_2 33.66 (7.45)	T_3 31.00 (8.50)
Heap	42.66	T_4 40.11 (8.70)	T_5 37.00 (7.67)	T_6 32.89 (8.08)
Sack	41.00	T_7 39.44 (8.63)	T_8 35.55 (7.53)	T_9 32.55 (7.73)

Figures in parenthesis indicates the percentage deviation
LSD (5% level): Fermentation methods (NS); Fermentation period (NS); Fermentation methods × Fermentation period (NS).

Table 11. Lipase activity of cocoa beans during fermentation

Fermentation methods	Lipase activity for different fermentation periods (µ Eq)			
	Initial	5th day	6th day	7th day
Basket	0.0027	T_1 0.0018 (24.18)	T_2 0.0014 (20.25)	T_3 0.0011 (30.27)
Heap	0.0022	T4 0.0014 (25.46)	T_5 0.0012 (25.30)	T_6 0.0005 (22.19)
Sack	0.0026	T_7 0.0019 (25.10)	T_8 0.0015 (20.12)	T_9 0.0012 (21.62)

Figures in parenthesis indicates the percentage deviation
LSD (5% level): Fermentation methods (NS); Fermentation period (NS); Fermentation methods × Fermentation period (NS)

4.1.2.8. Free fatty acid

The free fatty acid content of cocoa beans using different methods and periods of fermentation is presented in table 12. Based on the percentage deviation, the free fatty acid had a statistically significant response to fermentation methods, namely basket, heap and sack method and the difference in period of fermentation namely fifth, sixth and seventh were also significant. The three different types of fermentation had significant decrease with the maximum decrease in free fatty acid content in traditional heap method (42.96, 13.48 and 32.10% in 5th, 6th and 7th days respectively).

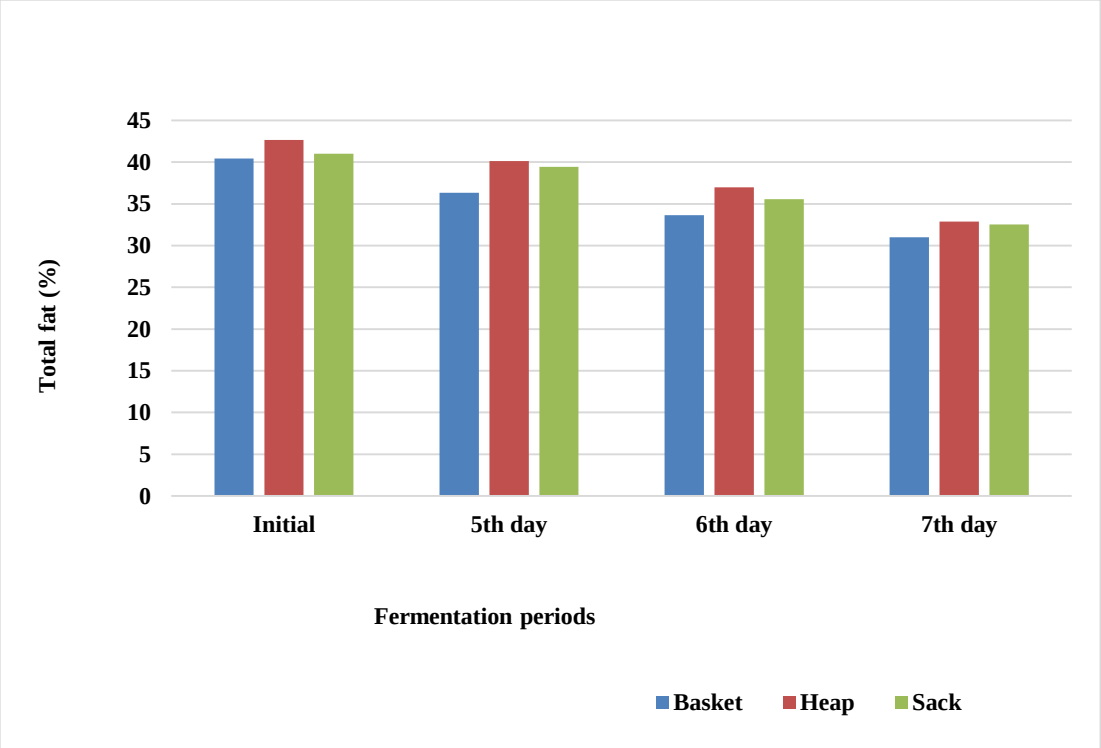


Fig.6. Total fat of fermented cocoa beans

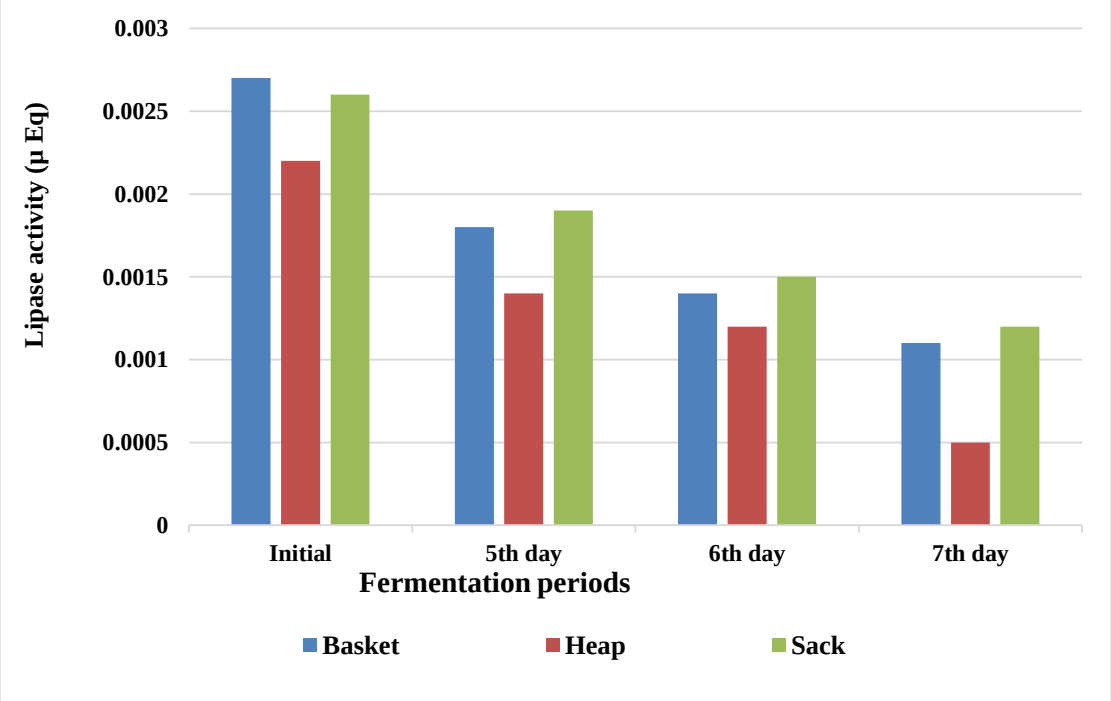


Fig.7. Lipase activity of fermented cocoa beans

However with regards to days of fermentation significant decreased changes were observable on all the three days of fermentation under observation with maximum observable decrease after five days of fermentation (40.07, 42.96 and 35.93 % in basket, heap and sack methods respectively) and the trend continued as there was a telescopic decreasing tendency noticeable even up to seven days of fermentation.

A mean free fatty acid content of 2.51 percent was recorded on the first day of fermentation and was reduced in to 1.05 per cent in basket method. Similarly the free fatty acid content of 2.39 per cent recorded on the first day in heap method was reduced to 0.80 per cent on fermentation at seventh day. The initial free fatty acid content of 2.52 per cent in sack method was reduced in to 1.18 per cent at the end of fermentation during seventh day. From the figure 8 it is clear that there is a drastic decrease in free fatty acid content in heap method at seventh day of fermentation compared to other two methods of basket and heap method.

Though the interaction effects of fermentation methods and fermentation periods were not significant, the heap method with seven days of fermentation combination was observed as with the lowest free fatty acid content (0.80%) and a higher percentage deviation (32.10 %).

4.1.2.9. Temperature during fermentation

The mean temperature of cocoa bean mass during fermentation using different methods and periods of fermentation is presented in table 13. The mean temperature of the fermented mass increase progressively as the fermentation advanced and reached to higher level during fourth day of fermentation. Thereafter the temperature decreased slightly.

Initially the temperature was 27^0C in basket method and 28^0C in heap and sack method. However with regards to periods of fermentation the temperature was increased during fermentation in all methods of fermentation. Where the maximum temperature reached was between 45^oC to 49^oC at fourth day of fermentation. From

fifth day on wards the temperature was slightly decreased up to seventh day of fermentation and it was 41^0C, 43^0C and 42^0C in basket, heap and sack method respectively at seventh day of fermentation. However the temperature at seventh day was more than that of the temperature at second and third day of fermentation.

An increase in temperature with advancement of fermentation periods was observed in all three fermentation methods. It is evident from the line graph (Fig. 10) that, the highest temperature of 49^0C was recorded for the heap method and 49^0C in basket and sack method at fourth day of fermentation.

Table 12. Percent free fatty acid of cocoa beans during fermentation with different fermentation methods

Fermentation methods	Per cent free fatty acid content for different fermentation periods			
	Initial	**5th day**	**6th day**	**7th day**
Basket	2.51	1.49 (40.07)	1.30 (12.27)	1.05 (18.35)
Heap	2.39	1.36 (42.96)	1.17 (13.48)	0.80 (32.10)
Sack	2.52	1.61 (35.93)	1.42 (11.78)	1.18 (19.91)

Figures in parenthesis indicates the percentage deviation
LSD (5% level): Fermentation methods (4.902); Fermentation period (4.902); Fermentation methods × Fermentation period (NS).

Table 13. Mean temperature of cocoa beans during fermentation with different fermentation methods

Fermentation methods	Temperature (^{0}C)						
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Basket	27^{0}C	32^{0}C	35^{0}C	45^{0}C	43^{0}C	42^{0}C	41^{0}C
Heap	28^{0}C	34^{0}C	40^{0}C	49^{0}C	45^{0}C	43^{0}C	43^{0}C
Sack	28^{0}C	33^{0}C	38^{0}C	45^{0}C	44^{0}C	42^{0}C	42^{0}C

Based on physico-chemical analysis the treatment T_6 (heap method at seventh day of fermentation) was selected for drying (Table.14). The free fatty acid content was much below the cut off value of 1.75per cent in heap method at seventh day of fermentation.

Table 14. Physico chemical parameters for selection of best method of fermentation

Fermentation methods	Days of fermentation	Cut test (%)	*Lipase* activity (µ eq)	Free fatty acid (%)
Basket	5- (T1)	28.99	0.0018	1.49
	6- (T_2)	59.77	0.0014	1.30
	7- (T_3)	72.22	0.0011	1.05
Heap	5- (T_4)	43.55	0.0014	1.36
	6- (T_5)	72.22	0.0012	1.17
	7- (T_6)	84.99	0.0005	**0.80**
Sack	5- (T_7)	29.88	0.0019	1.61
	6- (T_8)	54.66	0.0015	1.42
	7- (T_9)	69.10	0.0012	1.18

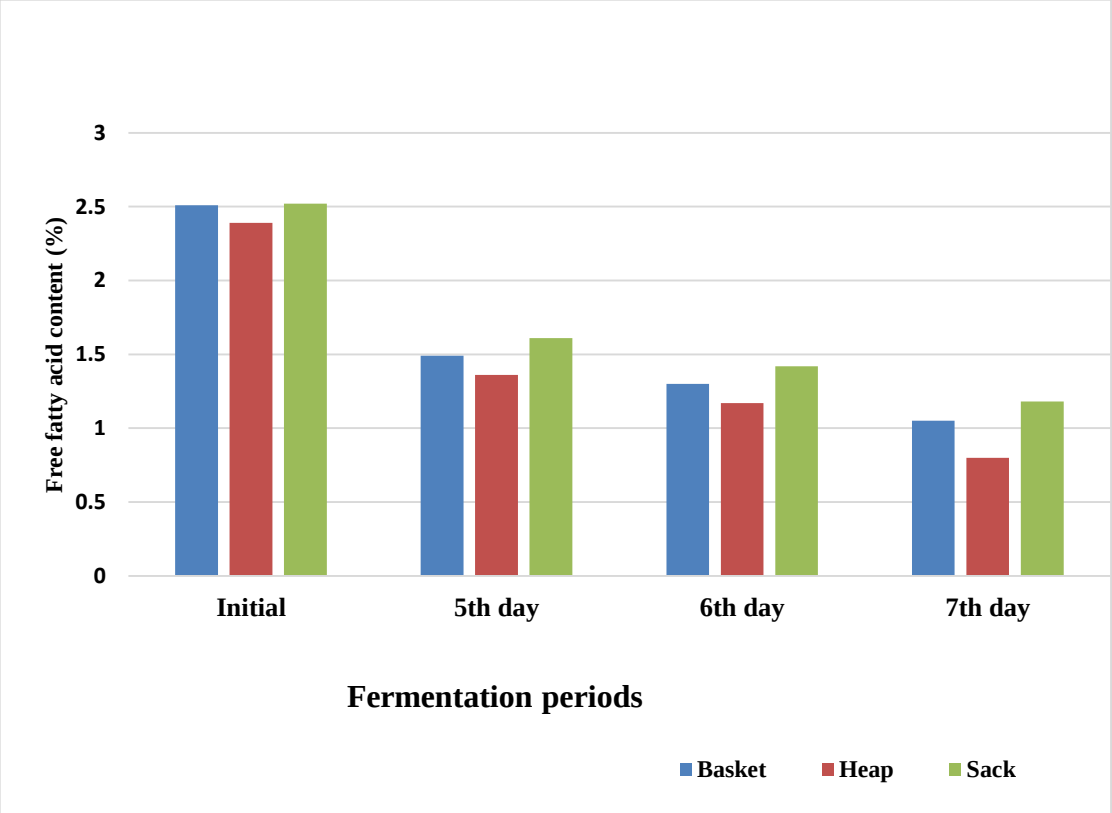


Fig.8. Free fatty acid content of fermented cocoa beans

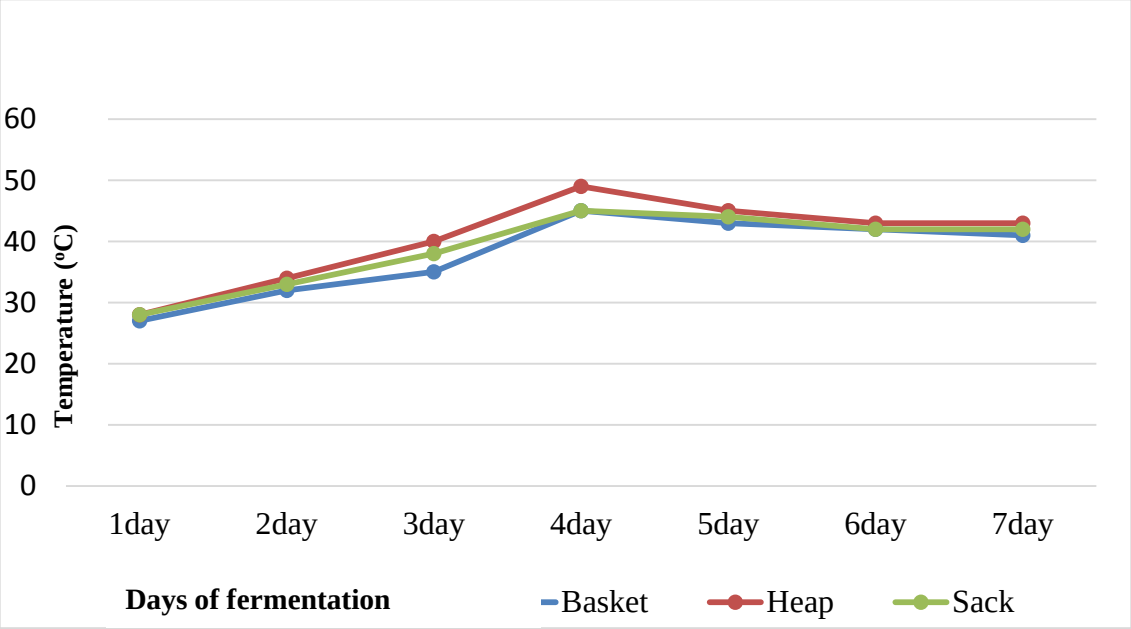


Fig.9. Temperature during fermentation

4.1.3. Standardisation of drying methods based on free fatty acid

The selected fermented beans (Heap method at seventh day of fermentation) was divided into two lots. One lot was subjected to sun drying and other for oven drying (65^0C). Drying methods compared of two treatments with twenty replications (Plates 7 and 8).

4.1.4. Physico-chemical analysis of dried beans

The physico-chemical qualities of the sun dried and oven dried cocoa beans were carried out using standard procedures. The analysis was carried out with three replications. The physico-chemical qualities of fermented dried beans such as bean recovery, moisture, pH, peroxide value, lipase activity, total fat, free fatty acid content and peroxide value were evaluated and are expressed in table 15.

4.1.4.1. Bean recovery

The bean recovery of dried beans was significantly high (41.00 %) in treatment T_1 (sun dried cocoa beans) as compared to treatment T_2 (oven dried beans) (40.12 %).

4.1.4.2. Moisture content

In the present study the moisture content was below 6 per cent in both sun dried and oven dried beans. However, the moisture content of 3.76 per cent was less in treatment T_2 (oven dried beans) than the moisture content of 4.22 per cent in treatment T_1 (sun dried cocoa beans). On statistical interpretation a significant difference was observed in the moisture content of sun dried and oven dried beans.

4.1.4.. pH value

A significant difference was observed in the pH value of sun dried and oven dried beans. The pH value of treatment T_1 (sun dried cocoa beans) was significantly higher (5.29) than the treatment T_2 (oven dried beans) of 4.89.

4.1.4.4. Fat content

The fat content of treatment T_1 (sun dried cocoa beans) was 48.33per cent and 47.93per cent in T_2 (oven dried beans).However, the treatments did not differ significantly.

4.1.4.5. Lipase activity

There was no significant difference in the lipase activity under the types of drying. However, a lesser lipase activity of 0.0018(µ eq) was observed in T_1 (sun dried cocoa beans) compared to lipase activity (0.0025µ eq) of oven dried beans (T_2).

4.1.4.6. Free fatty acid content

A significantly lesser (1.26 %) free fatty acid content was recorded in T1 (sun dried cocoa beans) compared to 1.47per cent in T_2 (oven dried beans). The sun drying proved to be adaptive as free fatty acid content was lower than oven dried beans and were below the cut off value of 1.75%.

4.1.4.7. Peroxide value

The peroxide value of fermented dried cocoa beans were not detected in sun dried and oven dried beans.

Plate 7. Sun drying method

Plate 8. Artificial drying method

Table.15. Physico-chemical analysis of sun dried and oven dried beans

Drying methods	Physico-chemical properties of dried beans					
	Bean recovery (%)	Moisture (%)	pH	Total fat (%)	Lipase activity (µ eq)	Free fatty acid (%)
Sun drying(T_1)	41.00	4.22	5.29	48.33	0.0018	1.26
Oven drying (T_2)	40.10	3.76	4.89	47.93	0.0025	1.47
T value	2.461*	3.701*	2.692*	0.321NS	3.487 NS	5.552 *
CD (0.05)	0.781	0.175	0.250	0.455	0.440	0.172

* Significant at 0.05 level

The free fatty acid content was below the cut off value of 1.75% in both sun dried and oven dried cocoa beans, but statistically the free fatty acid content was lower in sun dried beans compared to oven dried beans and had significant difference. So based on physico-chemical analysis the treatment T_1 (Sun dried beans) was selected for storage study.

4.1.5. Standardisation of storage methods of cocoa beans based on free fatty acid content

The selected sun dried cocoa beans was divided in to three parts and subjected to storage for a periods of six months. One part were stored in gunny bags (control), second part were stored in polythene covers and third part were stored in plastic containers under ambient condition for a period of six months with five replications (Plate 9,10 and 11).

4.1.6. Physico-chemical analysis of stored cocoa beans

The following physico-chemical qualities of cocoa beans stored in gunny bags, polythene covers and plastic containers were done initially and at an interval

Plate 9. Storage in gunny bag

Plate 10. Storage in polythene covers

Plate 11. Storage in food grade plastic containers

of one month for a duration of six months and the findings are given in tables 16, 17, 18, 19, 20 and 21 and interpreted using DMRT.

4.1.6.1. Bean recovery of stored cocoa beans

The bean recovery per cent of cocoa beans varied significantly between storage methods after first month of storage. The per cent bean recovery generally decreased from the first month to six month after storage (Table 17). From initial to first month of storage the bean recovery was hundred per cent in all three treatments. Based on one way ANOVA, significant variation in the bean recovery was observed between the treatments T_1, T_2 and T_3 from second month of storage to sixth month after storage.

The bean recovery was significantly superior in treatment T_3 (cocoa beans stored in plastic container) and was recorded to be the highest throughout the storage periods from initial to six month after storage. It was hundred percent bean recovery up to second month after storage in treatment T_3 and after that there was a slight decrease in bean recovery up to sixth month after storage (98%).

There was a significant difference observed in bean recovery from second month to sixth month after storage in treatment T_2 and it was 96.36 per cent bean recovery at sixth month after storage. However the treatment T_1 (gunny bag) recorded statistically significant low bean recovery percentage from second month of storage itself. The lowest bean recovery of 89.54 per cent was recorded in the treatment T_1 at sixth month after storage.

The change in bean recovery during storage period of six months was assessed using DMRT and line graph. Figure 10 shows that the bean recovery of cocoa beans during storage and a steady decrease in bean recovery was observed among three treatments. It is evident from the figure that a drastic decrease in bean recovery from fifth month after storage was noticed only in T_1 up to sixth month after storage.

Table.16. Per cent of bean recovery of stored cocoa beans

Treatments	Initial	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS
T_1(GB)	100[a] (89.595)	100[a] (89.595)	98.22[c] (82.335)	96.62[c] (79.407)	95.60[c] (77.920)	90.66[c] (72.205)	89.54[c()] (71.130)
T_2 (POC)	100[a] (89.595)	100[a] (89.595)	98.82[b] (83.767)	98.22[b] (82.335)	97.42[b] (80.758)	97.0[b] (80.026)	96.36[b] (79.003)
T_3 (PC)	100[a] (89.595)	100[a] (89.595)	100[a] (89.714)	99.4[a] (85.575)	98.70[a] (83.486)	98.50[a] (82.967)	98.02[a] (81.912)
CV (%)		-	0.193	0.346	0.548	40.172	0.203
CD (0.05)	NS	NS	0.227	0.393	0.610	0. 186	0.217

Superscripts indicates homogenous group between treatments
Values in parenthesis shows arc transformed value, MAS- Month after storage
GB – Gunny bags, POC - Polythene cover, PC- Plastic container

4.1.6.2. Moisture content of stored cocoa beans

The moisture content of the cocoa beans stored in different storage methods during the periods of six months was analysed and compared using one way ANOVA and are presented in table 17. With regard to the different storage methods, initially no significant difference in moisture content was observed between the treatments T_1, T_2 and T_3 and the moisture content of 4.22 per cent was recorded in all three treatments. During first month of storage the treatments T_1 and T_2 exhibited significant difference, but the treatments T_2 and T_3 were on par with each other when analysed statistically. After second month of storage up to sixth month after storage a statistically significant difference was observed in moisture content between the treatments T_1 (Gunny bags), T_2 (Polythene cover) and T_3 (Plastic container). The highest moisture content among all treatments from first month after storage to the end of six month of storage was recorded in cocoa beans stored in gunny bags (T_1) ranging from 4.23 to 5.21 percent. The least moisture content was recorded in treatment T_3 (Plastic container) as from 4.22 to 4.52 per cent from first month after storage to sixth month after storage. The cocoa beans stored in polythene cover (T_3) had the moisture content of 4.79 per cent at the end of sixth month of storage.

The change in moisture content of cocoa beans during storage period of six months is also expressed using line graph. Figure 11 shows that moisture content of cocoa beans stored in gunny bags, polythene covers and plastic containers gradually increased during storage. A steady increase in moisture content was observed in all three treatments. The maximum increase in moisture content was 5.12 per cent in T_1 which was recorded at the end of six month after storage.

4.1.6.3. pH of stored cocoa beans

The pH of stored cocoa beans are presented in Table 18 and Figure 12. Initially the pH of cocoa beans stored in gunny bags (T_1), polythene covers (T_2) and plastic containers (T_3) was 5.22, 5.23 and 5.22 respectively.

Table.17. Moisture content of stored cocoa beans

Treatments	Initial	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS
T_1(GB)	4.22	4.23^a	4.54^a	4.93^a	4.98^a	5.05^a	5.12^a
T_2 (POC)	4.22	4.22^b	4.24^b	4.34^b	4.40^b	4.54^b	4.79^b
T_3 (PC)	4.22	4.22^b	4.23^c	4.32^c	4.38^c	4.46^c	4.52^c
CV (%)	0.198	0.178	0.210	0.185	0.703	0.220	0.131
CD (0.05)	NS	0.010	0.013	0.012	0.045	0.014	0.009

Superscripts indicates homogenous group between treatments, MAS- Month after storage, GB – Gunny bags, POC - Polythene cover, PC- Plastic container

NS-Non significant

Table.18. pH of stored cocoa beans

Treatments	Initial	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS
T_1(GB)	5.22	5.22	5.25^{a}	5.26^{a}	5.28^{a}	5.30^{a}	5.31^{a}
T_2 (POC)	5.23	5.23	5.24^{ab}	5.25^{ab}	5.26^{b}	5.27^{b}	5.28^{b}
T_3 (PC)	5.22	5.22	5.23^{b}	5.24^{b}	5.25^{b}	5.26^{b}	5.27^{b}
CV (%)	0.188	0.188	0.160	0.159	0.159	0.158	0.158
CD (0.05)	NS	NS	0.012	0.012	0.012	0.012	0.012

Superscripts indicates homogenous group between treatments, MAS- Month after storage, GB – Gunny bags, POC - Polythene cover, PC- Plastic container
NS-Non significant

Based on one way ANOVA no significant difference in pH was noticed between the treatments T_1 (Gunny bags), T_2 (Polythene cover) and T_3 (Plastic container) during initially and first month after storage. During second month and third month of storage the treatment T_1 and T_2 and treatment T_2 and T_3 are statistically on par but significant changes were noted between the treatment T_1 and T_3.

From third month after storage to sixth month after storage, a significant changes were noticed in treatment T_1 with the treatments T_2, but no significant variation was noticed in the treatments T_2 and T_3 and they are on par with each other during storage periods. The highest pH of 5.31was observed in treatment T_1 (Gunny bags), lowest (5.27) in treatment T_3 and 5.28 was in treatment T_2 at sixth month after storage.

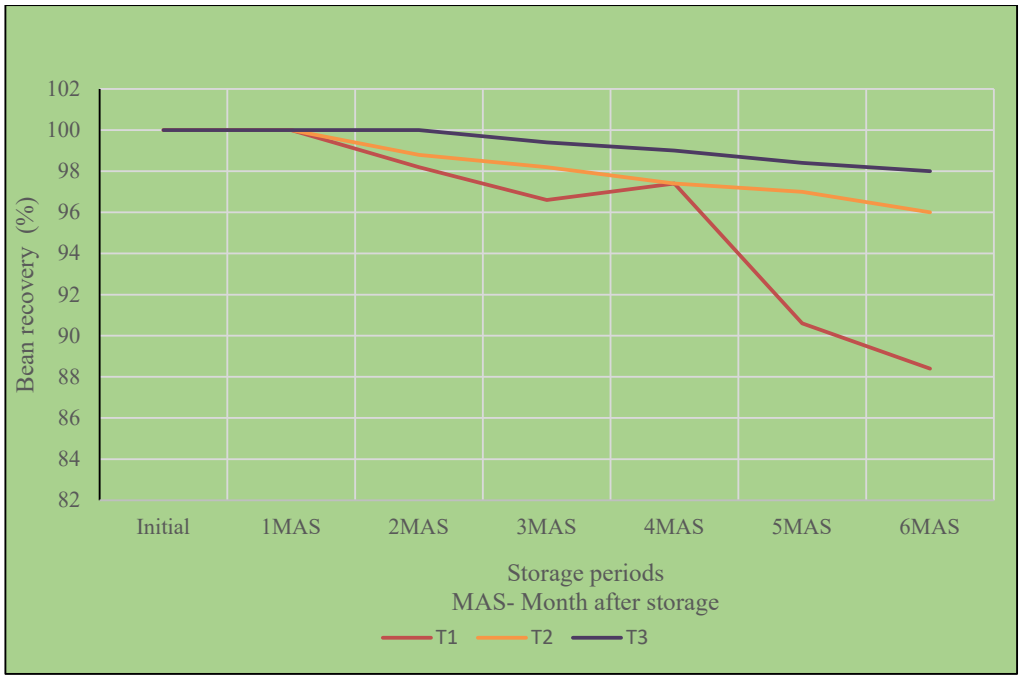


Fig.10. Bean recovery of stored cocoa beans

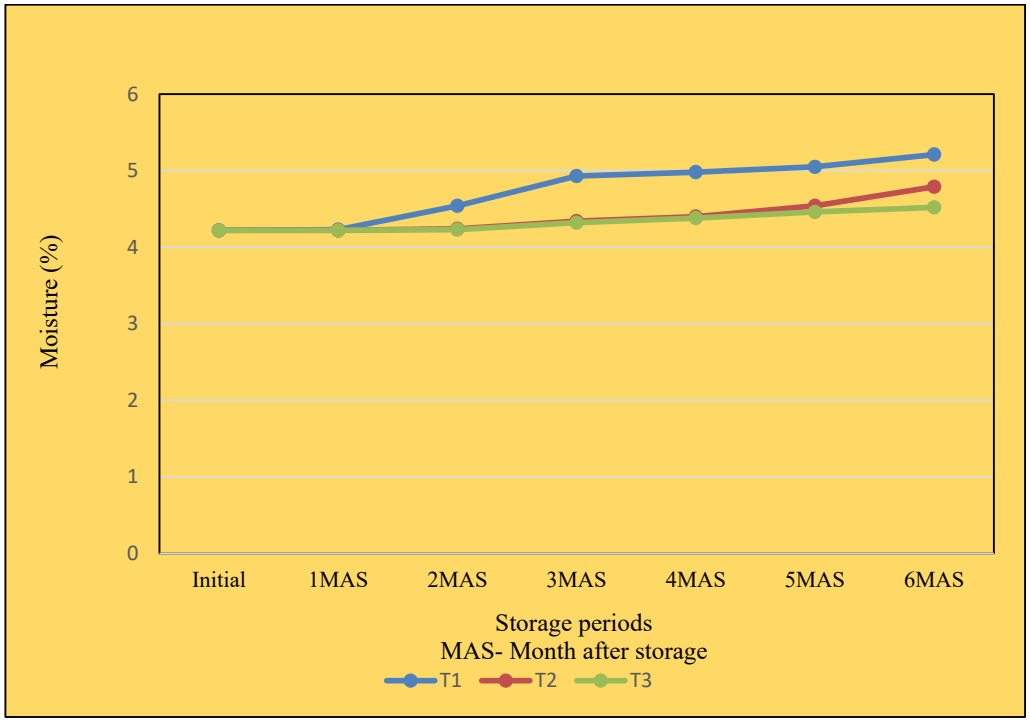


Fig. 11. Moisture content of stored cocoa beans

4.1.6.4. Total fat content of stored cocoa beans

The fat content of various treatmentsT_1, T_2 and T_3 during each month of storage was interpreted using DMRT (Table 19). Initially the fat content was in the range of 50.31 in treatment T_1 and 50.34 per cent in treatment T_2 and T_3 respectively. Generally a decreasing trend was recorded in all the three treatments from initial to six month after storage. Based on DMRT, initially no significant difference was observed in fat content in all three treatments and forms a homogenous group in all three treatments. On the basis of DMRT, the treatment T_1 and T_2 was on par during first month after storage, but significantly different with treatment T_3. Statistically significant difference was observed in all three treatments T_1, T_2 and T_3 from the second month to forth month after storage. But at fifth month after storage treatment T_2 and T_3 are statistically on par with each other and significantly different with treatment T_1. Significant difference was observed between all three treatments at sixth month after storage. The lowest fat content of 47.20 per cent was recorded in treatment T_1 (cocoa beans stored in gunny bags) and the highest fat content of 48.07 per cent was recorded in treatment T_3 (cocoa beans stored plastic container) at the end of six month after storage. From the figure 13, it is clear that there was a consistent reduction in fat content in all three treatments.

Table.19. Total fat content of stored cocoa beans

Treatments	Total fat (%)						
	Initial	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS
T_1(GB)	50.31^a	50.00^b	49.0^c	48.7^c	48.2^c	47.6^b	47.20^c
T_2 (POC)	50.34^a	50.00^b	49.30^b	48.85^b	48.54^b	48.05^a	47.68^b
T_3 (PC)	50.34^a	50.19^a	49.63^a	49.0^a	48.66^a	48.24^a	48.07^a
CV (%)	0.162	0.068	0.164	0.092	0.097	0.388	0.146
CD (0.05%)	NS	0.047	0.112	0.062	0.065	0.257	0.096

Superscripts indicates homogenous group between treatments GB – Gunny bags, POC - Polythene cover, PC- Plastic container, MAS- Month after storage, NS-Non significant

4.1.6.5. Lipase activity of stored cocoa beans

Lipase activity of stored cocoa beans is presented in Table 20. Initially the lipase activity in stored cocoa beans was 0.0017 µ eq in all three treatments like cocoa beans stored in gunny bags, polythene cover and plastic container. In cocoa beans stored in gunny bags (T_1) the lipase activity varied from 0.0017 to 0.0026 µ eq from initial to six month after storage. In cocoa beans stored in polythene cover (T_2) it varied from 0.0017 to 0.0020 µ eq and in cocoa beans stored in plastic container (T_3) it varied from 0.0017 to 0.0019 µ eq. Statistically no significant changes were noted from initial to second month after storage in all three treatments. Based on one way ANOVA, no significant difference in lipase activity was observed between the treatments T_2 and T_3, but significantly different with treatment T1 at third month of storage. From forth to sixth month after storage statistically significant difference was observed between the treatments T_1, T_2 and T_3.

As revealed in Table 20, generally the lipase activity of cocoa beans was increased during storage. The lipase activity was found to be highest (0.0026 µ eq) in treatment T_1 (Gunny bag), and the lowest lipase activity of 0.0019 µ eq was in treatment T_3 (Plastic container) at the end of sixth month of storage.

4.1.6.6. Free fatty acid content of stored cocoa beans

The free fatty acid content of stored cocoa beans in different treatments are presented in Table 21 and illustrated in Figure 15. Initially the free fatty acid content was 1.12 per cent in all three treatments T_1, T_2 and T_3. Generally an increasing trend in free fatty acid content was recorded during storage in all three treatments. Based on DMRT, statistically no significant difference was observed between the treatment T_2 and T_3 ie.,treatment T_2 was on par with treatment T_3 in first and second month after storage, but significantly different with treatment T_1.

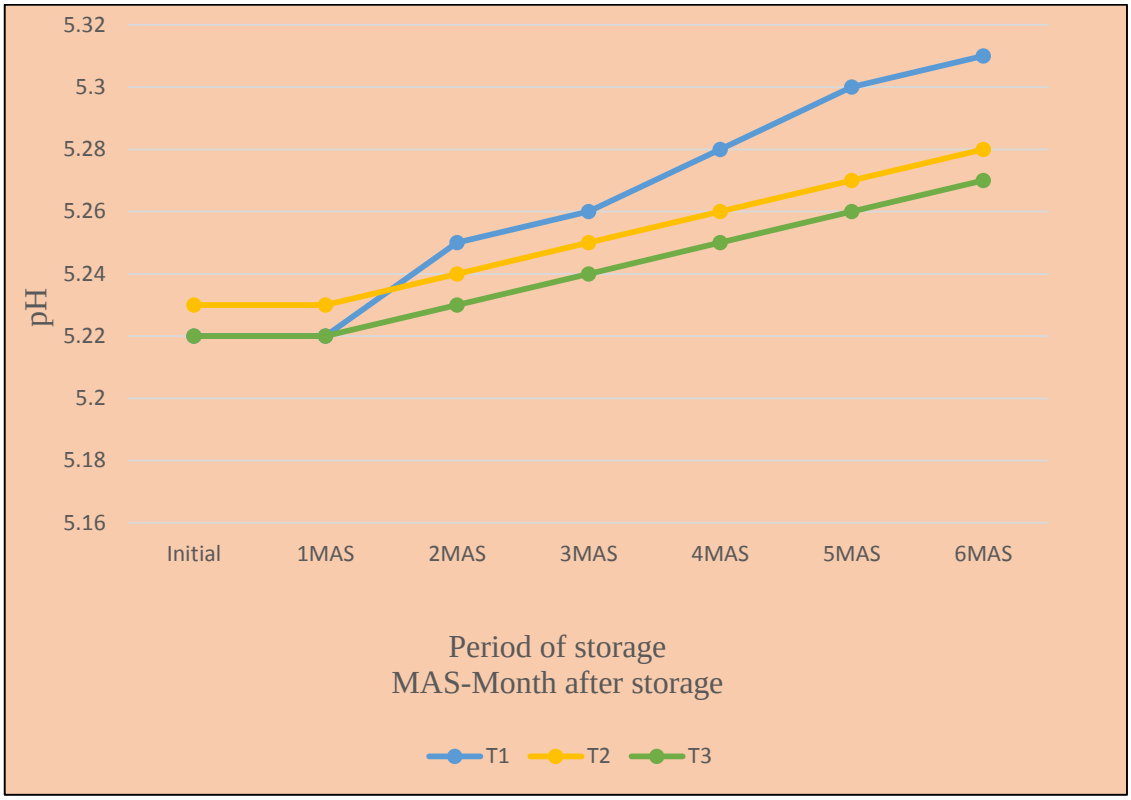


Fig.12.pH of stored cocoa bean

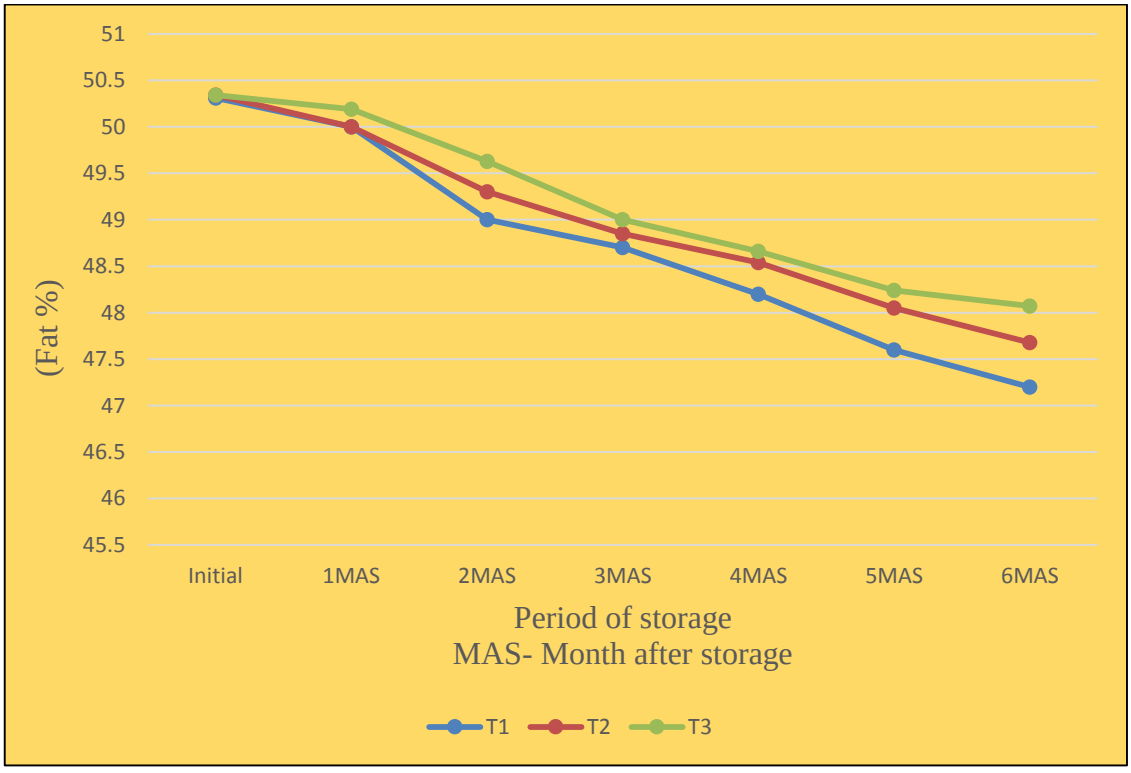


Fig.13. Fat content of stored cocoa beans

Statistically significant difference was observed between all the three treatments T_1 (Cocoa beans stored in gunny bags), T_2 (Cocoa beans stored in poythene cover) and T_3 (cocoa beans stored in plastic container) from third to sixth month after storage.

The highest free fatty acid content 2.80 per cent was recorded in treatment T_1 (Gunny bags) and the lowest free fatty acid content of 1.68per cent was in treatment T_3 (Plastic container) at sixth month after storage. The treatment T_3 was within the cut off value of free fatty acid content 1.75% at the end of six month of storage, but the treatment T_2 was within the cut off value at the fifth month of storage and treatment T_1 was within the cut off value of 1.75% at the end of third month of storage.

Table.20. Lipase activity of stored cocoa beans

Treatments	**Lipase activity (µ eq)**						
	Initial	**1 MAS**	**2 MAS**	**3 MAS**	**4 MAS**	**5 MAS**	**6 MAS**
T_1(GB)	0.0017	0.0017	0.0018	0.0019[a]	0.0020[a]	0.0022[a]	0.0026[a]
T_2 (POC)	0.0017	0.0017	0.0018	0.0018[b]	0.0019[b]	0.0020[b]	0.0020[b]
T_3 (PC)	0.0017	0.0017	0.0018	0.0018[b]	0.0018[c]	0.0019[c]	0.0019[c]
CV (%)	3.148	3.148	3.928	4.091	3.683	3.654	3.432
CD (0.05%)	NS	NS	NS	0.000	0.000	0.000	0.000

Superscripts indicates homogenous group between treatments,MAS-Month after storage,GB – Gunny bags, POC - Polythene cover, PC- Plastic container,

Table.21. Free fatty acid content of stored cocoa beans

Treatments	**Free fatty acid (%)**						
	Initial	**1 MAS**	**2 MAS**	**3 MAS**	**4 MAS**	**5 MAS**	**6 MAS**
T_1(GB)	1.12	1.28[a]	1.51[a]	1.55[a]	2.12[a]	2.46[a]	2.8[a]
T_2 (POC)	1.12	1.12[b]	1.40[b]	1.51[b]	1.60[b]	1.79[b]	2.01[b]
T_3 (PC)	1.12	1.12[b]	1.40[b]	1.40[c]	1.51[c]	1.68[c]	1.68[c]
CV (%)	0.486	0.464	0.389	0.364	0.308	0.284	0.252
CD (0.05)	NS	0.008	0.008	0.008	0.008	0.008	0.008

Superscripts indicates homogenous group between treatments, MAS-Month after storage NS- Non significant GB – Gunny bags, POC - Polythene cover, PC- Plastic container

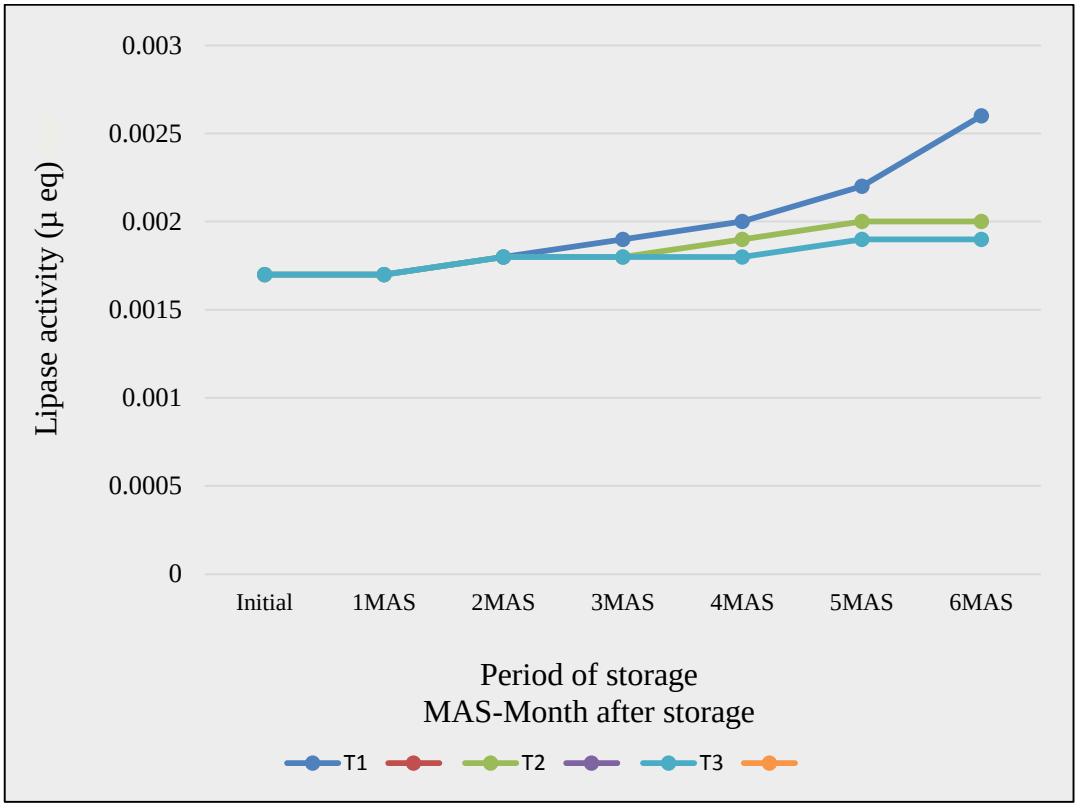


Fig.14. Lipase activity stored cocoa beans

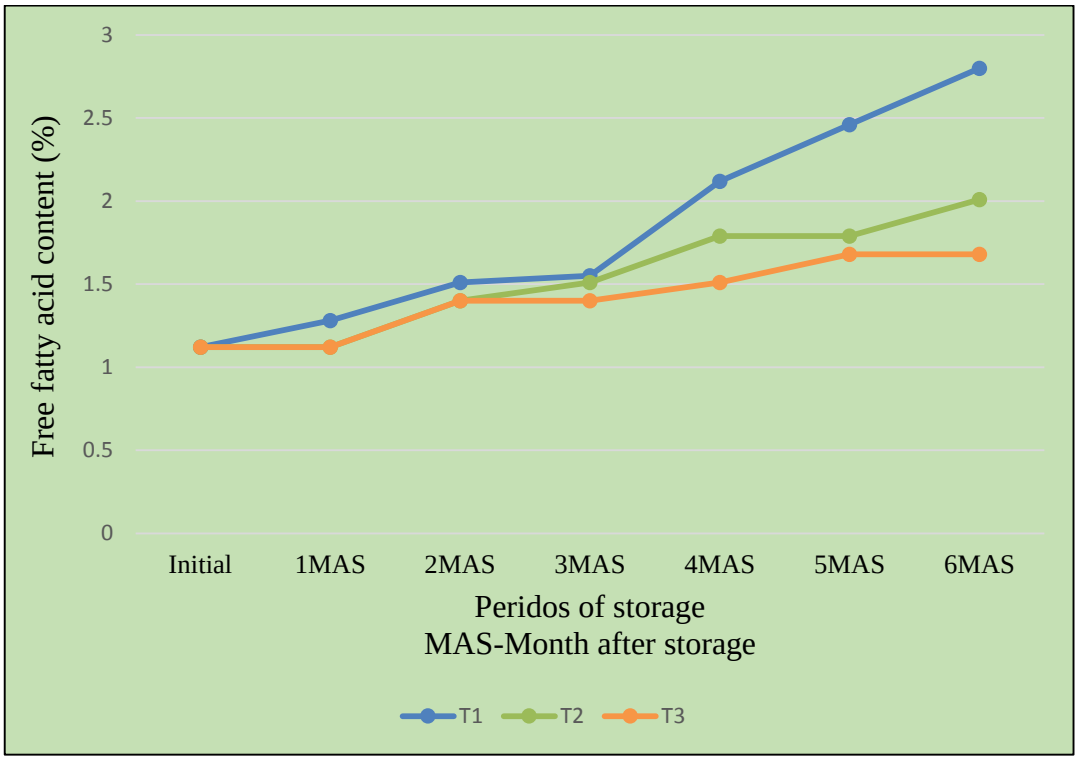


Fig.15. Free fatty acid content of stored cocoa beans

4.1.7. Microbial load on cocoa beans during storage

The microbial population (bacterial and fungal count) was recorded initially and at the end of sixth month of storage of cocoa beans subjected to different storage treatments. The results are given in Tables 22.

Initially, the lowest bacterial population of l.2 x 10^4 cfu g-1 was recorded in beans stored in plastic container (T_3) followed by beans stored polythene cover i.e. T_2 (1.4 x 10^4cfu g-1). The maximum bacterial count of 1.6 x 10^4 cfu g-1was recorded with control (T_1) i.e. the cocoa beans stored in gunny bags. After six month storage the bacterial count was increased in all three treatments. After six month after storage, the treatment T_3 (6 .0 x 10^4 cfu g-1) and T_2 (7.2 x 10^4 cfu g-1) recorded the lower bacterial count. The maximum bacterial count of (10.4 x 10^{-4}cfu g-1) was observed in control (T_1) at the end of storage.

At the initial stage of observation the fungal population was lowest in treatment T_2 (1 x 10^{-3} cfu g-1) and T_3 (1 x 10^{-3} cfu g-1) and followed in treatment T1 (1.2 x 10^{-3} cfu g-1). The fungal population was found to increase in general at the end of storage. The highest fungal count of (13.6 x 10^{-3} cfu g-1) was recorded in cocoa beans stored in gunny bags (T_1), and the lowest fungal count of 2.2 x 10^{-3} cfu g-1and 2.0 x 10^{-3} cfu g-1in T_2 and T_3 respectively.

Table. 22. Effect of storage on microbial population

Treatments	**Storage periods**			
	Bacteria (x 10^4 cfu g-1)		Fungus x 10^{-3} cfu g-1)	
	Initial	6 MAS	Initial	6 MAS
T_1-Gunny bag	1.6 x 10^{-4}	10.4 x 10^{-4}	1.2 x 10^{-3}	13.6x 10^{-3}
T_2-Polythene cover	1.4 x 10^{-4}	7.2 x 10^{-4}	1 x 10^{-3}	2.2 x 10^{-3}
T_3-Plastic container	1.2 x 10^{-4}	6.0 x 10^{-4}	1 x 10^{-3}	2.0 x 10^{-3}

MAS-Month after storage

4.2. Alkalisation of stored cocoa beans

The cocoa beans stored in plastic container was selected for secondary processing of cocoa. Secondary processing of cocoa include alkalisation, roasting and chocolate making of cocoa. Based on free fatty acid content, the lowest free fatty acid content (1.68%) was observed in cocoa beans stored in plastic container. The stored cocoa beans in plastic container were divided into two lots. One lot was used as such and the second lot was subjected to alkalisation. The following steps were done in alkalisation,

Soaking 1kg cocoa beans in 1litre water for three hours

⇩

Add one per cent sodium bicarbonate

⇩

Drain the water

⇩

Dried the drained cocoa beans

4.3. Standardisation of time and temperature for chocolate making using machine

The chocolates were prepared with alkalised and non alkalised beans at different time and temperature by the procedure standardised by Cocoa Research Centre, Kerala Agricultural University, Vellanikkara and is detailed below (Plate 12, 13 and 14). All the ingredients (Table 1) needed for chocolate making was fed in to tempering and conching machine (Plate 15). Twelve sets of chocolates were prepared at different time periods of 7, 9 and 11 hours at a temperature of 60^0C and 70^0C (detailed earlier in Table 2). The twelve sets of chocolates prepared using machine is depicted in plate 16,17,18,19,20,21,22,23,24,,25,26, and 27.

Plate 12. Roasting of cocoa beans

Plate 13. Dehusking (Knibbling) of roasted cocoa beans

Plate 14. Grinding of cocoa nibs

Plate 15. Tempering and conching machine

Alkalised chocolates

Plate 16. T_1 (60^0C-7hrs)

Plate 17. T_2 (60^0C-9hrs)

Plate 18. T_3 (60^0C-11hrs)

Plate 19. T_4 (70^0C-7hrs)

Plate 20. T_5 (70^0C-9hrs)

Plate 21. T_6 (70^0C-11hrs)

Non alkalised chocolates

Plate 22. T_7 (60^0C-7hrs)

Plate 23. T_8 (60^0C-9hrs)

Plate 24. T_9 (60^0C-11hrs)

Plate 25. T_{10} (70^0C-7hrs)

Plate 26. T_{11} (70^0C-9hrs)

Plate 27. T_{12} (70^0C-11hrs)

Roasting (120^{0}C for 30 minutes)
and dehusking of cocoa beans

Grading

Grinding the cocoa nibs

Ingredients fed in to the tempering and conching machine

After the preparation of different types of alkalised and non alkalised chocolates in tempering and conching machine, they were subjected to analysis of physico-chemical qualities, organoleptic evaluation and for enumeration of microflora.

4.3.1. Physico-chemical analysis of chocolates

The physico-chemical qualities of alkalised and non alkalised chocolates prepared in tempering and conching machine are given in table 23 and 24 and the findings are interpreted using DMRT.

4.3.1.1. Textural properties

The textural properties of the alkalised and non alkalised chocolates were evaluated in freshly prepared chocolates. The results of the textural quality parameters such as hardness, cohesiveness, adhesiveness and gumminess of the chocolates were observed and the results are detailed in Table 23.

The hardness of the alkalised chocolates varied between 72.87N and 97.70N. Based on one way ANOVA, statistically significant difference was found in hardness between the treatments. The maximum hardness of 97.07N was observed in treatment T_4 (chocolate prepared at 70^{0}C for 7 hours) and minimum (72.87N) in treatment T_3 (chocolate prepared at 60^{0}C for 11 hours).

In non alkalised chocolates the hardness varied from 72.51 N to 99.11N. The highest value for hardness was observed in treatment T_{10} (chocolate prepared at 70^0C for 7 hours) and lowest in T_{12} (chocolate prepared at 70^0C for 11 hours). In both alkalised and non alkalised chocolates, hardness of chocolates reduced with increasing tempering and conching time.

Cohesiveness of chocolates varied in alkalised chocolates from 0.008N to 0.021N. Statistically the highest cohesiveness of 0.021N was observed in chocolates prepared at 70^0C for 11 hours (T_6) and lowest (0.008N) in treatment T_1.Statistically the cohesiveness of chocolates significantly varied between the treatments. But the treatments T_2 and T_4 was on par with each other.

The maximum cohesiveness of 0.022N was found in non alkalised chocolates in treatment T_{12} (chocolate prepared at 70^0C for 11 hours) and the minimum of 0.009N in treatment T_7 (chocolate prepared at 60^0C for 7 hours). There was a sharp decline in cohesiveness in all chocolates during tempering and conching.

The adhesiveness in alkalised chocolates was significantly different between the treatments. The adhesiveness of 0.0027N was found to be highest in treatment T_6 (chocolate prepared at 60^0C for 11 hours) and lowest in treatment T_1 (chocolate prepared at 60^0C for 7 hours).

In non alkalised chocolates, the adhesiveness varied from 0.0007N to 0.0021N. The maximum adhesiveness of 0.0021N was found to be in treatment T_{12} and lowest value of 0.0007N in treatment T_7 and $T_{10.}$ There was a slight increase in adhesiveness of chocolates with the duration of tempering and conching.

Table.23. Textural attributes of chocolates

Treatments	Textural attributes			
	Hardness (N)	**Cohesiveness (N)**	**Adhesiveness (N)**	**Gumminess (N)**
T_1 (AC)	93.93^{b}	0.008^{e}	0.0006^{f}	0.9482^{e}
T_2 (AC)	80.38^{d}	0.010^{d}	0.0014^{d}	1.2605^{c}
T_3 (AC)	72.87^{f}	0.016^{b}	0.0021^{b}	1.3782^{a}
T_4 (AC)	97.70^{a}	0.010^{d}	0.0007^{e}	0.8976^{f}
T_5(AC)	84.34^{c}	0.015^{c}	0.0015^{c}	1.2145^{d}
T_6 (AC)	77.55^{e}	0.021^{a}	0.0027^{a}	1.3079^{b}
CV (%)	**0.023**	**4.225**	**0.000**	**0.005**
CD (0.05)	**0.035**	**0.001**	**2.839**	**0.000**
T_7 (NAC)	95.12^{b}	0.009^{d}	0.0007^{e}	1.0620^{f}
T_8 (NAC)	86.49^{c}	0.011^{c}	0.0014^{d}	1.1617^{d}
T_9 (NAC)	79.74^{e}	0.016^{bc}	0.0020^{b}	1.3201^{a}
T_{10} (NAC)	99.11^{a}	0.010^{cd}	0.0007^{e}	1.0929^{e}
T_{11} (NAC)	81.46^{d}	0.017^{b}	0.0015^{c}	1.1755^{c}
T_{12} (NAC)	72.51^{f}	0.022^{a}	0.0021^{a}	1.2471^{b}
CV (%)	**0.007**	**5.584**	**16.282**	**0.021**
CD (0.05)	**0.010**	**0.001**	**0.000**	**0.000**

Superscripts indicates homogenous group between treatments

T_1-60^0 C for 7hours T_2- 60^0 C for 9 hours T_3- 60^0C for 11hours AC-Alkalised chocolates T_4 - 70^0 Cfor 7hours T_5- 70^{0C} for 9hours T_6- 70^0 C for 11hours NAC-Non alkalised chocolates T_7-60^0C for 7hours T_8- 60^0C for 9hours T_9- 60^0C for 11hours T_{10} - 70^0C for 7hours T_{11}- 70^0C for 9hours T_{12}- 70^0C for 11hours

The gumminess of chocolates was varied with different treatments in alkalised and non alkalised chocolates. The maximum gumminess of 1.3782N was recorded in alkalised chocolates prepared at 60^0C for 11 hours (T_3) and minimum (0.9482N) in treatment T_4 (chocolate prepared at 70^0C for 7 hours).

In non alkalised chocolates, statistically the highest gumminess of 1.3201 N was recorded in treatment T_9 (chocolate prepared at 60^0C for 11 hours) and lowest (1.0620N) in treatment T_7 (chocolate prepared at 60^0C for 7 hours). Gumminess

was slightly decreased in alkalized and non alkalised chocolates with the duration of tempering and conching.

4.3.1.2. Moisture

The moisture content in the alkalised chocolates differed significantly between the treatments. The treatment T_1 (chocolate prepared at 60^0C for 7 hours) had the maximum moisture content (1.33%) and T_6 (chocolate prepared at 70^0C for 11 hours) had recorded the lowest moisture content of 1.18% followed by the treatment T_3 (chocolate prepared at 60^0C for 11 hours) having the moisture content of 1.20%. The moisture content generally decreased with increasing duration of tempering and conching with in a temperature range. Again the moisture content also decreased with increasing temperature (T_4, T_5 and T_6 as compared to T_1, T_2 and T_3).

In non alkalised chocolates the highest moisture content of 1.34per cent was observed in treatment T_7 (chocolate prepared at 60^0C for 7 hours) followed by treatment T_8 (chocolate prepared at 60^0C for 9 hours) having the moisture content of 1.30per cent. The lowest moisture content in non alkalised chocolates was recorded in chocolates prepared at 70^0C for 11 hours in treatment T_{12} (1.23%) followed by treatment $T_{11.}$ Based on DMRT the moisture content between the treatments differed significantly, but the treatment T_8 (Chocolate prepared at 60^0C for 9 hours) and T_{11} (chocolate prepared at 70^0C for 9 hours) was on par with each other in moisture content. In non alkalised chocolates also the moisture content decreased with increasing the tempering and conching time and temperature.

4.3.1.3. Energy

The calorific value or energy content of the alkalised and non alkalised chocolates are given in Table.24. The lower calorific value of 539.80 K.cal was noticed in treatment T_1 (chocolate prepared at 60^0C for 7 hours) followed by treatment T_2 (543.14 K.cal). The highest calorific value of 579.04 and 579.82 was recorded in chocolates prepared at 60^0c and 70^0C for 11 hours in treatment T_3 and

T_6 respectively, but the treatments T_3 and T_6 are significantly different. Based on DMRT significant changes were observed in calorific value or in energy content between the treatments.

Based on DMRT in non alkalised chocolates the caloric value differed significantly between the treatments. The highest energy content of 579.62 K.cal was noticed in treatment T_{12} (chocolate prepared at 70^0C for 11 hours) followed by treatment T_9 (chocolate prepared at 60^0C for 11 hours) with 575.04 K.cal. The lowest calorific value of 539.00 K.cal was noticed in treatment T_7 followed by T_8 (543.14 K.cal).

There is a general trend in energy value in both alkalised and non alkalised chocolates, the energy content was generally increased with increasing duration of tempering and conching with in a temperature range. Again the energy content also increased with increasing the temperature of conching and tempering of chocolates. The energy content of treatment T_4, T_5 and T_6 (chocolates prepared at 70^0C) was higher than the energy value of T_1, T_2 and T_3 (chocolates prepared at 60^0C) in alkalised chocolates. The same trend was observed in non alkalised chocolates also, the calorific value was more in treatment $T1_0$, $T1_1$ and $T1_2$ (chocolates prepared at 70^0C) compared to the energy value of T_7, T_8 and T_9 (chocolates prepared at 60^0C).

4.3.1.4. TSS

A decrease in TSS content was seen in alkalised and non alkalised chocolates with increasing temperature and also with the duration of tempering and conching. However the difference was statistically non significant. In alkalised chocolates the highest TSS of 68^0 Bx was recorded in treatment T_1 (Chocolate prepared at 60^0C for 7 hours) followed by 67^0Bx in treatment T_2 (Chocolate prepared at 60^0C for 9 hours) and T_3 (chocolate prepared at 60^0C for 11 hours). The same trend was also observed in non alkalised chocolates and the treatments are statistically not significant.

4.3.1.5. Total sugar

In alkalised chocolates the highest total sugar content of 48.02 g/100g was noticed in T_6 (chocolates prepared at 70^0C for 11 hours) which was closely followed by 48.01g/100g in T_3 (chocolates prepared at 60^0C for 11 hours). Based on DMRT, T_6 and T_3 was on par with each other. The lowest total sugar content of 46.7g/100g and 46.8g/100g was observed in T_1 (chocolates prepared at 60^0C for 7hours) and T_4 (chocolates prepared at 70^0C for 7hours) respectively and had a statistically significant difference. The total sugar content increased with increasing the duration of tempering and conching of chocolates.

In non alkalised chocolates the total sugar content varied significantly between the treatments. The highest total sugar content of 48.02g/100g was recorded in chocolates prepared at 60^0C for 11 hours (T_9) and 70^0C for 11 hours (T_{12}). Based on DMRT treatment T_9 and $T1_2$ are statistically on par. The lowest total sugar content of 46.6 g/100g and 46.7 g/100g was recorded in treatment T_7 (Chocolates prepared at 60^0C for 7hours) and T_{10} (Chocolates prepared at 70^0C for 7hours) respectively, But statistically they were on par with each other.

4.3.1.6. Reducing sugar

The reducing sugar content in alkalised chocolates varied significantly. Based on one way ANOVA highest reducing sugar content of 5.1g/100gm was recorded in treatment T_6 (70^0C for 11 hours) followed by treatment T_3(5.0g/100gm), chocolate prepared at 60^0C for 11 hours. The lowest reducing sugar content of 4.91g/100gm was observed in treatment T_4 (70^0C for 7 hours). Statistically the treatments T_1, T_2 and T_5 was on par with each other.

In non alkalised chocolates the highest reducing sugar content of 5.3g/100g was recorded in treatment T_9 (60^0C for 11 hours) followed by 5.0g/100g in treatment T_8 (chocolates prepared at 60^0C for 9 hours). But a statistically significant difference was observed between them. The treatment T_{10}, T_{11} and T_{12} was

statistically on par. The lowest reducing content of 4.8g/ 100g was observed in chocolates prepared at 60^0C for 7 hours (T_7).

Increase in the reducing sugar content with increasing the duration of tempering and conching of chocolates were observed in both alkalised and non alkalised chocolates.

4.3.1.7. Protein

The protein content of alkalised chocolates varied between 7.30 and 10.45g/100g with the lowest protein in T_6 (70^0C for 11 hours) and highest in T_1 (60^0C for 7 hours). Significant difference was observed in protein content of alkalised chocolates in all treatment, with T_1 having significantly high protein content followed by T_2 (9.80g/100g). There was a reduction in protein content in alkalised chocolates with increasing the time of tempering and conching process of chocolates.

In non alkalised chocolates also the protein content significantly varied between the treatments. The highest protein content of 9.45g/100g was noticed in treatment T_7 (60^0C for 7 hours) followed by 9.12g/100g in T_{10} (70^0C for 7 hours). The lowest protein content of 7.58g/100g was observed in T_{12} (70^0C for 11 hours). In non alkalised chocolates also there was a reduction in protein content with increasing the duration of tempering and conching of chocolates.

4.3.1.8. Total fat

The total fat content of alkalised chocolates ranged from 41.1 per cent to 44.5 per cent with the lowest and highest total fat content in treatment T_1 (60^0C for 7 hours) and T_6 (70^0C for 11 hours) respectively. There was no significant variation in fat content of the treatments T_3 (60^0C for 11 hours), T_5 (70^0C for 9 hours) and T_6 (70^0C for 11 hours). The total fat content of the treatment T_6 was significantly high when compared to all other treatments.

As revealed in Table 26, in non alkalised chocolates, the total fat content was found to be highest in T_{12} (46.50 per cent) and lowest in treatment T_7 (42.0 per cent). Statistically, significant difference in fat content of different treatments were observed. Based on one way ANOVA the treatments T_{11} and T_{12} was on par with each other and treatments T_7, T_8 and T_9 was also on par with each other. In alkalised and non alkalised chocolates the total fat content increased with increasing the duration of tempering and conching.

4.3.1.9. Total polyphenol

The total polyphenol content of alkalised chocolates varied significantly between the treatments. Based on one way ANOVA the highest total polyphenol of 0.21g/100g was found to be highest in treatment T_1 (Chocolates prepared at 60^0C for 7hours) and lowest 0.16g/100g of polyphenol in chocolate prepared at 70^0C for 11 hours (T_6).

In non alkalised chocolates also the total polyphenol content varied significantly between the treatments. The treatment T_7 had the maximum polyphenol content (0.23g/100g) and T_{11}, T_{12} had the lowest polyphenol content (0.20g/100g). Generally the polyphenol content was decreased with increasing temperature and the duration of tempering and conching of chocolates.

4.3.1.10. Total ash

In alkalised chocolates the total ash content varied significantly between the treatments. Based on DMRT the maximum total ash content of 1.45g/100g was recorded in treatment T_6 (70^0C for 11 hours) followed by chocolates prepared at 70^0C for 9 hours (1.44g/100g). The minimum total ash content of 1.41g/100g was observed in alkalised chocolates for treatment T1 (60^0C for 7 hours).

The total ash content in non alkalised chocolates was maximum (1.43g/100g) in treatments T_9 (60^0C for 11 hours), T_{11} (70^0C for 9 hours) and T_{12} (70^0C for 11 hours). The minimum total ash content of 1.40 g/100g was in treatment T_7 (60^0C for 7 hours). Statistically the the total ash content vary between the

treatments. The total ash content was increased with increasing temperature and the duration of tempering and conching of chocolates.

4.3.1.11. Calcium

The treatments T_3 and T_6 had slightly high calcium content (0.04g/100gm) compared to other treatments (0.03g/100gm).

In non alkalised chocolates the calcium content was more in treatment T_8, T_9 and $T1_2$ (0.04g/100gm) and were statistically on par. The lowest calcium content of 0.03mg/100gm was recorded in treatment T_7, T_{10} and T_{11} with 0.03g/100gm.

4.3.1.12. Phosphorus

The phosphorus content in alkalised chocolates had slight difference between the treatments. No significant difference in phosphorus content was observed between the treatments T_1, T_2, T_3, T_5 and T_6. The maximum phosphorus content of (0.17g/100g) was recorded in treatment T_3 and T_6 followed by T_1, T_2 and T_5 (0.16g/100g). In non alkalised chocolates the phosphorus content was maximum in treatment T_{12} (0.17g/100g) followed by treatments T_9 and T_{11} (0.16g/100g).

4.3.1.13. Iron

The iron content in alkalised chocolates differed significantly between the treatments. Based on one way ANOVA the maximum iron content of 18.45 mg/100gm was observed in the treatment T_3 followed by T_2 (17.93mg/100gm). The lowest iron content of 15.09 mg/100gm was found to be in chocolate prepared at 70^0C for 7 hours. In non alkalised chocolates, slight difference was observed in iron content between the treatments. The iron content was highest in treatment T_9 (16.89mg/100gm). But statistically significant variation was observed in iron content between the treatments.

4.3.1.14. Lipase activity

In alkalised chocolates the lipase activity was decreased with increasing the temperature and duration of tempering and conching of chocolates. The lipase activity of alkalized chocolates ranged from 0.0010 to 0.0015 µ eq with the lowest and highest lipase activity in treatment T_5 (70^0C for 9 hours) and T_6 (70^0C for 11 hours) and highest in T_1 (60^0C for 7 hours) respectively. Statistically treatment T_5 and T_6 were on par and T_2, T_3 and T_4 were also on par with each other. In non alkalised chocolates the highest lipase activity was recorded in T_7 (0.0017 µ eq) and lowest in treatment T_{12} (0.0010 µ eq).

4.3.1.15. Free fatty acid

The free fatty acid content in the alkalised chocolates differed significantly between the treatments. The treatment T_1 (Chocolate prepared at 60^0C for 7 hours) had the maximum free fatty acid content (2.80 %) followed by T_2 (Chocolate prepared at 60^0C for 9 hours). The treatment T_6 (Chocolate prepared at 60^0C for 11 hours) had recorded the lowest free fatty acid content of 1.67per cent, which is a desirable property for good chocolate. The free fatty acid content generally decreased with increasing duration of tempering and conching. The free fatty acid content also decreased with increasing temperature (T_4, T_5 and T_6 as compared to T_1, T_2 and T_3).

In non alkalised chocolates the highest free fatty acid content of 2.61per cent was observed in treatment T_7 (Chocolate prepared at 60^0C for 7 hours) followed by treatment T_8 (Chocolate prepared at 60^0C for 9 hours) having the free fatty acid content of 2.43 per cent. The lowest free fatty acid content in non alkalised chocolates was also recorded in chocolates prepared at 70^0C for 11 hours in treatment T_{12} (1.68%) followed by treatment T_{11} (2.05%). Based on DMRT, in non alkalised chocolates, the free fatty acid content varied significantly between the treatments. In non alkalised chocolates also the free fatty acid content decreased with increasing the tempering and conching time and temperature.

Table.24. Physico chemical analysis of alkalised and non alkalised chocolates

Treatments	Physico-chemical analysis															
	Alkalkised chocolates						CV (%)	CD (0.05)	Non alkalised chocolates						CV (%)	CD (0.05)
	Temperature-60^{0}C			Temperature-70^{0}C					Temperature-60^{0}C			Temperature-70^{0}C				
	T1 (7hrs)	T_2 (9hrs)	T_3 (11hrs)	T_4 (7hrs)	T_5 (9hrs)	T_6 (11hrs)			T_7 (7hrs)	T_8 (9hrs)	T_9 (11hrs)	$T1_0$ (7hrs)	$T1_1$ (9hrs)	$T1_2$ (11hrs)		
Moisture (%)	1.33^a	1.28^c	1.20^e	1.30^b	1.26^d	1.18^f	0.795	0.018	1.34^a	1.30^c	1.25^d	1.32^b	1.29^c	1.23^e	0.731	0.017
Energy (K.cal)	539.80^f	543.14^e	579.04^b	550.68^d	554.30^c	579.82^a	0.010	0.103	539.00^f	543.14^e	575.04	550.68^d	550.78^c	579.82^a	0.005	0.045
TSS (^{0} Brix)	68	67	67	67	66	66	1.496	NS	68	67	67	67	66	66	1.496	NS
Total sugar (g/100g)	46.70^d	47.84^b	48.01^a	46.80^d	47.40^c	48.02^a	0.172	0.145	46.60^d	47.70^b	48.02^a	46.70^d	47.50^c	48.02^a	0.132	0.112
Reducing sugar (g/100gm)	4.92^{bc}	4.94^{bc}	5.00^b	4.91^c	4.96^{bc}	5.10^a	0.839	0.074	4.80^c	5.00^b	5.30^a	4.91^b	4.95^b	4.99^b	1.168	0.104
Protein (g/100gm)	10.45^a	9.80^b	7.47^e	9.50^c	8.60^d	7.30^f	0.844	0.133	9.45^a	8.36^c	7.76^e	9.12^b	8.20^d	7.58^f	0.497	0.074
Fat (%)	41.10^b	42.00^b	44.00^a	41.90^b	43.50^a	44.50^a	1.361	1.037	42.00^c	43.33^b	44.00^b	43.80^b	45.50^a	46.50^a	1.316	1.035

Contd…

Treatments	Physico-chemical analysis														
	Alkalkised chocolates						CV (%)	CD (0.05)	Non alkalised chocolates						CV (%)	CD (0.05)
	Temperature-60^{0}C			Temperature-70^{0}C					Temperature-60^{0}C			Temperature-70^{0}C				
	T1 (7hrs)	T_2 (9hrs)	T_3 (11hrs)	T_4 (7hrs)	T_5 (9hrs)	T_6 (11hrs)			T_7 (7hrs)	T_8 (9hrs)	T_9 (11hrs)	$T1_0$ (7hrs)	$T1_1$ (9hrs)	$T1_2$ (11hrs)		
Polyphenol (g/100gm)	0.21^{a}	0.19^{b}	0.17^{cd}	0.20^{b}	0.18^{bc}	0.16^{d}	8.108	0.018	0.23^{a}	0.22^{ab}	0.21^{bc}	0.21^{bc}	0.20^{cd}	0.20^{cd}	6.742	0.018
Total ash (mg 100 /g)	1.41^{c}	1.43^{b}	1.44^{ab}	1.43^{b}	1.44^{ab}	1.45^{a}	0.698	0.018	1.40^{c}	1.41^{bc}	1.43^{a}	1.42^{ab}	1.43^{a}	1.43^{a}	0.704	0.018
Calcium (mg 100/ g)	0.03^{b}	0.03^{b}	0.04^{a}	0.03^{b}	0.03^{b}	0.04^{a}	43.03	0.023	0.003^{b}	0.004^{a}	0.004^{a}	0.003^{b}	0.003^{b}	0.004^{a}	43.033	0.023
Phosphorus (mg 100 /g)	0.16^{ab}	0.16^{ab}	0.17^{a}	0.15b	0.16^{ab}	0.17^{a}	6.186	0.017	0.15^{b}	0.15^{b}	0.16^{ab}	0.15^{b}	0.16^{ab}	0.17^{a}	6.742	0.018
Iron (mg 100 /g)	17.05^{d}	17.93^{b}	18.45^{a}	15.09^{f}	16.26^{e}	17.55^{c}	0.059	0.018	16.78^{c}	16.81^{b}	16.89^{a}	16.67^{e}	16.71^{d}	16.82^{b}	0.063	0.018
Lipase (µ eq)	0.0015^{a}	0.0012^{b}	0.0012^{b}	0.0012^{b}	0.0010^{c}	0.0010	7.714	0.000	0.0017^{a}	0.0015	0.0012^{c}	0.0015^{b}	0.0012^{c}	0.0010^{d}	6.762	0.000
Free fatty acid (%)	2.80^{a}	2.61^{b}	2.05^{c}	2.80^{a}	2.05^{c}	1.67^{d}	2.555	0.106	2.61^{a}	2.43^{b}	2.05^{d}	2.24^{c}	2.05^{d}	1.68^{e}	0.541	0.021

Superscripts indicates homogenous group between treatment

4.3.2. Organoleptic evaluation of chocolates

Chocolates were prepared at different time periods of 7, 9 and 11 hours at a temperature of 60^0C and 70^0C respectively in tempering and conching machine. Organoleptic evaluation of alkalised and non alkalised chocolates were carried out using score card by a panel of fifteen judges. The mean scores obtained for various organoleptic qualities of chocolates such as appearance, colour, flavor, texture, taste and overall acceptability are presented in Table 25 and 26 and plate 16-21. The different quality attributes were ranked based on their mean score using Kendall's coefficient (w).

As revealed in Table 25, the means score for appearance of alkalised chocolates varied from 8.02 (T_4) to 8.87 (T_3) with mean rank scores in the range of 3.17 to 3.77. The chocolate prepared at 70^0C for 11 hours (T_3) in tempering and conching machine had the highest mean score (8.87) and lowest in T_4 (8.02) for appearance.

The highest mean score for colour (8.84) was noticed in T_6 and the lowest mean score for colour was observed in T_4 (7.96). The mean rank scores for colour of various treatments ranged between 1.93 and 5.03.

The mean score for flavour of different alkalised chocolates ranged from 7.71 to 8.71. The lowest mean score of 7.71 was noticed in chocolate prepared at 70^0C for 7 hours (T_4) and the highest score of 8.71 for the treatment T_6 (chocolate prepared at 70^0C for 11 hours).

Among different treatments tried for the preparations of alkalised chocolates, the highest mean score for texture (8.89) was recorded for T_6. The lowest mean score of 7.60 for texture was noticed in T_1 (chocolate prepared at 60^0C for 7 hours).

The mean score for taste varied from 8.00 to 9.00. The lowest mean score for taste recorded in treatment T_1 and the highest score of 9.00 was recorded in

treatment T_3 (chocolate prepared at 60^0C for 11 hours) and T_6 (chocolate prepared at 70^0C for 11 hours) respectively.

Among the different treatments tried for the alkalised chocolates, the highest rank score for overall acceptability was noticed in T_3 (8.84) followed by T_6 (8.87). The lowest mean score of 7.83 was recorded for T_1 (chocolate prepared at 60^0C for 7 hours).

The highest total score of 53.10 was recorded in treatment T_6 and followed by in treatment T_3 (52.92).

From the, organoleptic evaluation of different treatments, based on colour, flavour, texture, taste and total score the best rated treatment T_6 (Alkalised chocolates prepared at 70^0C for 11 hours) were selected for further studies (plate21).

Table 25. Mean scores for organoleptic evaluation of alkalised chocolates

Treatments	Parameters						
	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability	Total score
T_1	8.51 (3.17)	8.27 (3.33)	8.13 (2.83)	7.60 (1.13)	8.00 (1.53)	7.83 (1.57)	48.34
T_2	8.53 (3.77)	8.47 (3.97)	8.49 (3.63)	8.04 (2.63)	8.33 (2.43)	8.20 (2.80)	50.06
T_3	**8.87** (3.77)	8.76 (4.80)	8.67 (4.77)	8.78 (5.30)	**9.00** (5.10)	**8.84** (5.37)	52.92
T_4	8.02 (3.30)	7.96 (1.93)	7.71 (1.40)	8.00 (2.87)	8.50 (2.07)	8.07 (2.33)	48.26
T_5	8.62 (3.57)	8.22 (1.93)	8.36 (3.47)	8.24 (3.50)	8.67 (4.57)	8.38 (3.40)	50.49
T_6	8.82 (3.43)	**8.84** (5.03)	**8.71** (4.90)	**8.89** (5.57)	**9.00** (5.30)	**8.87** (5.53)	**53.13**
Kendall's value	0.033**	0.640**	0.526**	0.854**	0.845**	0.855**	

Values in parentheses is mean rank score based on Kendall's W

** Significant at 1% level

T_1-60^0 C for 7hours T_2- 60^0C for 9 hours T_3- 60^0 C for 11hours

T_4 - 70^0Cfor 7hours T_5- 70^0 C for 9hours T_6- 70^0 C for 11hours

Mean scores for organoleptic evaluation of nonalkalised chocolates

The mean scores of the non alkalised chocolates prepared in tempering and conching machine at different temperatures of 60^0C and 70^0C for the duration of 7, 9 and 11 hours are presented in Table 26 and Plate 22- 27.

As revealed in Table 26 the mean score for the appearance of non alkalised chocolates varied from 8.02 (T_7) to 8.73 (T_{12}) with mean rank scores in the range of 2.53 to 6.00. Among different treatments tried for the preparation of chocolates prepared, the highest mean scores of 8.73 for appearance was noticed in T_{12} and the lowest in T_7 (8.02).

Among different treatments tried for the preparations of non alkalised chocolates, the highest mean score for colour (8.80) was recorded for T_{12} (70^0C for 11 hours). The lowest mean score of 7.93 for colour was noticed in T_7 (60^0C for 7 hours).

The mean score for flavour of non alkalised chocolates ranged from 7.80 to 8.84. The highest mean score of 8.84 was obtained for the chocolates prepared at 70^0C for 11 hours (T_{12}) and the lowest mean score of 7.80 was noticed in chocolates prepared at 60^0C for 7 hours (T_7).

The highest mean score of 8.62 for texture was recorded for T_{12} which was immediately followed with a mean score of 8.55 by T_9 (60^0C for 11 hours). The lowest mean score of 7.53 was noticed in T_7 (60^0C for 7 hours).

The mean scores for taste of different chocolates ranged from 7.62 to 8.82 with highest score for T_9 (60^0C for 11 hours). Among the different treatments tried, the lowest mean score of 7.62 for taste was noticed in chocolates T_7 and T_{10} prepared at 60^0C for 7 hours (T_7) and 70^0C for 7 hours (T_{10}). The treatments T_8, T_{11} and T_{12} obtained the mean scores for taste of 8.04, 8.24 and 8.73 respectively

The scores for overall acceptability ranged from 7.55 to 8.95. The chocolates (T_7 and T_{10}) attained the lowest mean score of 7.55 and 7.86. The

chocolates prepared at 70^0C for 11 hours (T_{12}) attained the maximum score of 8.95 for overall acceptability. The treatments T_8, T_9 and T_{11} obtained the mean scores for taste as 8.31, 8.91 and 8.44 respectively.

Based on total score (52.63) obtained for organoleptic qualities and based on appearance, colour, flavour, texture and overall acceptability, the best rated treatment T_{12} (non alkalised chocolate prepared at 70^0C for 11 hours) were selected for further studies. The treatment T_{12} selected for further studies.

Comparison of organoleptic scores based on tempering and conching temperatures of selected chocolates

Table 27, reveales the comparison of organoleptic scores like appearance colour, flavour, texture, taste, overall acceptability and total score based on temperature during tempering and conching of chocolates. In alkalised chocolates the maximum score for colour (8.84), flavour (8.71), texture (8.89), taste(9.00), overall acceptability(8.87) and total score (53.13) was obtained for the treatment T_6 (70^0 for 11hours). In non alkalised chocolates the maximum score for sensory attributes like, appearance (8.73) colour (8.80), flavour (8.84), texture (8.62), overall acceptability(8.95) and total score (52.63) was obtained for the treatment T_{12} (70^0 for 11hours). So the temperature of 70^0C was suitable for tempering and conching of chocolate preparation.

Table 26. Mean scores for organoleptic evaluation of non alkalised chocolates

Treatments	Parameters						
	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability	Total Score
T_7	8.02(2.13)	7.93(1.70)	7.80(2.0)	7.51(1.3)	7.62(1.80)	7.55(1.23)	46.43
T_8	8.17(2.73)	8.35(3.07)	8.08(2.7)	8.00(3.0)	8.04(3.17)	8.31(3.27)	49.03
T_9	8.66(4.93)	8.77(4.93)	8.55(4.7)	8.55(5.0)	8.82(5.50)	8.91(5.27)	52.30
T_{10}	8.08(2.57)	7.97(1.83)	7.91(2.0)	7.84(2.3)	7.62(1.60)	7.86(2.07)	47.28
T_{11}	8.31(3.63)	8.68(4.50)	8.40(3.7)	8.31(3.3)	8.24(3.67)	8.44(3.73)	50.38
T_{12}	**8.73**(5.00)	**8.80**(4.97)	**8.84**(5.0)	**8.62**(5.0)	**8.73**(5.27)	**8.95**(5.43)	**52.63**
Kendall's value	0.548	0.703	0.721	0.760	0.824	0.870	

Values in parentheses is mean rank score based on Kendall's W

** Significant at 1%

T_7-60^0C for 7hours T_8- 60^0C for 9hours T_9- 60^0 C for 11hours

T_{10} - 70^0C for 7hours T_{11}- 70^0 C for 9hours T_{12}- 70^0 C for 11hours

Table 27. Comparison of scores based on tempering and conching temperatures

Sensory Parameter	Alkalised chocolates		Non alkalised chocolates	
	T_3	T_6	T_9	T_{12}
Apearance	8.87	8.82	8.66	8.73
Colour	8.76	8.84	8.77	8.80
Flavour	8.67	8.71	8.55	8.84
Texture	8.78	8.89	8.55	8.62
Taste	9.00	9.00	8.82	8.73
Overall acceptability	8.84	8.87	8.91	8.95
Total score	52.92	53.13	52.30	52.63

T_3- 60^0 C for 11hours T_6- 70^0 C for 11hours T_9- 60^0 C for 11hours

T_{12}- 70^0 C for 11hours

4.3.3. Enumeration of total micro flora in alkalised and non alkalised chocolates

The microbial population (bacterial, fungal and yeast count) of different types of chocolates was analysed after the preparation of chocolates at different temperature and duration of tempering and conching time. From the table.28 it is found that the bacterial, fungal and yeast population was not detected in all treatments ie, chocolates prepared with alkalised and non-alkalised cocoa beans.

Table 28. Enumeration of total micro flora in alkalised and non alkalised chocolates

Treatments	Enumeration of total microflora		
	Bacteria (10^{-4} cfu g-1)	**Fungus (10^{-2} cfu g-1)**	**Yeast (10^{-2} cfu g-1)**
T_1 (AC)	ND	ND	ND
T_2 (AC)	ND	ND	ND
T_3 (AC)	ND	ND	ND
T_4 (AC)	ND	ND	ND
T_5 (AC)	ND	ND	ND
T_6 (AC)	ND	ND	ND
T_7 (NAC)	ND	ND	ND
T_8 (NAC)	ND	ND	ND
T_9 (NAC)	ND	ND	ND
T_{10} (NAC)	ND	ND	ND
T_{11} (NAC)	ND	ND	ND
T_{12} (NAC)	ND	ND	ND

T1-60^0 C for 7hours T_2- 60^0 C for 9 hours T_3- 60^0C for 11hours AC-Alkalised chocolates
T_4 - 70^0 C for 7hours T_5- 70^0C for 9hours T_6- 70^0Cfor 11hours NAC-Non alkalised chocolates
T_7-60^0C for 7hours T_8- 60^0C for 9hours T_9- 60^0 C for 11hours
T_{10} - 70^0C for 7hours T_{11}- 70^0C for 9hours T_{12}- 70^0C for 11hours , ND –Not detected

Based on total score through sensory evaluation and the free fatty acid content of chocolates, the best treatments T_6 (70^0C for 11 hours) and T_{12} (70^0C for

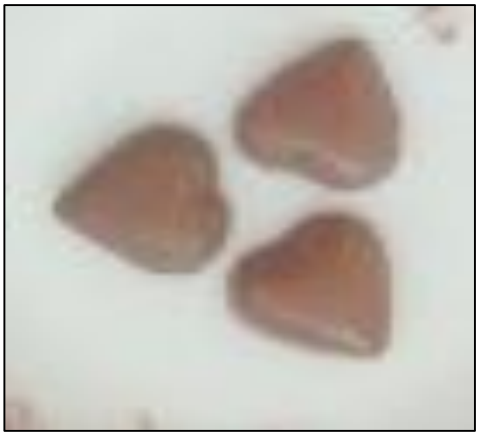


Plate 28. Control (100 % chocolate)

Plate 29. 5 % chocolate + 5% dehydrated grapes

Plate 30. 95 % chocolate + 5% dehydrated dates

Plate 31. 95% chocolates + 5% Osmodehydrated jackfruit

Plate 32. 95 %chocolate + 5% osmodehydrated pineapple

Plate 33. 95 % chocolate + 5% cashew nut

Plate 34. 95 % chocolate + 5% almonds

Plate 35. 95% chocolate + 5% white pepper powder

Plate 36. 97 % chocolate + 3% dehydrated mint powder

11 hours) (one each from alkalised and non alkalised) were selected for further studies or for the preparation of blended chocolates (Table 29).

Table.29. Comparison of important parameters of alkalised and non alkalised chocolates

Parameters	Alkalised chocolates (T_6) (70^0 for 11hours)	Non alkalised chocolates (T_{12}) (70^0 for 11hours)
Total score	53.13	52.63
Free fatty acid (%)	1.67	1.68

4.4. Blending of chocolates with other ingredients

Chocolates prepared from alkalised beans and subjected to tempering and conching operations at 70^0C for 11 hours were used for the preparation of blended chocolates. Chocolates were blended with dehydrated grapes, dehydrated dates, osmodehydrated jacks, osmodehydrated pineapple, almond, cashew nut, powdered dehydrated mint leaves and white pepper powder (Plates 28, 29,30,31,32,33,34,35 and 36). All the above mentioned combinations were also done in chocolate prepared from non alkalised beans. The rate of incorporation of mint powder and white pepper powder were standardised using preliminary studies.

4.4.1. Standardisation of dehydrated mint powder and white pepper powder in chocolates

Standardisation of mint powder and white pepper powder to be added in the chocolates were standardised based on organoleptic evaluation.

4.4.1.1. Organoleptic evaluation of chocolates blended with different concentration of powdered dehydrated mint leaves

Mint powder was incorporated at different levels in alkalised chocolates. Oganoleptic evaluation of mint powder blended chocolates was carried out using score card by a panel of fifteen judges. The mean scores obtained for various quality attributes of chocolates prepared by incorporating dehydrated mint powder are

presented in Table 30. The different quality attributes were ranked based on their mean score using Kendall's coefficient (w) test.

The mean score for appearance of different mint powder blended chocolates ranged from 7.64 to 8.89. The appearance score was recorded highest for treatment T_3 (97% C+ 3% DMP) followed by treatment T_2 (98% C+ 2% DMP) among the different treatments tried for the mint blended chocolates the lowest mean score for appearance was obtained for treatment T_5 (95%C+ 5%DMP).

Among different treatments tried for the preparations of mint blended chocolates, the highest mean score for colour (8.93) was recorded for T_3. The lowest mean score of 7.44 for colour was noticed in chocolate prepared with 95% chocolates and 5per cent dehydrated mint powder.

The mean score for flavour of different dehydrated mint powder chocolate ranged from 7.56 to 8.89. The lowest mean score of 7.56 was noticed in chocolate prepared using 95% chocolate and 5% dehydrated mint powder (T_5) and the highest score for T_3 (97%C+ 3%DMP).

Among different treatments tried for the preparations of dehydrated mint powder blended chocolates, the highest mean score for texture (8.82) was recorded for T_3. The lowest mean score of 7.22 for texture was noticed in T_5 (95%C+ 5%DMP).

The mean scores for taste of chocolates ranged from 7.64 to 8.98 with highest score for T_3 followed by T_2. Among the different treatments tried, the lowest mean score of 7.64 for taste was noticed in T_5 (95%C+ 5%DMP).

Among the different treatments tried for the chocolates, the highest rank score for overall acceptability was noticed in T_3 (8.89) followed by T_2 (8.63). The lowest mean score of 7.71 was recorded for T_5 (95%C+ 5%DMP).

The total score of organoleptic qualities was also highest (53.40) for treatment T_3 (97% Chocolate + 3% dehydrated mint powder). Hence, T_3 which

contained 97per cent per cent chocolate and 3 per cent dehydrated mint powder was selected for further studies. From the organoleptic evaluation of different treatments, the best rated treatment T_3 (97%C+ 3%DMP) was standardised for further studies.

Based on Kendall's (w) value, significant agreement among judges was noticed in the evaluation of different quality attributes of dehydrated mint powder blended chocolates.

Table. 30. Mean rank score for organoleptic evaluation for standardisation of chocolates blended with powdered dehydrated mint leaves

Treatments	Parameters						
	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability	Total score
T_1 (99% C+ 1%DMP)	8.49 (2.87)	8.49 (2.83)	8.36 (2.60)	8.33 (2.73)	8.22 (2.53)	8.31 (2.80)	50.2
T_2(98% C+ 2%DMP	8.67 (3.60)	8.69 (3.77)	8.67 (4.23)	8.69 (3.97)	8.67 (4.33)	8.63 (3.83)	52.02
T_3 (97% C+ 3%DMP	8.89 (4.67)	8.93 (4.57)	8.89 (4.57)	8.82 (4.58)	8.98 (4.60)	8.89 (4.57)	**53.40**
T_4 (96% C+ 4%DMP	8.49 (2.87)	8.36 (2.83)	8.31 (2.60)	8.33 (2.73)	8.22 (2.53)	8.22 (2.80)	49.93
T_5 (95% C+ 5%DMP	7.64 (1.00)	7.44 (1.00)	7.56 (1.00)	7.22 (1.00)	7.64 (1.00)	7.71 (1.00)	45.21
Kendall's value	0.882**	0.777**	0.925**	0.886**	0.968**	0.878**	

Values in parentheses is mean rank score based on Kendall's W
DMP- Dehydrated Mint leaf powder ** Significant at 1%

4.4.1.2. Organoleptic evaluation of chocolates blended with different concentration of white pepper powder

Organoleptic evaluation of chocolates blended with white pepper powder was carried out using score card by a panel of fifteen judges. The mean scores obtained for various quality attributes of chocolates prepared using chocolate and white pepper are presented in Table 31. The different quality attributes were ranked based on their mean score using Kendall's coefficient (w) test.

The mean scores obtained for the organoleptic evaluation varied from 8.02 (T_1) to 8.90 (T_4) for appearance. The colour and flavour had mean scores ranging from 7.93 (T1) to 8.90 (T_4) and 7.80 (T_1) to 8.71 (T_5) respectively. The texture and taste of the white pepper powder blended chocolates obtained mean scores of 7.51 (T_1) to 8.89 (T_5) and 7.62 (T_1) to 9.00 (T_5) respectively. The mean scores for overall acceptability differs from 7.55 to 8.89.

The mean rank scores, varied from 2.13 to 3.60 for appearance, 1.70 to 5.03 for colour, 2.00 to 4.90 for flavour, 1.23 to 5.57 for texture, 1.80 to 5.30 for taste and 1.23 to 5.53 for overall acceptability.

The treatment T_5 had the highest mean score of 8.71 (flavour), 8.89 (texture), 9.00 (taste) and 8.89 (overall acceptability). The treatment T_4 (96% chocolate + 4 % white pepper powder) had the highest mean score for appearance and colour (8.89). The total score of organoleptic qualities was also highest (53.13) for treatment T_5 (95% Chocolate + 5 % White pepper powder). Hence, T_5 which contained 95% per cent chocolate and 5 per cent white pepper was selected for further studies.

The Kendall's value showed a significant agreement among the judges for all quality attributes of white pepper blended chocolates.

4.4.1.3. Organoleptic evaluation of alkalised blended chocolates

The mean score and mean rank score obtained for different quality attributes of alkalised blended chocolates prepared using alkalised cocoa nibs and different dehydrated fruits like grapes, dates, osmodehydrated pineapple and osmodehydrated jackfruit; almond and cashew nuts; dehydrated mint leaves powder and white pepper powder at various levels and including control (chocolates prepared with alkalised cocoa nibs only) are presented in Table 32.

Table. 31. Mean rank score for organoleptic evaluation for standardisation of chocolates blended with white pepper powder

Treatments	Parameters						
	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability	Total score
T_1 (99 % C+ 1% WPP)	8.02 (2.13)	7.93 (1.70)	7.80 (2.00)	7.51 (1.23)	7.62 (1.80)	7.55 (1.23)	46.43
T_2 (98% C + 2 % WPP)	8.17 (2.73)	8.35 (3.07)	8.08 (2.57)	8.08 (3.20)	8.04 (3.17)	8.31 (3.27)	49.03
T_3 (97% C + 3 % WPP)	8.62 (3.57)	8.22 (1.93)	8.36 (3.47)	8.24 (3.50)	8.67 (4.57)	8.38 (3.40)	50.49
T_4(96% C + 4 % WPP)	8.90 (2.65)	8.90 (2.70)	8.40 (2.35)	8.60 (2.80)	8.80 (2.70)	8.85 (2.80)	52.45
T_5 (95% C + 5 % WPP)	8.82 (3.43)	8.84 (5.03)	8.71 (4.90)	8.89 (5.57)	9.00 (5.30)	8.89 (5.53)	**53.16**
Kendall's value	0.823**	0.800**	0.591**	0.884**	0.786**	0.864**	

Values in parentheses is mean rank score based on Kendall's W
C- Control, WPP- White Pepper Powder ** Significant at 1%

The mean score for appearance of alkalised blended chocolates ranged from 7.64 to 9.00. The appearance score was highest (9.00) for T_1 (95 % alkalised chocolates +5% dehydrated grapes) and T_3 (95 % alkalised chocolates +5% osmodehydrated jack fruit), and the lowest mean score of 7.64 was obtained for chocolate prepared using 97% alkalised chocolates and 3 % dehydrated mint leaves powder (T_7). The mean rank score for appearance varied from 1.00 to 7.20.

Among different treatments tried for the preparations of blended chocolates, the highest mean score for colour (9.00) was recorded for T_6 (95 % AC+5% A). The lowest mean score of 7.44 for colour was noticed in treatment T_7 ((97 % AC+3% DMP) and the mean rank score varied from 1.00 to 7.53.

The mean score for flavour of different alkalised blended chocolates ranged from 7.56 to 9.00. The highest mean score of 9.00 was obtained for treatment T_3, T_6 and T_8 and the lowest mean score of 7.56 was noticed in chocolate prepared

using 97% alkalised chocolates and 3 % dehydrated mint leaves powder (T_7). The mean rank score for flavour varied from 1.07 to 7.57.

The same trend were observed in the case of texture, taste and overall acceptability, the maximum mean score of 9.00 was observed in treatments T_3 (95 % AC+5% ODJ), T_6 (95 % AC+5% B) and T_8 (95 % AC+5% WPP). The lowest mean score for texture, taste and overall acceptability were recorded as 7.22, 7.64 and 7.71 respectively in treatment T_7 ((97 % AC+3% DMP). The mean rank score for texture varied from 1.00 to 7.47, for taste it varied from 1.13 to 7.33 and for overall acceptability varied from 1.07 to 7.10.

The total scores for organoleptic qualities was highest (54.00) for treatments T_3 (95 % alkalised chocolates +5% osmodehydrated jack fruit), T_6 (95 % alkalised chocolates +5% almond) and T_8 (95 % alkalised chocolates +5% white pepper). Hence, the treatments T_3, T_6 and T_8 were selected for further studies. Based on Kendall's (w) value, significant agreement among the judges was noticed in the evaluation of different quality attributes of chocolates.

4.4.1.4. Organoleptic evaluation of non alkalised blended chocolates

The mean score and mean rank score obtained for different quality attributes of non alkalised blended chocolates prepared using non alkalised cocoa nibs and different dehydrated fruits like grapes, dates, pineapple and jackfruit; almond and cashew nuts; dehydrated mint powder and white pepper powder at various levels, including control (chocolates prepared with non alkalised cocoa nibs only) are presented in Table 33.

As revealed in Table 33, the means score for appearance of blended non alkalised chocolates varied from 7.64 (T_{15}) to 9.00 (T_{14} and T_{16}) with mean rank scores in the range of 1.00 to 7.43. The chocolate blended with 95 % non alkalised chocolates and 5% almond (T_{14}) and the chocolates blended with 95 % non alkalised chocolates and 5% white pepper (T_{16}) had the highest mean score (9.00) and lowest in T_{15} (7.64) for appearance.

Table.32. Mean scores obtained for organoleptic evaluation of blended alkalised chocolates

Treatments	Parameters						
	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability	Total score
T_0 - (100% AC)	8.49 (3.13)	8.36 (3.70)	8.31 (3.33)	8.33 (3.13)	8.22 (2.57)	8.22 (2.70)	49.93
T_1 - (95 % AC+5% DG)	9.00 (7.20)	8.62 (5.00)	8.84 (6.17)	8.87 (6.37)	8.82 (5.83)	8.93 (6.40)	53.08
T_2- (95 % AC+5% DD)	8.56 (3.87)	8.38 (3.90)	8.49 (3.77)	8.47 (3.63)	8.53 (4.27)	8.64 (4.83)	51.07
T_3-(95 % AC+5% ODJ)	9.00 (7.20)	8.43 (3.93)	9.00 (7.57)	8.81 (3.47)	9.00 (7.33)	9.00 (7.10)	**53.24**
T_4- (95 % AC+5% ODP)	8.51 (3.63)	8.42 (4.13)	8.13 (2.80)	8.27 (3.07)	8.38 (3.47)	8.38 (3.63)	50.09
T_5-- (95 % AC+5% CN)	8.69 (4.57)	8.58 (4.67)	8.64 (5.17)	8.69 (5.40)	8.78 (5.73)	8.76 (5.07)	52.14
T_6 - (95 % AC+5% A)	8.70 (4.61)	9.00 (7.53)	9.00 (7.57)	9.00 (7.47)	9.00 (7.33)	9.00 (7.10)	**53.70**
T_7 -(97 % AC+3% DMP)	7.64 (1.00)	7.44 (1.00)	7.56 (1.07)	7.22 (1.00)	7.64 (1.13)	7.71 (1.07)	45.21
T_8 - (95 % AC+5% WPP)	8.69 (4.20)	8.50 (4.53)	9.00 (7.57)	9.00 (7.47)	9.00 (7.33)	9.00 (7.10)	**53.19**
Kendall's value	0.764	0.702	0.751	0.854	0.803	0.778	

Values in parentheses is mean rank score based on Kendall's W
** Significant at 1% level
AC- Alkalised Chocolates, ODJ – Osmodehydrated Jack fruit, ODP- Osmodehydrated Pineapple, CN- Cashew Nut, A-Almond, DMP- Dehydrated Mint leaves Powder, WPP- White Pepper Powder

The mean score for colour varied from 7.74 to 8.93.The highest mean score for colour (8.93) was noticed in T_{11} (95 % non alkalised chocolates +5% osmodehydrated jack fruit) and T_{16} (95 % non alkalised chocolates +5% white pepper) and the lowest mean score of 7.74 was obtained for chocolate prepared using 97% non alkalised chocolates and 3 % dehydrated mint leaves powder (T_{15}). The mean rank scores for colour of various treatments ranged between 1.03 and 7.67. The mean score for flavour of different blended non alkalised chocolates ranged from 7.56 to 9.00. The lowest mean score of 7.56 was noticed in chocolate blended with 97% non alkalised chocolates and 3 % dehydrated mint leaves powder

(T_{15}) and the highest score of 9.00 for the treatment T_{16} (95 % non alkalised chocolates +5% white pepper) followed by treatment T_{11} (95 % non alkalised chocolates +5% osmodehydrated jack fruit) with mean score of 8.96. The mean rank scores for flavour varied from 1.07 to 7.87.

Among different treatments tried for the preparations of non alkalised blended chocolates, the highest mean score for texture (8.96) was recorded for T_{16}. The lowest mean score of 7.24 for texture was noticed in T_{15} (97% non alkalised chocolates and 3 % dehydrated mint powder). The mean rank score varied from 1.03 to 7.57.

The mean score for taste varied from 7.62 to 9.00. The lowest mean score for taste recorded in treatment T_{15} (97% non alkalised chocolates and 3 % dehydrated mint leaves powder) and the highest score of 9.00 was recorded in treatment T_{11} (95 % non alkalised chocolates +5% osmodehydrated jack fruit) T_{14} (chocolate blended with 95 % non alkalised chocolates and 5% almond) and T_{16} (95 % non alkalised chocolates and 5% white pepper powder) respectively. The mean rank score for taste varied from 1.20 to 7.73.

In non alkalised chocolates, the highest rank score of 9.00 for overall acceptability was noticed in T_{11} (95 % non alkalised chocolates +5% osmodehydrated jack fruit) and in $T1_4$ (chocolate blended with 95 % non alkalised chocolates and 5% almond) and followed by T_{16} (95 % non alkalised chocolates and 5% white pepper powder) with mean score of 8.98.. The lowest mean score of 7.56 was recorded for T_{15} (97% non alkalised chocolates and 3 % dehydrated mint leaves powder). The mean rank score for overall acceptability varied between from 1.13 to 7.23.

The total scores for organoleptic qualities was 53.77, 53.80 and 53.78 was highest for treatments T_{11} (95 % non alkalised chocolates +5% osmodehydrated jack fruit), T_{14} (chocolate blended with 95 % non alkalised chocolates and 5% almond) and T_{16} (95 % non alkalised chocolates and 5% white pepper powder) respectively. Hence, the treatments T_{11}, T_{14} and T_{16} were selected for further

studies. Based on Kendall's (w) value, significant agreement among the judges was noticed in the evaluation of different quality attributes of chocolates.

Table.33. Mean scores obtained for organoleptic evaluation of blended non alkalised chocolates

Treatments	Parameters						
	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability	Total score
T_0 - (100% NAC)	8.33 (2.83)	8.29 (3.50)	8.33 (3.60)	8.20 (3.07)	8.27 (2.80)	8.04 (2.30)	49.47
T_9 - (95 % NAC+5% DG)	8.89 (6.50)	8.89 (4.93)	8.56 (6.47)	8.80 (6.30)	8.89 (5.23)	8.84 (6.37)	52.87
T_{10}- (95 % NAC+5% DD)	8.51 (3.80)	8.51 (4.07)	8.44 (3.87)	8.44 (3.93)	8.60 4.13)	8.60 (4.77)	51.11
T_{11}-(95 % NAC+5% ODJ)	8.98 (7.20)	8.93 (7.50)	8.96 (7.43)	8.90 (7.42)	9.00 (7.73)	9.00 (7.23)	**53.77**
T_{12}- (95 % NAC+5% ODP)	8.53 (3.97)	8.42 (4.17)	8.27 (2.93)	8.38 (3.13)	8.38 (3.20)	8.38 (3.87)	50.11
T_{13}-- (95 % NAC+5% CN)	8.67 (4.83)	8.49 (4.60)	8.56 (4.77)	8.60 (5.20)	8.73 (5.43)	8.73 (5.10)	51.78
T_{14} - (95 % NAC+5% A)	9.00 (7.43)	8.96 (7.67)	9.00 (7.87)	8.85 (7.40)	9.00 (7.53)	9.00 (7.23)	**53.80**
T_{15} -(95 % NAC+5% DMP)	7.64 (1.00)	7.44 (1.03)	7.56 (1.07)	7.24 (1.03)	7.62 (1.20)	7.56 (1.13)	45.07
T_{16} - (95 % NAC+5% WPP)	9.00 (7.43)	8.93 (7.53)	8.91 (7.00)	8.96 (7.57)	9.00 (7.73)	8.98 (7.00)	**53.78**
Kendall's value	0.840	0.719	0.810	0.801	0.832	0.783	

Values in parentheses is mean rank score based on Kendall's W
** Significant at 1% level
NAC- Non Alkalised Chocolates, ODJ – Osmodehydrated Jack fruit, ODP- Osmodehydrated Pineapple, CN- Cashew Nut, A- Almond, DMP- Dehydrated Mint leaves Powder, WPP- White Pepper Powder

As revealed in Table. 34 the best six treatments (three each from alkalised and non alkalised) along with control (alkalised and non alkalised) were selected for further storage studies. From the, organoleptic evaluation of different treatments, based on appearance, colour, flavour, texture, taste, overall acceptability and total score the best rated treatments from alkalised chocolates were T_3 (95 % alkalised chocolates +5% osmodehydrated jack fruit), T_6 (95 % alkalised chocolates

+5% almond) and T_8 (95 % alkalised chocolates +5% white pepper) and from non alkalised chocolates the treatments T_{11} (95 % non alkalised chocolates +5% osmodehydrated jack fruit), T_{14} (chocolate blended with 95 % non alkalised chocolates and 5% almond) and T_{16} (95 % non alkalised chocolates and 5% white pepper powder) were selected for further storage studies.

Table.34. Blended chocolates selected for storage study

Alkalised chocolates	Non akalised chocolates
T_0 - (100% AC)	**T_0** - (100% NAC)
T_1-(95 % AC+5% ODJ)	**T_4**- (95 % AC+5% ODJ)
T_2 - (95 % AC+5%A)	**T_5** - (95 % AC+5%A)
T_3 - (95 % AC+5% WPP)	**T_6** - (95 % AC+5% WPP)

AC- Alkalised chocolates, NAC- Non alkalised chocolates, ODJ – Osmodehydrated jack fruit, A –Almond, WPP- White Pepper Pepper

4.4.2. Quality evaluation of selected blended chocolates

The selected blended chocolates (alkalised and non alkalised) and control (alkalised and non alkalised) were subjected to quality evaluation studies. The chocolates were wrapped in aluminium foil and stored for six months in refrigerated conditions for further studies (Plate 37 and 38).

4.4.2.1. Physico- chemical qualities of selected blended chocolates during storage

The selected blended chocolates from alkalised and non alkalised chocolates were packed in aluminium foils and stored in refrigerator for six months. The chocolates were analysed for its physical and chemical qualities initially and during sixth month of storage. The textural properties were analysed initially and at the end of storage and all other constituents like moisture, energy, TSS, total sugar, reducing sugar, protein, fat, polyphenol, total ash, minerals like calcium, phosphorus, iron, lipase and free fatty acids were analysed initially and also during sixth month of storage. Among the treatments, variations in chemical constituents were statistically analysed by applying one way ANOVA, and changes in chemical

Plate 37. Chocolates wrapped in aluminium foil

Plate 38. Storege of chocolates

constituents in each treatments due to storage was analysed by applying‘t’ test and the results are presented in Table 35 to 49.

4.4.2.1.1. Textural properties of blended chocolates

The textural properties of blended chocolates were evaluated initially and at the end of six month of storage and it was compared with commercial chocolates. The results of the textural quality parameters such as hardness, cohesiveness, adhesiveness and gumminess of blended chocolates (alkalised and non alkalised) were observed (Table.35-38). The textural properties of commercial chocolates (Dairy milk, Dairy milk silk and For U (KAU milk chocolates) were also evaluated and compared with blended chocolates.

Initially the hardness of blended chocolates varied from 74.41N to 99.46N. Based on one way ANOVA the treatments were statistically significant in hardness, but the treatments T_2 and T_5 were statistically on par and similarly the treatments T_1 and T_4 were also on par in hardness. The lowest hardness of 74.41N was recorded in control (T_0 - NAC), chocolates prepared with 100% non alkalised beans followed by treatment T_0 (76.71N) with 100% alkalised beans. The highest hardness of 99.46N was recorded in treatment T_5 (95 % NAC+5% A), followed by 99. 11N in treatment T_2 (95 % AC+5% A).

At the end of six month of storage the lowest hardness of 77.16N was observed in treatment T_0 (100% non alkalised chocolates), followed by control (100 % alkalised chcocolates). The highest hardness of 102.51N was found in treatment T_2 (95 % AC+5% A) followed by treatment T_5 (95 % NAC+5% B) (Table 37).

Based on t test, regarding storage periods, the hardness of all blended chocolates and controls (alkalised and non alkalised chocolates) significantly

increased at the sixth month of storage compared to initial hardness (Figure16).

The initial hardness of commercial chocolates dairy milk, dairy milk (silk) and For U (KAU chocolate) was 37.46N, 29.34N and 66.09N, respectively. At the end of storage the hardness was increased to 38.95N, 30.21N and 68.24N respectively (Table 35).

Initial cohesiveness of blended chocolates varied from 0.022N to 0.028N. Statistically the highest cohesiveness of 0.028N was observed in chocolates prepared with 95% non alkalised chocolates and 5% almond (T_5) and lowest (0.022N) in treatment T_0 (100% AC) and T_3 (95 % AC+5% WPP). Based on one way ANOVA, the treatments are significantly varied in cohesiveness, but all the treatments are on par with treatment T_1 (95 % AC+5% ODJ). Statistically the cohesiveness of blended chocolates significantly varied between the treatments (Table 36).

The maximum cohesiveness of 0.031N was recorded at the sixth month of storage in treatment T_5 (95 % NAC+5% A) followed by 0.029N in treatment T_4 - (95 % NAC+5% ODJ). The lowest cohesiveness of 0.025N was in treatment T_0 (100% AC) and T_3 (95 % AC+5% WPP). Based on t test, statistically the cohesiveness of blended chocolates are non significant during storage.

The initial cohesiveness of commercial chocolates was 0.021N, 0.012N and 0.015N and at the end of six month of storage cohesiveness increased to 0.023N, 0.014N and 0.017N in dairy milk, dairy milk (silk) and For U(KAUchocolates)respectively. From the figure 17 it is clear that the cohesiveness of blended chocolates and commercial chocolates increased with storage.

The initial adhesiveness of blended chocolates varied between 0.0017N and 0.0028N. The maximum adhesiveness of 0.0028N was recorded in chocolates prepared with 100% alkalised chocolates ie; T_0 (100% AC) and the lowest adhesiveness of 0.0017N recorded in treatment T_5 (95 % NAC+5% alomond)

followed by 0.0018N in treatment T_2 (95 % AC+5% B). Based on one way ANOVA, statistically significant difference was observed in adhesiveness between the treatments but treatment T_1 (95 % AC+5% ODJ) and T_4 (95 % NAC+5% ODJ) were significantly on par in adhesiveness.

At the end of sixth month of storage the highest adhesiveness was found to 0.0026N in treatment T_0 (100% AC) and the lowest adhesiveness of 0.0014N in treatment T_2 (95 % AC+5% almond) and T_5 (95 % NAC+5% almond). At the end of storage, based on one way ANOVA, statistically significant difference was recorded between the treatments but the treatments T_1 and T_4 were statistically on par and similarly T_2 and T_5 was also on par and T_3, T_0 (100% NAC) and T_6 was also statistically on par. The adhesiveness of blended chocolates decreased from the initial to the end of six month of storage (Figure 18). Regarding to storage, based on t test, no significant difference was observed in all treatments, except the treatment T_1 (95 % AC+5% ODJ) and T_4 (95 % NAC+5% ODJ).The adhesiveness of commercial chocolates also found to be reduced from 0.0035N, 0.0039N and 0.0031N to 0.0031N, 0.0035N and 0. 0029N in Dairy Milk, Dairy milk (silk) and For U (KAU chocolate) respectively.

The initial gumminess of blended chocolates varied from 1.8813N to 1.2401N. Statistically, treatment T_2 (95 % AC+5% A), T_3 (95 % AC+5% WPP), T_4 (95 % NAC+5% ODJ) and T_5 (95 % NAC+5% A) were on par with each other and superior in gumminess. The highest gumminess of 1.8813N was found to be in treatment T_4 (95 % NAC+5% ODJ) and lowest in treatment T_6 (95 % AC+5% WPP). At the end of storage the maximum gumminess of 1.9841 N was recorded in T_4 (95 % NAC+5% ODJ) followed by 1.9541N in T_5 (95 % NAC+5% A). The lowest gumminess of 1.3798 N was recorded in T_3 (95 % AC+5% WPP). Based on one way ANOVA, statistically significant difference was recorded in gumminess at sixth month of storage between the treatments. The gumminess of blended chocolates slightly increased at sixth month of storage (Figure19). Based on t test, at sixth month of storage, the gumminess of all blended chocolates was non

significant except the treatment T_0 (100% NAC and) T_4 (95 % NAC+5% ODJ).

The gumminess of commercial chocolates also increased from 1.198N, 1.123N and 1.238N to 1.289N, 1.254N and 1.299N in Dairy Milk, Dairy milk (silk) and For U (KAU chocolates)respectively. The chemical constituents of stored blended chocolates, analysed initially and monthly intervals for a periods of six months.

Table. 35. Hardness (N) of blended chocolates during storage

Treatments	Initial	6th Month	't' value
T_0 (100% AC)	76.71^{e}	79.37^{e}	7.270**
T_1 -(95 % AC+5% ODJ)	84.33^{b}	87.32^{ab}	5.872*
T_2 (95 % AC+5% A)	99.11^{a}	102.51^{a}	2.776*
T_3 (95 % AC+5% WPP)	81.75^{c}	84.88^{c}	2.776*
T_0 (100% NAC)	74.41^{f}	77.16^{f}	12.824*
T_4 -(95 % NAC+5% ODJ)	83.58^{b}	86.39^{b}	4.133**
T_5 (95 % NAC+5% A)	99.46^{a}	101.49^{a}	2.776**
T_6 (95 % AC+5% WPP)	80.16^{d}	83.55^{d}	4.328**
CV (%)	**0.673**	**0.819**	-
CD (0.05)	**0.990**	**1.245**	-
Dairy milk	37.46	38.95	-
Dairy milk (silk)	29.34	30.21	-
For U (KAU chocolate)	66.09	68.24	-

AC- Alkalised Chocolates, NAC – Non alkalised chocolates, ODJ – Osmodehydrated Jack fruit, A- almond, WPP- White Pepper Powder
DMRT column wise comparison
Figures with same superscripts no significant difference
t test row wise comparison
*- Significant at1% level
**- Significant at 5% level, NS-Not significant

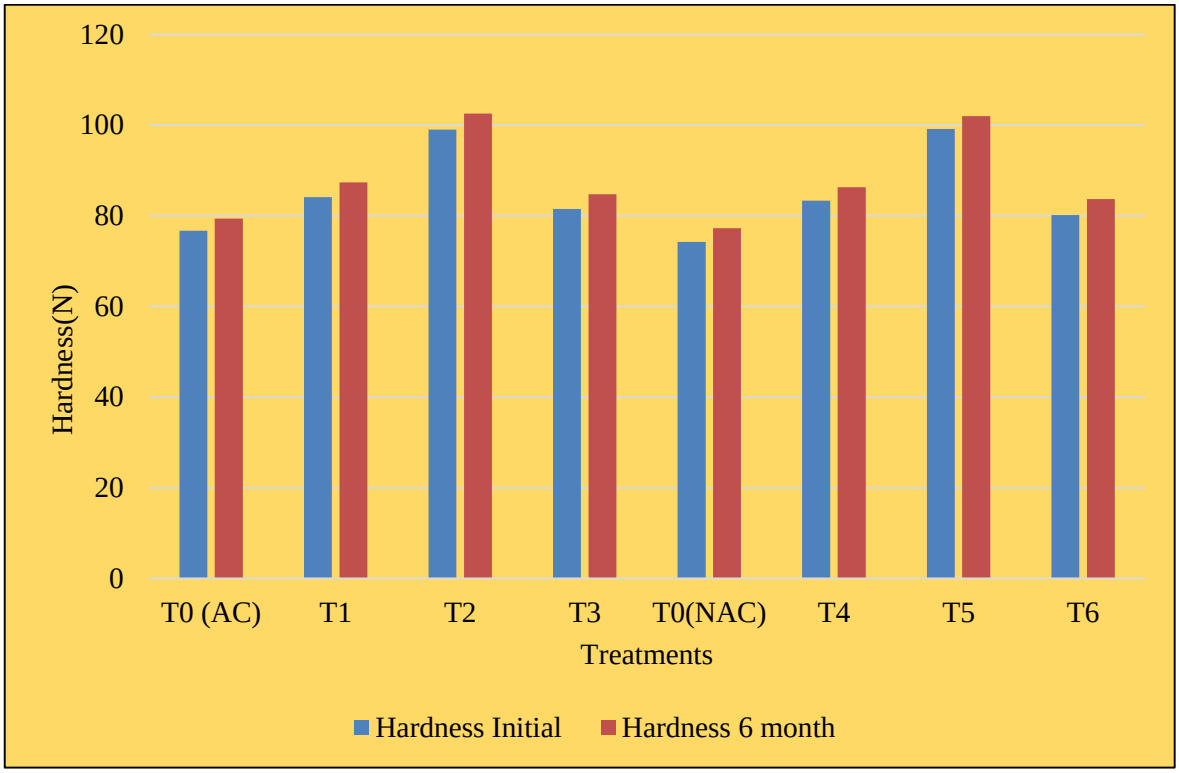


Fig.16. Hardness of blended chocolates during storage

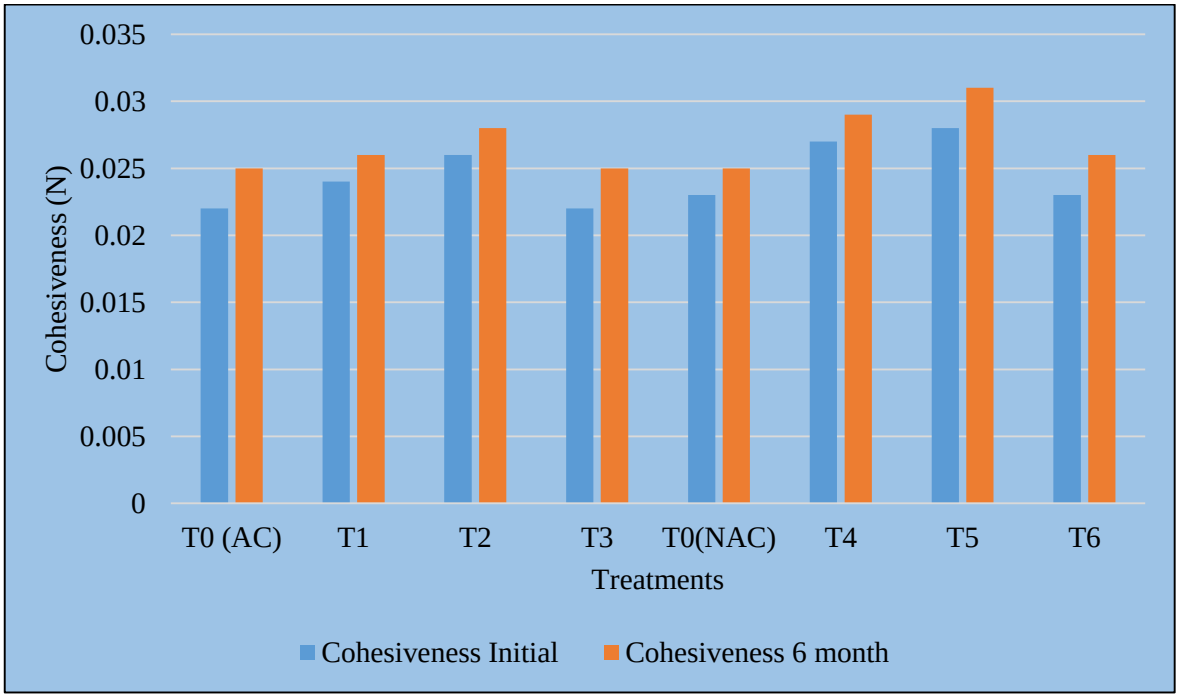


Fig.17. Cohesiveness of blended chocolates during storage

Table. 36. Cohesiveness (N) of blended chocolates during storage

Treatments	Initial	6th Month	't' value
T_0 (100% AC)	0.022^{c}	0.025^{c}	0.281^{NS}
T_1 -(95 % AC+5% ODJ)	0.024^{abc}	0.026^{bc}	2.449^{NS}
T_2 (95 % AC+5% A)	0.026^{a}	0.028^{bc}	0.658^{NS}
T_3 (95 % AC+5% WP)	0.022^{c}	0.025^{c}	2.530^{NS}
T_0 (100% NAC)	0.023^{bc}	0.025^{c}	2.530^{NS}
T_4 -(95 % NAC+5% ODJ)	0.027^{ab}	0.029^{ab}	1.890^{NS}
T_5 (95 % NAC+5% A)	0.028^{a}	0.031^{a}	2.776^{NS}
T_6 (95 % AC+5% WPP)	0.023^{bc}	0.026^{bc}	2.500^{NS}
CV (%)	**8.411**	**7.519**	-
CD(0.05)	**0.021**	**0.033**	-
Dairy milk	0.021	0.023	-
Dairy milk (silk)	0.012	0.014	-
For U (KAU chocolates)	0.015	0.017	-

AC- Alkalised Chocolates, NAC – Non alkalised chocolates, ODJ – Osmodehydrated Jack fruit, A-Almond, WPP- White Pepper Powder
DMRT column wise comparison
Figures with same superscripts no significant difference
t test row wise comparison
*- Significant at1% level
**- Significant at 5% level, NS-Not significant

Table.37 Adhesiveness (N) of blended chocolates during storage

Treatments	Initial	6th Month	't' value
T_0 (100% AC)	0.0028[a]	0.0026[a]	1.225 NS
T_1 -(95 % AC+5% ODJ)	0.0019[e]	0.0015[d]	4.899*
T_2 (95 % AC+5% A)	0.0018[f]	0.0014[e]	2.774 NS
T_3 (95 % AC+5% WPP)	0.0025[b]	0.0020[bc]	1.567 NS
T_0 (100% NAC)	0.0023[d]	0.0020[bc]	1.567 NS
T_4 -(95 % NAC+5% ODJ)	0.0019[e]	0.0015[d]	5.500*
T_5 (95 % NAC+5% A)	0.0017[g]	0.0014[e]	2.776 NS
T_6 (95 % AC+5% WPP)	0.0024[c]	0.0021[b]	1.492 NS
CV (%)	**7.344**	**14.828**	-
CD(0.05)	**0.000**	**0.000**	-
Dairy milk	0.0035	0.0031	-
Dairy milk (silk)	0.0039	0.0035	-
For U (KAU chocolates)	0.0031	0.0029	

AC- Alkalised Chocolates, NAC – Non alkalised chocolates, ODJ – Osmodehydrated Jack fruit, A-Almond, WPP- White Pepper Powder
DMRT column wise comparison
Figures with same superscripts no significant difference
t test row wise comparison
*- Significant aT1% level
**- Significant at 5% level, NS-Not significant

Table.38. Gumminess (N) of blended chocolates during storage

Treatments	Initial	6th Month	't' value
T_0 (100% AC)	1.3422^{cd}	1.4735^{cd}	1.990 NS
T_1 (95 % AC+5% ODJ)	1.7552^{b}	1.8666^{b}	1.687 NS
T_2 (95 % AC+5% A)	1.8490^{ab}	1.9286^{ab}	1.689 NS
T_3 (95 % AC+5% WPP)	1.2916^{ab}	1.3798^{de}	3.203 NS
T_0 (100% NAC)	1.3647^{cd}	1.4976^{c}	4.516**
T_4 -(95 % NAC+5% ODJ)	1.8813^{a}	1.9841^{a}	5.753*
T_5 (95 % NAC+5% A)	1.8697^{ab}	1.9541^{ab}	3.736 NS
T_6 (95 % AC+5% WPP)	1.2409^{d}	1.3418^{e}	1.193 NS
CV (%)	**4.424**	**3.928**	-
CD (0.05)	**0.121**	**0.011**	-
Dairy milk	1.1985	1.2897	-
Dairy milk (silk)	1.1232	1.2544	-
For U (KAU chocolates)	1.238	1.2999	-

AC- Alkalised Chocolates, NAC – Non alkalised chocolates, ODJ – Osmodehydrated Jack fruit, A-Almond, WPP- White Pepper Powder

DMRT column wise comparison

Figures with same superscripts no significant difference

t test row wise comparison

*- Significant at 1% level

**- Significant at 5% level, NS-Not significant

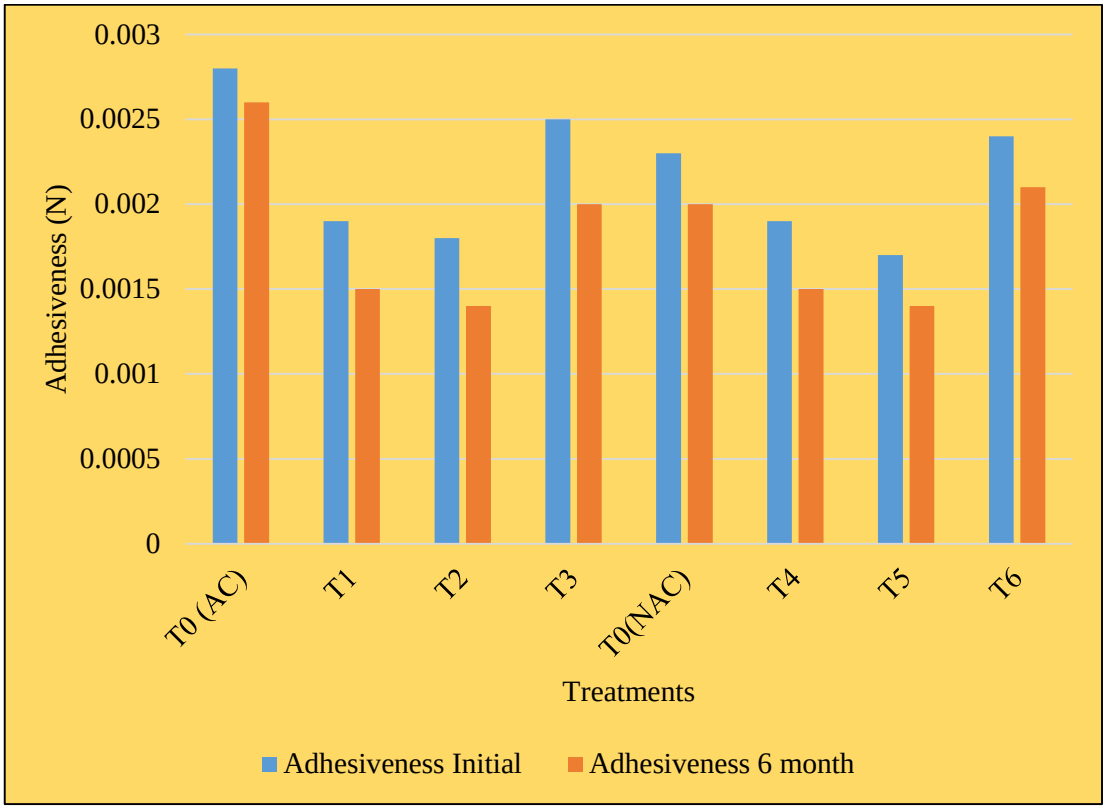


Fig.18. Adhesiveness of blended chocolates during storage

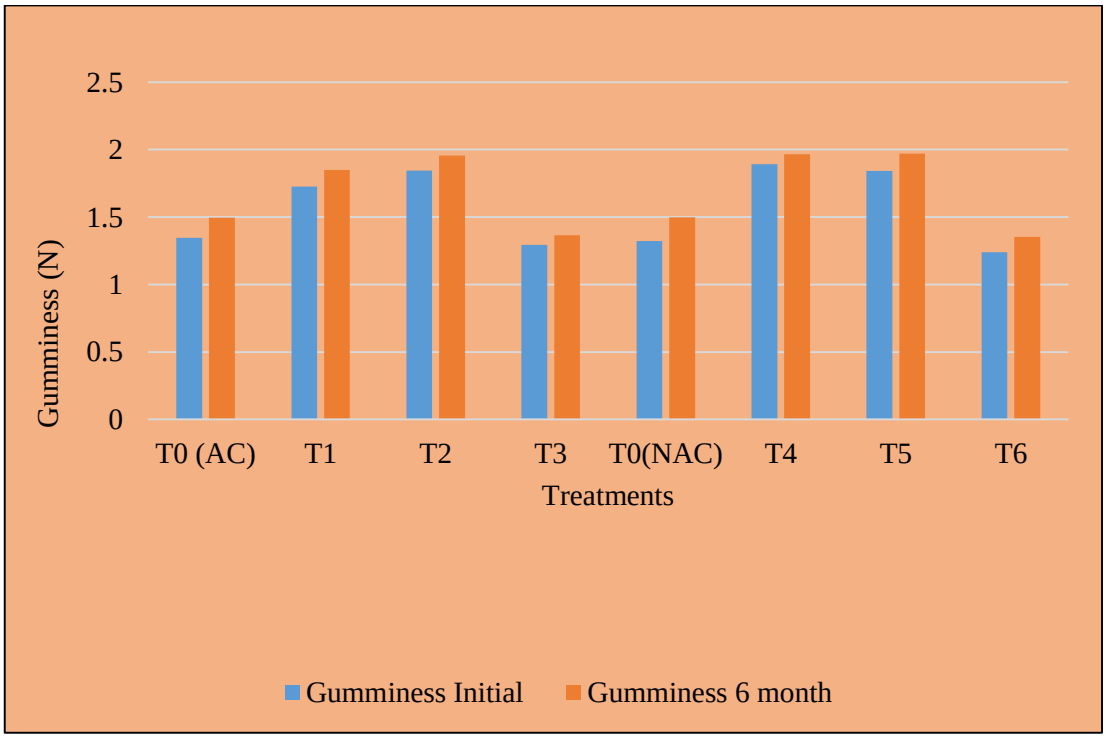


Fig.19. Gumminess of blended chocolates during storage

4.4.2.1.2. Moisture

As revealed in table 39, initially the moisture content in blended chocolates ranged between 1.16 to 1.24 per cent with highest moisture content of 1.24 per cent in treatment T_4 (95 % NAC+5% ODJ) and lowest (1.16 per cent) in T_2 (95 % AC+5% A). Based on DMRT, significant difference in moisture content was not observed between the treatments from initial up to fifth month of storage. During sixth month of storage the highest moisture content of 1.52 per cent was recorded in T_1 (95 % AC+5% ODJ) and T_4 (95 % NAC+5% ODJ) and lowest (1.43%) in T_2 (95 % AC+5% B). Statistically treatments, T_0 (100% AC), T_1 (95 % AC+5% ODJ), T_0 (100% NAC), T_4 (95 % NAC+5% ODJ) and T_5 (95 % NAC+5% B) were statistically on par, similarly treatments T_2 (95 % AC+5% A), T_3 (95 % AC+5% WPP) and T_6 (95 % AC+5% WPP) was also on par in moisture content.

Statistically significant increase in moisture content was recorded in blended chocolates during storage. Based on t test, the effect of storage on moisture content of blended chocolates in each treatment showed no significant difference up to two months of storage. After two months of storage, statistically significant increase in moisture content was observed in all treatments.

4.4.2.1.3. Energy

Based on DMRT, significant difference in energy content among blended chocolates was observed in all treatments with highest energy content (582.98K.cal) in treatment T_5 (95 % NAC+5% A) followed by 582.49 K.cal in treatment T_2 (95 % AC+5% A). Statistically T_2 and T_5, T_2 and T_4 T_1, T_3, T_4 and T_6 were on par in energy content. The lowest energy content (579.67K.cal) was recorded in treatment T_0 (100% AC) followed by 579.77Kcal in treatment T_0 (100% NAC). Decrease in energy content was recorded in all treatments during storage. At the end of sixth month of storage the maximum energy content of 580.15 Kcal was found to in treatment T_5 (95 % NAC+5% A) followed by T_2 (95 % AC+5% A). There was no significant difference in energy content in treatment T_2 and T_5

similarly in T_1 and T_4 and also in T_3 and T_6. The treatments T_0 (100% AC) and T_0 (100% NAC) was also statistically on par in energy content (Table 40).

Based on t test, no significant difference was observed in energy content of blended chocolates in each treatment up to three months of storage, but T_1 and T_4 was non significant up to fourth month of storage. During fourth month of storage up to sixth month of storage significant decrease in energy content was recorded in all treatments. But at the end of sixth month of storage the treatment T_2 and T_5 are superior in energy content. Effect of storage on energy content of blended chocolates is illustrated in figures 21.

4.4.2.1.4. TSS

The TSS content of blended chocolates are presented in table 41. Initially among treatments, the highest TSS content of $68B^0$was observed in chocolates blended with osmodehydrated jackfruit ie, in treatments T_1 (95 % AC+5% ODJ) and T_4 (95 % NAC+5% ODJ) and the TSS of all other blended chocolates are $67B^0$. Based on DMRT, statistically no significant difference was observed in TSS content of blended chocolates in all treatments initially and during six month of storage. There was an increase in TSS content of blended chocolates was recorded in all treatments during storage (Figure 22). During sixth month of storage, the highest TSS of $72B^0$was in treatments T_1 (95 % AC+5% ODJ). Based on t test, statistically with respect to storage periods no significant difference in TSS was observed up to forth month of storage. During fifth month of storage significant difference in TSS observed in all treatments except in T_2, T_3 and T_6. At the end of sixth month of storage significant difference in TSS was observed in all treatments compared to initial TSS.

Table. 39. Moisture content of blended chocolates during storage

Treatments	Moisture (%)						
	Initial	**1st Month**	**2nd month**	**3rd month**	**4th month**	**5th month**	**6th month**
T_0 (100% AC)	1.18 (6.24)	^{NS}1.19 (6.27)	^{NS}1.22 (6.34)	**1.30 (6.53)	**1.34 (6.64)	*1.42 (6.85)	*1.51^{ab} (7.05)
T_1 -(95 % AC+5% ODJ)	1.19 (6.27)	^{NS}1.21 (6.29)	^{NS}1.25 (6.47)	**1.31 (6.57)	*1.37 (6.73)	*1.44 (6.88)	*1.52^{a} (7.08)
T_2 (95 % AC+5% A)	1.16 (6.20)	^{NS}1.19 (6.22)	^{NS}1.22 (6.34)	**1.26 (6.43)	*1.31 (6.58)	*1.35 (6.66)	*1.43^{d} (6.86)
T_3 (95 % AC+5% WPP)	1.17 (6.20)	^{NS}1.19 (6.21)	^{NS}1.23 (6.34)	**1.27 (10.83)	*1.33 (6.22)	*1.38 (6.75)	*1.45^{bcd} (6.91)
T_0 (100% NAC)	1.23 (6.37)	^{NS}1.25 (6.38)	^{NS}1.26 (6.45)	**1.29 (5.34)	*1.36 (6.40)	*1.42 (6.84)	*1.51^{abc} (7.04)
T_4 -(95 % NAC+5% ODJ)	1.24 (6.38)	NS1.26 (6.39)	^{NS}1.29 (6.53)	*1.33 (6.61)	*1.39 (6.76)	*1.44 (6.90)	*1.52^{a} (7.08)
T_5 (95 % NAC+5% A)	1.19 (6.27)	^{NS}1.21 (6.29)	^{NS}1.26 (6.45)	*1.32 (6.59)	*1.36 (6.91)	*1.43 (6.86)	*1.50^{ab} (7.05)
T_6 (95 % AC+5% WPP)	1.2 (6.30)	^{NS}1.21 (6.32)	^{NS}1.25 (6.42)	*1.29 (6.53)	*1.38 (6.72)	*1.41 (6.81)	*1.45^{cd} (6.90)
CV (%)	1.404	2.249	1.014	4.312	1.260	1.469	1.136
CD(0.05)	NS	NS	NS	NS	NS	NS	0.138

AC- Alkalised Chocolates, NAC – Non alkalised chocolates, ODJ – Osmodehydrated Jack fruit, A-Almond, WPP- White Pepper Powder, Figures with same superscripts no significant difference, DMRT column wise comparison, t test row wise comparison

*- Significant at1% level, **- Significant at 5% level, NS-Not significan

Table. 40. Energy content of blended chocolates during storage

Treatments	Energy (K.cal)						
	Initial	**1st month**	**2 nd month**	**3rd month**	**4th month**	**5th month**	**6th month**
T_0 (100% AC)	579.67^{e}	^{NS}579.13^{c}	^{NS}578.75^{c}	^{NS}578.28^{d}	**577.37^{e}	**577.09^{c}	**576.74^{e}
T_1 (95 % AC+5% ODJ)	581.14^{c}	^{NS}580.98^{b}	^{NS}580.68^{ab}	^{NS}580.14^{bc}	^{NS}579.67^{bc}	**579.13^{ab}	**578.90^{b}
T_2 (95 % AC+5% A)	582.49^{ab}	^{NS}582.32^{a}	^{NS}581.65^{a}	^{NS}581.0^{ab}	*580.96^{a}	*580.33^{a}	*579.67^{a}
T_3 (95 % AC+5% WPP)	580.96^{cd}	^{NS}580.48^{b}	^{NS}579.67^{bc}	^{NS}579.19^{cd}	*578.65^{cd}	*578.18^{bc}	*577.55^{c}
T_0 (100% NAC)	579.77^{de}	^{NS}579.23^{c}	^{NS}578.88^{c}	^{NS}578.28^{d}	**577.44^{de}	**577.15^{c}	**576.82^{de}
T_4 -(95 % NAC+5% ODJ)	581.29^{bc}	^{NS}581.04^{b}	^{NS}580.99^{ab}	^{NS}580.26^{b}	^{NS}579.97^{ab}	**579.13^{ab}	**578.90^{b}
T_5 (95 % NAC+5% A)	582.98^{a}	^{NS}582.28^{a}	^{NS}581.73^{a}	^{NS}581.27^{a}	*580.94^{a}	*580.34^{a}	*580.15^{a}
T_6 (95 % AC+5% WPP)	580.33^{cde}	^{NS}580.07^{b}	^{NS}579.30	^{NS}579.15^{d}	*578.65^{cd}	*578.20^{bc}	*577.49^{cd}
CV (%)	0.121	0.119	0.133	0.098	0.124	0.121	0.073
CD(0.05)	1.219	1.199	1.334	0.981	1.242	1.215	0.728

AC- Alkalised Chocolates, NAC – Non alkalised chocolates, ODJ – Osmodehydrated
Jack fruit, A-Almond, WPP- White Pepper Powder
DMRT column wise comparison
Figures with same superscripts no significant difference
t test row wise comparison
*- Significant aT1% level**- Significant at 5% level, NS-Not significant

Table. 41. TSS of blended chocolates during storage

Treatments	TSS (^{0}Brix)						
	Initial	1st month	2nd month	3rd month	4th month	5th month	6th month
T_0 (100% AC)	67	^{NS}67	^{NS}68	^{NS}69	^{NS}69	**70	*71
T_1 (95 % AC+5% ODJ)	68	^{NS}68	^{NS}69	^{NS}69	^{NS}70	**71	*72
T_2 (95 % AC+5% A)	^{NS} 67	^{NS} 67	^{NS} 68	^{NS} 68	^{NS} 69	^{NS} 69	*70
T_3 (95 % AC+5% WPP)	^{NS} 67	^{NS} 67	^{NS} 68	^{NS} 68	^{NS} 68	^{NS} 69	*70
T_0 (100% NAC)	^{NS} 67	^{NS} 67	^{NS} 68	^{NS} 69	^{NS} 69	**70	*71
T_4 -(95 % NAC+5% ODJ)	^{NS} 68	^{NS} 68	^{NS} 69	^{NS} 69	^{NS} 70	**71	*71
T_5 (95 % NAC+5% A)	^{NS} 67	^{NS} 67	^{NS} 68	^{NS} 68	^{NS} 69	**70	*70
T_6 (95 % AC+5% WPP)	^{NS} 67	^{NS} 67	^{NS} 68	^{NS} 68	^{NS} 68	^{NS} 69	*70
CV (%)	1.108	1.487	1.406	1.471	1.457	1.439	1.416
CD (0.05)	NS	NS	NS	NS	NS	NS	NS

AC- Alkalised Chocolates, NAC – Non alkalised chocolates, ODJ – Osmodehydrated Jack fruit, A-Almond, WPP- White Pepper Powder

DMRT column wise comparison

Figures with same superscripts no significant difference

t test row wise comparison

*- Significant at1% level

**- Significant at 5% level, NS-Not significant

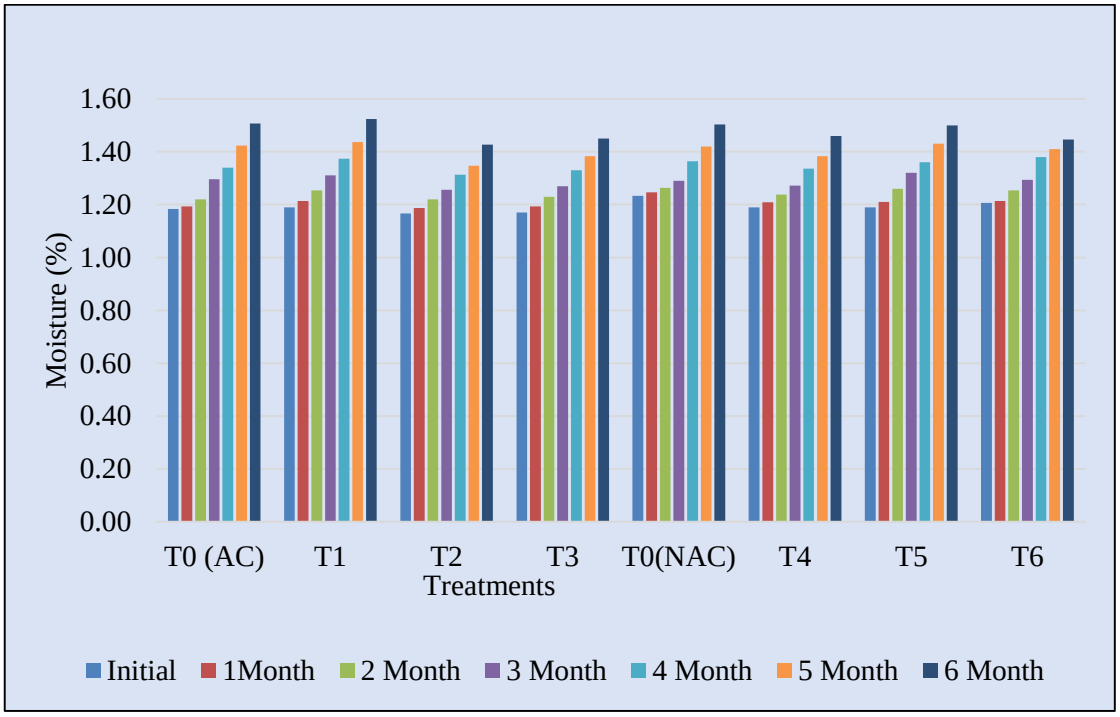


Fig.20. Moisture content of blended chocolates during storage

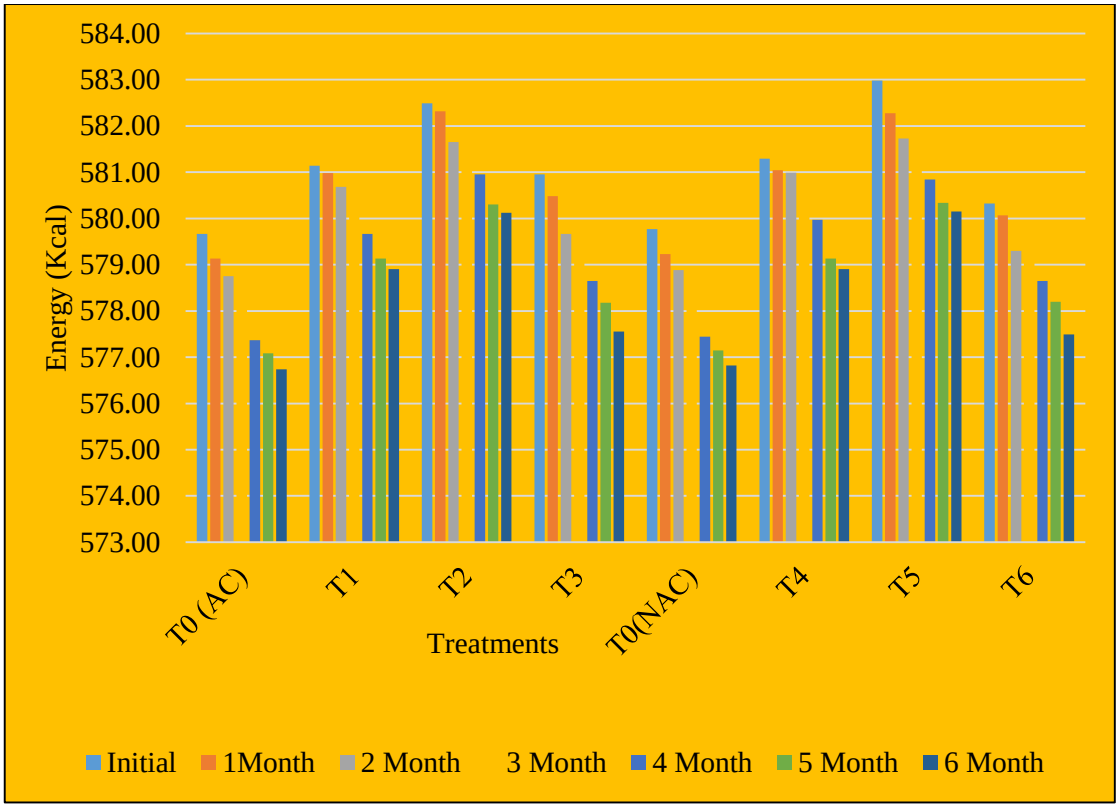


Fig.21. Energy content of blended chocolates during storage

4.4.2.1.5. Total sugar

Initially the total sugar content of blended chocolates varied from 40.23 g/100gm to 41.59 g/100gm with the lowest in treatment T_6 (95 % AC+5% WPP) and highest in treatment T_1 (95 % AC+5% ODJ). Based on DMRT, treatment wise there was no significant difference was observed among the blended chocolates in total sugar content during each month of storage (Table 42). Regarding storage periods, t test shows statistically significant increase in total sugar content of blended chocolates during storage periods, but from initial to second month of storage no significant variation was observed in total sugar content of blended chocolates. From third month to sixth month of storage statistically significant difference was recorded in all treatments due to storage. At the end of sixth month of storage the maximum total sugar content of 47.81g/100gm was recorded in T_1 (95 % AC+5% ODJ) followed by 47.74g/100gm in T_4 (95 % NAC+5% ODJ). From initial to sixth month of storage the maximum total sugar was in T_1 (95 % AC+5% ODJ). Effect of storage on total sugar content of blended chocolates is illustrated in figures 23.

4.4.2.1.6. Reducing sugar

The reducing sugar content of various treatments during six month of storage is furnished in Table 43. Initially based on one way ANOVA, statistically on par the treatments T_1 (95 % AC+5% ODJ) and T_4 (95 % NAC+5% ODJ) in reducing sugar content and similarly all other treatments are also non significant. Initially the reducing sugar content of blended chocolates varied from 5.23g/100g to 6.35 g/100g. The highest reducing sugar content among all treatment from initial to the end of six months of storage was recorded in T_1 which is reduced from 6.35g/100g to 5.60g/100g. The treatments T_1 and T_4 are superior in reducing sugar content at sixth month of storage. Among the blended chocolates, the lowest reducing content of 4.30g/100g was observed in T_2 (95 % AC+5% A) at the end of storage (Table 43).

Table. 42. Total sugar of blended chocolates during storage

Treatments	Total sugar(g/100gm)						
	Initial	1st month	2nd month	3rd month	4th month	5th month	6th month
T_0 (100% AC)	41.52	^{NS} 42.63	^{NS} 44.14	**45.25	**46.45	*47.02	*47.21
T_1 (95 % AC+5% ODJ)	41.59	^{NS} 42.45	^{NS} 43.71	**44.18	*46.26	*47.05	*47.81
T_2 (95 % AC+5% A)	40.56	^{NS} 42.38	^{NS} 43.66	**44.06	**45.56	*46.84	*47.15
T_3 (95 % AC+5% WPP)	40.38	^{NS} 42.30	^{NS} 43.54	**44.07	* 45.53	*46.86	*47.09
T_0 (100% NAC)	41.49	^{NS} 42.58	^{NS} 44.26	**45.36	** 46.51	*47.07	*47.25
T_4(95% NAC+5% ODJ)	41.58	^{NS} 42.48	^{NS} 43.90	**44.17	** 46.27	*47.17	*47.74
T_5(95% NAC+5% A)	40.64	^{NS} 42.29	^{NS} 43.56	**44.17	** 45.59	*46.82	*47.15
T_6 (95 % AC+5% WPP)	40.23	^{NS} 42.49	^{NS}43.43	^{NS} 44.39	** 45.57	*46.70	*47.05
CV (%)	2.277	^{NS} 2.457	2.244	2.250	2.262	2.163	2.212
CD (0.05)	NS	NS	NS	NS	NS	NS	NS

AC- Alkalised Chocolates, NAC Non alkalised chocolates, ODJ Osmodehydrated Jack fruit, A-Almond, WPP- White Pepper Powder
DMRT column wise comparison
Figures with same superscripts no significant difference
t test row wise comparison
*- Significant at 1% level
**- Significant at 5% level, NS-Not significant

A decreasing in reducing sugar content was observed in all treatments during storage (Figure 24). Based on t test, no significant difference was observed up to third month of storage in all treatments except T_4 (95 % NAC+5% ODJ). From forth month onwards significant decrease in reducing sugar content was noticed in all treatments except in T_0 (100% AC), T_1 (95 % AC+5% ODJ) and T_3 (95 % AC+5% WPP) at fourth month of storage. During fifth and sixth month of storage significant difference was recorded in all treatments.

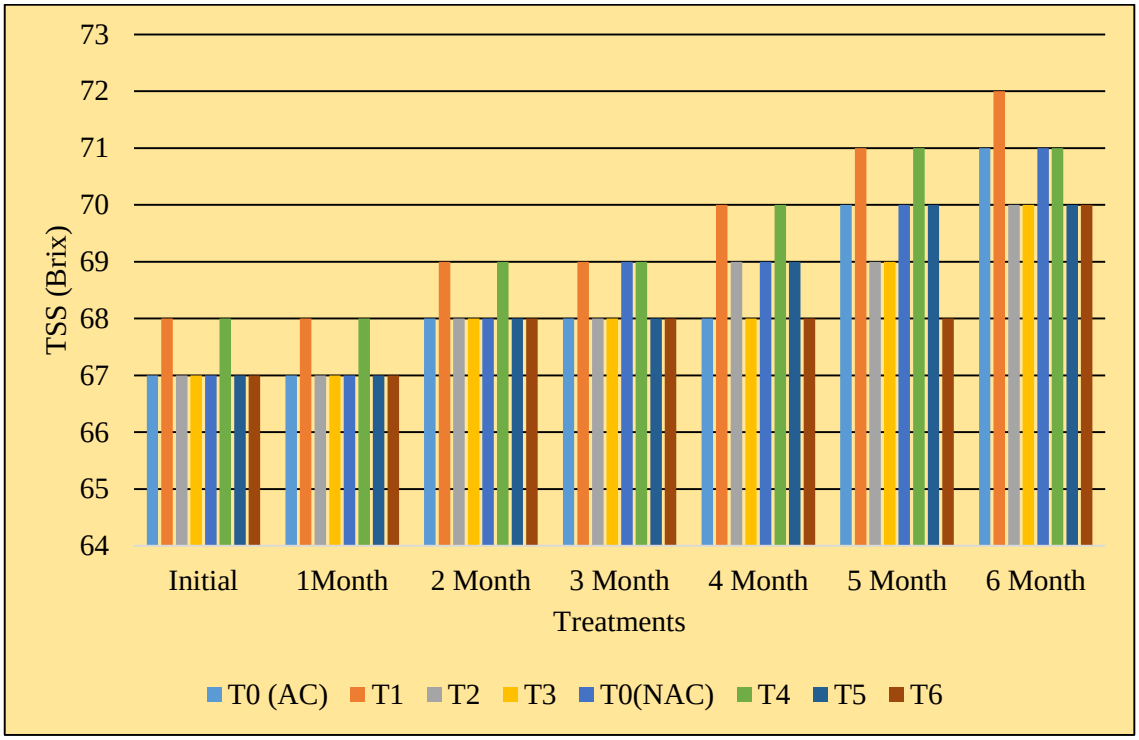


Fig.22.TSS content of blended chocolates during storage

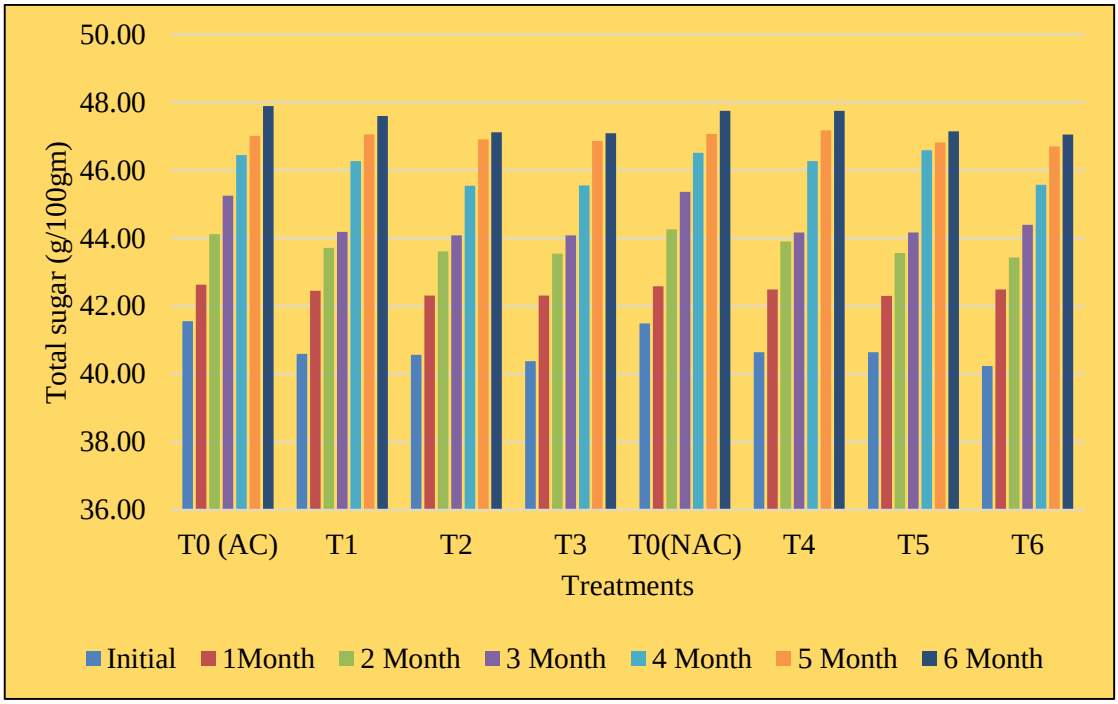


Fig.23.Total sugar content of blended chocolates during storage

4.4.2.1.7. Protein

Initially and during storage, the protein content of blended chocolates is represented in Table 44. Initially the maximum protein content (7.74 g/100g) was noticed in treatment T_2 (95 % AC+5% A) and T_5 (95 % NAC+5% A) and lowest (7.31g/100g) in T_0 (100% AC). Based on one way ANOVA, initially and till the end of second month of storage no significant difference was noticed between the treatments. But from third month to sixth of storage a significant difference was noticed in protein content of blended chocolates among the treatments. Statistically based on one way ANOVA the treatments T_2 and T_5 was on par and treatments T_0 (alkalised), T_1, T_3, T_0 (non alkalised), T_4 and T_6 was also on par in protein content from third month to sixth month of storage.

Based on t test, effect of storage on protein content of blended chocolates in each treatment showed no significant difference up to third month of storage. A decreasing trend in protein content was noticed in all treatments during storage (Figure 25). After third months of storage significant decrease in protein content was observed in all treatments, but the treatments T_0 (alkalised), T_2 and T_1 are non significant throughout the storage periods. The chocolates blended with almond (T_2 and T_5) are superior in protein content throughout the storage periods.

Table. 43. Reducing sugar of blended chocolates during storage

Treatments	Protein (g/100gm)						
	Initial	1st month	2nd month	3rd month	4th month	5th month	6th month
T_0 (100% AC)	7.31 (15.68)	^{NS}7.24 (15.48)	^{NS} 7.22 (15.683)	^{NS} 7.11^{b} (15.44)	^{NS} 7.09^{b} (15.442)	^{NS}7.01^{bc} (15.39)	^{NS} 6.97^{ab} (15.30)
T_1 (95 % AC+5% ODJ)	7.35 (15.73)	^{NS} 7.31 (15.67)	^{NS} 7.27 (15.68)	^{NS} 7.16^{b} (15.44)	^{NS} 7.09^{b} (15.442)	^{NS} 6.91^{b} (15.501)	^{NS} 6.88^{bc} (15.564)
T_2 (95 % AC+5% A)	7.74 (16.16)	^{NS} 7.69 (15.42)	^{NS} 7.64 (15.87)	^{NS} 7.59^{a} (15.91)	^{NS} 7.51^{a} (15.912)	^{NS} 7.47^{a} (15.911)	^{NS}7.44^{a} (15.524)
T_3 (95 % AC+5% WPP)	7.49 (15.87)	^{NS} 7.40 (16.38)	^{NS} 7.33 (15.54)	^{NS} 7.24^{b} (15.49)	**7.12^{b} (15.472)	*6.95^{bc} (15.438)	*6.87^{bc} (15.37)c
T_0 (100% NAC)	7.39 (15.77)	^{NS} 7.33 (15.27)	^{NS} 7.24 (15.34)	^{NS}7.12^{b} (15.28)	*6.95^{b} (15.225)	*6.86^{c} (15.472)	*6.78 ^{c} (15.68)
T_4 (95 % NAC+5% ODJ)	7.42 (15.80)	^{NS} 7.38 (15.694)	^{NS} 7.29 (15.64)	^{NS} 7.20^{b} (15.50)	**7.14^{b} (15.57)	*7.02^{bc} (15.43)	*6.98^{ab} (15.21)
T_5 (95 % NAC+5% A)	7.74 (16.16)	^{NS} 7.65 (16.96)	^{NS} 7.63 (15.98)	^{NS} 7.58^{a} (15.91)	**7.51^{a} (15.89)	**7.47^{a} (15.89)	**7.40^{a} (15.821)
T_6 (95 % AC+5% WPP)	7.50 (15.90)	^{NS} 7.44 (15.75)	^{NS} 7.36 (15.78)	^{NS} 7.26^{b} (15.43)	**7.11^{b} (15.54)	*6.98^{bc} (15.48)	*6.89^{bc} (15.32)
CV (%)	1.307	1.243	1.430	1.533	0.803	0.951	1.078
CD (0.05)	NS	NS	NS	0.308	0.225	0.255	0.272

AC- Alkalised Chocolates, NAC – Non alkalised chocolates, ODJ – Osmodehydrated Jack fruit, A-Almond, WPP- White Pepper Powder

DMRT column wise comparison

Figures with same superscripts no significant difference

t test row wise comparison

*- Significant at 1% level

Table. 44. Protein content of blended chocolates during storage

Treatments	Reducing sugar (g/100gm)						
	Initial	1st month	2nd month	3rd month	4th month	5th month	6th month
T_0 (100% AC)	5.29^{b}	$^{NS}5.17^{bc}$	$^{NS}5.12^{bc}$	$^{NS}5.09^{bc}$	$^{NS}4.86^{b}$	$*4.79^{bc}$	$*4.54^{b}$
T_1 (95 % AC+5% ODJ)	6.35^{a}	$^{NS}6.21^{a}$	$^{NS}6.10^{a}$	$^{NS}6.04^{a}$	$^{NS}5.84^{a}$	$*5.72^{a}$	$*5.60^{a}$
T_2 (95 % AC+5% A)	5.23^{b}	$^{NS}5.12^{bc}$	$^{NS}4.91^{bc}$	$^{NS}4.81^{bcd}$	$**4.65^{b}$	$**4.45^{c}$	$**4.30^{b}$
T_3 (95 % AC+5% WPP)	5.24^{b}	$^{NS}5.12^{bc}$	$^{NS}4.90^{bc}$	$^{NS}4.73^{cd}$	$^{NS}4.61^{b}$	$** 4.47^{c}$	$*4.45^{b}$
T_0 (100% NAC)	5.48^{b}	$^{NS}5.38^{b}$	$^{NS}5.22^{b}$	$^{NS}5.12^{b}$	$**5.02^{b}$	$*4.92_{b}$	$**4.79^{b}$
T_4 -(95 % NAC+5% ODJ)	6.31^{a}	$^{NS}6.17^{a}$	$^{NS}5.16^{b}$	$*5.06^{bc}$	$*4.83^{b}$	$*4.71^{bc}$	$*4.52^{b}$
T_5 (95 % NAC+5% A)	5.23^{b}	$^{NS}5.06^{c}$	$^{NS}4.82^{c}$	$^{NS}4.60^{d}$	$**4.55^{b}$	$*4.44^{c}$	$*4.34^{b}$
T_6 (95 % AC+5% WPP)	5.35^{b}	$^{NS}5.16^{bc}$	$^{NS}4.91^{b}$	$^{NS}4.76^{bcd}$	$**4.70^{b}$	$**4.50^{bc}$	$*4.36^{b}$
CV (%)	3.022	1.682	1.870	2.231	2.896	2.776	3.610
CD (0.05)	0.7132	0.392	0.426	0.500	0.639	0.604	0.774

AC- Alkalised Chocolates, NAC – Non alkalised chocolates, ODJ – Osmodehydrated Jack fruit, A-Almond, WPP- White Pepper Powder

DMRT column wise comparison

Figures with same superscripts no significant difference

t test row wise comparison *- Significant at 1% level, **- Significant at 5% level, NS-Not significant

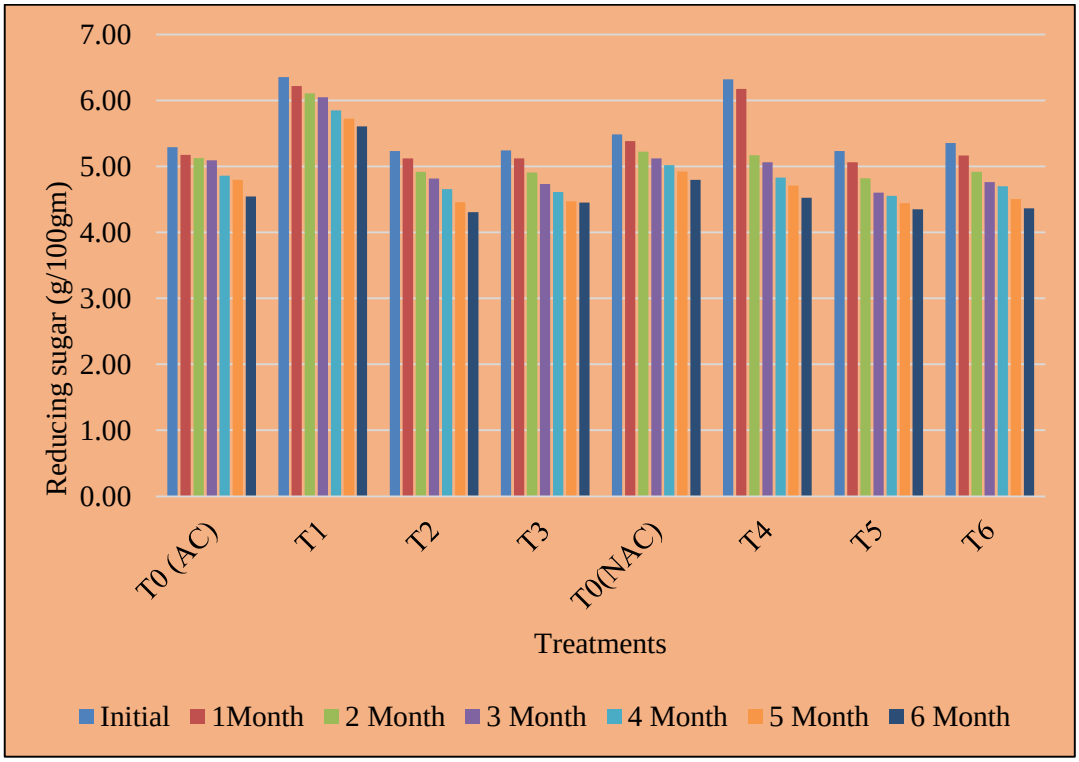


Fig.24.Reducing sugar content of blended chocolates during storage

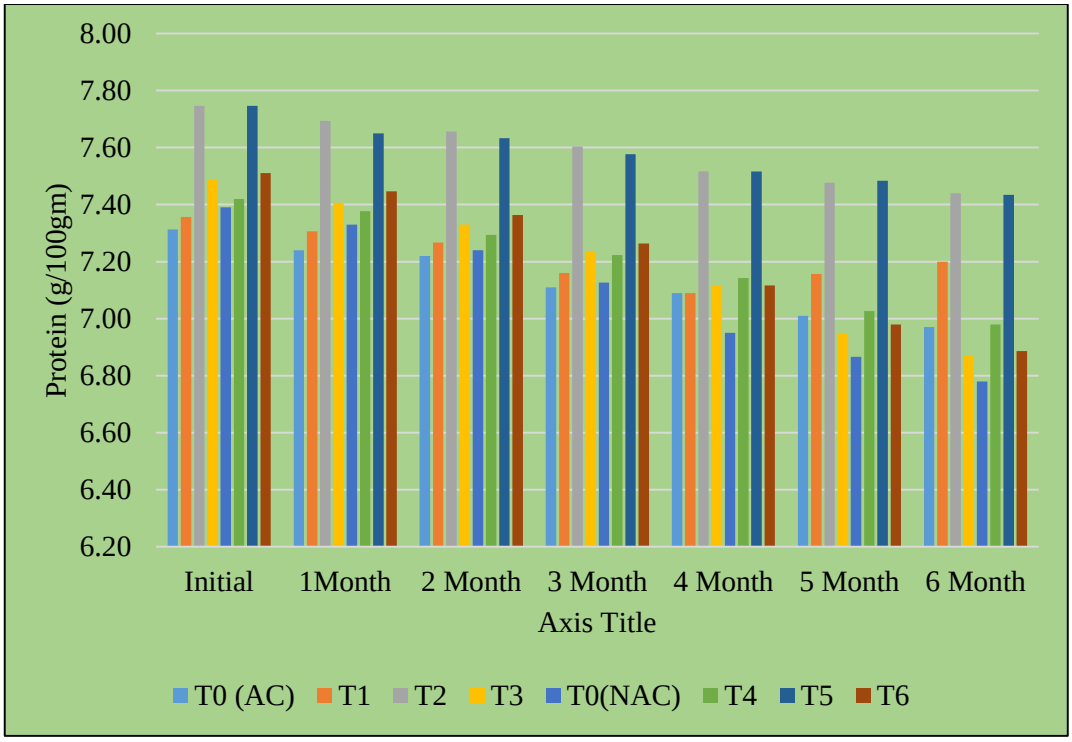


Fig.25.Protein content of blended chocolates during storage

4.4.2.1.8. Fat

The fat content of various blended chocolates initially and during each month of storage was interpreted using one way ANOVA and two sample t test (Table 45). Initially, based on one way ANOVA, the treatment T_5 (95 % NAC+5% A) had significantly high fat (48.35 %) content and T_2 (95 % AC+5% A) was found to be on par with T_5. The lowest (46.40%) fat content was recorded in T_4 (95 % NAC+5% ODJ) and was found to be on par with T_1 (95 % AC+5% ODJ). A steady decrease in fat content during storage was observed among all treatments (Figure 26). Based on one way ANOVA, the fat content was significantly varied among the treatments throughout the storage periods. Regarding to storage periods, t test shows no significant difference was observed in fat content among all treatments from initial to sixth month of storage, except in T_0 (100% AC) at fifth and sixth month of storage. The treatments T_2 and T_5 are superior in fat content throughout the storage periods.

4.4.2.1.9. Total polyphenol

The total polyphenol content of blended chocolates are presented in Table 46 and Figure 26.The initial polyphenol content of different blended chocolates varied from 0.18g/100g to 23g/100g with highest in treatment T_0 (100% NAC) and lowest in treatment T_3 (95 % AC+5% WPP). Based on one way ANOVA, initially and throughout the storage periods significant difference was observed among the treatments, but from initial to second month of storage, the treatment T_0 (100% AC), T_1, T_2 and T_3 was on par and similarly T_0 (100% NAC), T_4 and T_5 was also statistically on par. A decreasing in poly phenol content with advancement of storage periods was observed in all treatments. Among the treatments the highest polyphenol content ranging from 0.23g/100g to 0.19g/100g was seen in chocolates prepared with hundred per cent nonalkalised chocolates (T_0 -100% NAC) throughout the storage periods. With regards to periods of storage, t test shows no statistical difference in polyphenol content in all treatments up to third month of storage. At the fourth month of storage no significant difference was observed in all treatments except T_4 and T_5.

Table. 45. Fat content of blended chocolates during storage

Treatments	Fat (g/100gm)						
	Initial	**1st month**	**2nd month**	**3rd month**	**4th month**	**5th month**	**6th month**
T_0 (100% AC)	47.56^{ab}	$^{NS}47.42^{ab}$	$^{NS}47.30^{ab}$	$^{NS}47.20^{ab}$	$^{NS}46.58^{b}$	$^{**}46.36^{abc}$	$^{**}46.10^{b}$
T_1 (95 % AC+5% ODJ)	46.50^{b}	$^{NS}46.35^{b}$	$^{NS}46.10^{c}$	$^{NS}45.80^{c}$	$^{NS}45.72^{c}$	$^{NS}45.45^{c}$	$^{NS}45.26^{c}$
T_2 (95 % AC+5% A)	48.28^{a}	$^{NS}48.24^{a}$	$^{NS}48.09^{a}$	$^{NS}47.80^{a}$	$^{NS}47.60^{ab}$	$^{NS}47.40^{ab}$	$^{NS}47.30^{a}$
T_3 (95 % AC+5% WPP)	$^{NS}47.45^{ab}$	$^{NS}47.28^{ab}$	$^{NS}47.00^{bc}$	$^{NS}46.85^{abc}$	$^{NS}46.50^{b}$	$^{NS}46.20^{bc}$	$^{NS}46.00^{bc}$
T_0 (100% NAC)	$^{NS}47.80^{a}$	$^{NS}47.60^{ab}$	$^{NS}47.37^{ab}$	$^{NS}47.10^{abc}$	$^{NS}46.88^{bc}$	$^{NS}46.60^{abc}$	$^{NS}46.40^{b}$
T_4 -(95 % NAC+5% ODJ)	$^{NS}46.40^{b}$	$^{NS}46.38^{b}$	$^{NS}46.20^{c}$	$^{NS}46.10^{bc}$	$^{NS}45.91^{c}$	$^{NS}45.76^{c}$	$^{NS}45.61^{c}$
T_5 (95 % NAC+5% A)	$^{NS}48.31^{a}$	$^{NS}48.32^{a}$	$^{NS}48.20^{a}$	$^{NS}48.05^{a}$	$^{NS}47.80^{a}$	$^{NS}47.65^{a}$	$^{NS}47.50^{a}$
T_6 (95 % AC+5% WPP)	$^{NS}47.50^{ab}$	$^{NS}47.47^{ab}$	$^{NS}47.26^{ab}$	$^{NS}47.06^{abc}$	$^{NS}46.90^{bc}$	$^{NS}46.77^{a}$	$^{NS}46.60^{b}$
CV (%)	1.508	1.436	1.284	1.651	1.761	1.605	1.832
CD (0.05)	1.239	1.157	1.049	1.344	1.138	1.296	1.254

AC- Alkalised Chocolates, NAC – Non alkalised chocolates, ODJ – Osmodehydrated Jack fruit, A- Almond, WPP- White Pepper Powder

DMRT column wise comparison

Figures with same superscripts no significant difference

t test row wise comparison

*- Significant at 1% level **- Significant at 5% level, NS-Not significant

Table. 46. Poly phenol content of blended chocolates during storage

Treatments	Polyphenol (g/100gm))						
	Initial	1st month	2nd month	3rd month	4th month	5th month	6th month
T_0 (100% AC)	0.19^{c}	$^{NS}0.19^{bc}$	$^{NS}0.19^{bc}$	^{NS} 0.18cd	$^{NS}0.18^{bcd}$	$^{NS}0.18^{bc}$	$^{NS}0.17^{bc}$
T_1 (95 % AC+5% ODJ)	0.18^{c}	$^{NS}0.18^{c}$	^{NS} 0.18c	^{NS} 0.17d	$^{NS}0.17^{bcd}$	$^{NS}0.16^{d}$	$^{NS}0.16^{c}$
T_2 (95 % AC+5% A)	0.19^{c}	$^{NS}0.18^{c}$	$^{NS}0.18^{c}$	^{NS} 0.18cd	$^{NS}0.17^{d}$	$*0.17^{cd}$	$**0.16^{c}$
T_3 (95 % AC+5% WPP)	0.18^{c}	$^{NS}0.18^{c}$	$^{NS}0.18^{c}$	^{NS} 0.18cd	$^{NS}0.17^{d}$	$^{NS}0.17^{cd}$	$**0.16^{c}$
T_0 (100% NAC)	0.23^{a}	$^{NS}0.23^{a}$	$^{NS}0.22^{a}$	^{NS} 0.22a	$^{NS}0.22^{a}$	$*0.20^{a}$	$*0.19^{a}$
T_4 -(95 % NAC+5% ODJ)	0.22^{ab}	$^{NS}0.22^{b}$	$^{NS}0.22^{a}$	^{NS} 0.21ab	$**0.20^{b}$	$*0.20^{a}$	$*0.19^{ab}$
T_5 (95 % NAC+5% A)	0.22^{ab}	$^{NS}0.21^{ab}$	$^{NS}0.20^{abc}$	^{NS} 0.20a	$**0.19^{ab}$	$**0.19^{ab}$	$*0.18^{ab}$
T_6 (95 % AC+5% WPP)	0.21^{b}	$^{NS}0.21^{ab}$	$^{NS}0.20^{abc}$	^{NS} 0.20a	$^{NS}0.19^{ab}$	$**0.19^{ab}$	$*0.18^{ab}$
CV (%)	3.267	5.793	5.793	5.206	6.104	4.494	4.570
CD (0.05)	0.013	0.014	0.015	0.017	0.024	0.014	0.014

AC- Alkalised Chocolates, NAC – Non alkalised chocolates,
ODJ – Osmo dehydrated Jack
*- Significant at1% level
**- Significant at 5% level, NS-Not significant
A-Almond, WPP- White Pepper Powder
DMRT column wise comparison
Figures with same superscripts no significant difference
t test row wise comparison

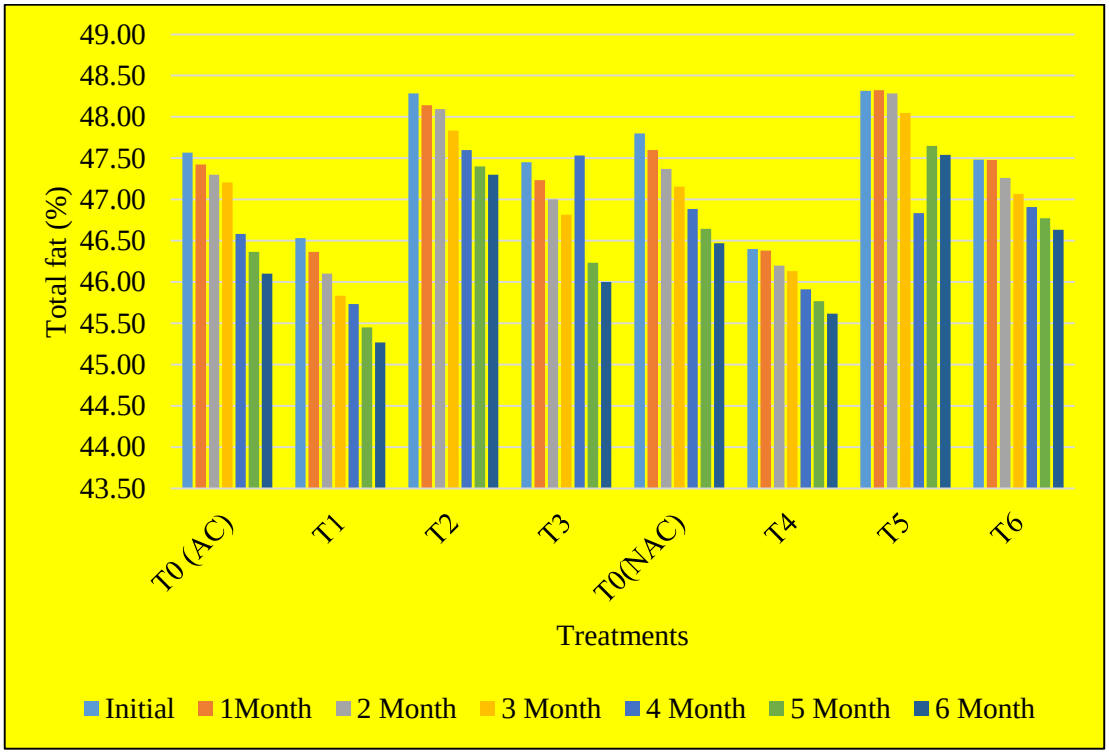


Fig.26.Fat content of blended chocolates during storage

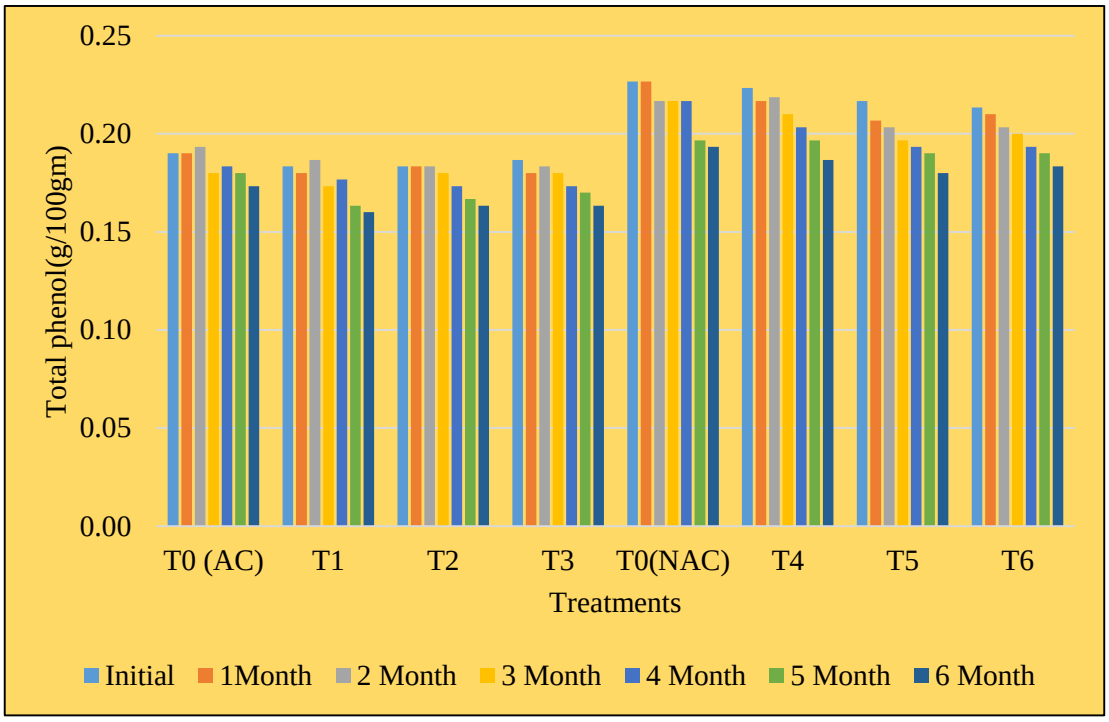


Fig.27.Total phenol content of blended chocolates during storage

But in the fifth and sixth month highly significant difference was observed in poly phenol content in all treatments except T_0 (100% AC), T_1 and T_3 in fifth month and T_0 (100% AC) and T_1 in sixth month of storage.

4.4.2.1.10. Total ash

The total ash content in blended chocolates is given in Table 47. Initially, the total ash content of blended chocolates are varied from 1.47g/100g to 1.81g/100g with highest in treatment T_3 (95 % AC+5% WPP) and lowest in T_0 in treatment (100% AC). Based on one way ANOVA the treatments T_1, T_2, T_3, T_4, T_5 and T_6 are statistically on par and the controls T_0 (100% AC) and T_0 (100% NAC) was also on par in total ash content. Statistically significant decrease was observed in total ash content of blended chocolates during storage periods (Figure 27). Statistically significant difference was observed between the treatments during storage. At sixth month of storage the maximum ash content (1.67g/100g) was in T_3 (95 % AC+5% WPP) and T_6 (95 % NAC+5% WPP).

With respect to storage periods based on t test, no significant difference in total ash content was recorded in all blended chocolates up to third month of storage. But in fourth and fifth month of storage, significant difference was noticed in ash content in treatments T_1, T_4, T_5 and T_6. During sixth month of storage statistically significant difference was observed in all treatments.

4.4.2.1.11. Calcium

Initially and during storage the calcium content of blended chocolates are presented in Table 48 and Figure 27. Initially, the calcium content of blended chocolates varied from 0.53g/100g to 0.80g/100g with lowest in T_0 (100% AC) and highest in T_0 (100% AC) and T_5 (95 % NAC+5% A) and showed statistically significant difference among the treatments. After storage, a reduction in calcium content was observed in all treatments and at the end of sixth month calcium content varied from 0.39g/100g to 0.63g/100g, lowest in T_0 (100% AC) and T_0 (100% NAC) and highest in T_5 (95 % NAC+5% A).

During storage, t test shows, no significant difference up to second month of storage in all treatments. After third month of storage to sixth month of storage, significantly different variations was found in calcium content in all treatments except in T_0 (100% AC) during third and fourth month of storage.

Table. 47. Total ash content of blended chocolates during storage

Treatments	Total ash (g/100gm))						
	Initial	1st month	2nd month	3rd month	4th month	5th month	6th month
T_0 (100% AC)	1.47^{b}	^{NS} 1.45^{b}	^{NS} 1.43^{c}	^{NS} 1.43^{b}	^{NS} 1.38^{c}	^{NS} 1.37^{c}	$^{**}1.34^{d}$
T_1 (95 % AC+5% ODJ)	1.75^{a}	^{NS} 1.74^{a}	^{NS} 1.69^{b}	^{NS} 1.67^{a}	$^{**}1.63^{b}$	$^{**}1.62^{b}$	$^{**}1.58^{c}$
T_2 (95 % AC+5% A)	1.74^{a}	^{NS} 1.74^{a}	^{NS} 1.73^{ab}	^{NS} 1.69^{a}	^{NS} 1.66^{ab}	$^{NS}1.63^{ab}$	$^{**}1.59^{c}$
T_3 (95 % AC+5% WPP)	1.81^{a}	$^{NS}1.80^{a}$	^{NS} 1.78^{a}	^{NS} 1.74^{a}	^{NS} 1.71^{a}	^{NS} 1.67^{ab}	$^{**}1.67^{a}$
T_0 (100% NAC)	1.48^{b}	^{NS} 1.43^{b}	^{NS} 1.43^{c}	^{NS} 1.43^{b}	^{NS} 1.38^{c}	^{NS} 1.37^{c}	$^{**}1.35^{d}$
T_4 (95 % NAC+5% ODJ)	1.74^{a}	^{NS} 1.73^{a}	^{NS} 1.71^{b}	^{NS} 1.67^{a}	$^{*}1.62^{b}$	$^{*}1.62^{b}$	$^{*}1.60^{bc}$
T_5 (95 % NAC+5% A)	1.78^{a}	^{NS} 1.75^{a}	^{NS} 1.73^{ab}	$^{NS}1.71^{a}$	$^{*}1.68^{ab}$	$^{*}1.65^{ab}$	$^{*}1.63^{abc}$
T_6 (95 % AC+5% WPP)	1.80a	^{NS} 1.80^{a}	^{NS} 1.78^{a}	^{NS} 1.73^{a}	$^{**}1.71^{a}$	$^{*}1.70^{a}$	* 1.67^{a}
CV (%)	3.146	2.953	2.327	3.069	2.493	2.514	2.472
CD (0.05)	0.092	0.086	0.067	0.087	0.069	0.069	0.066

AC- Alkalised Chocolates, NAC – Non alkalised chocolates, ODJ – Osmodehydrated Jack fruit A-Almond, WPP- White Pepper Powder

DMRT column wise comparison, t test row wise comparison*- Significant at1% level **- Significant at 5% level, NS-Not significant

Figures with same superscripts no significant difference

Table. 48. Calcium content of blended chocolates during storage

Treatments	Calcium (g/100gm))						
	Initial	**1st month**	**2nd month**	**3rd month**	**4th month**	**5th month**	**6th month**
T_0 (100% AC)	0.53^{c}	$^{NS}0.52^{c}$	$^{NS}0.48^{c}$	$^{NS}0.47^{e}$	$^{NS}0.46^{d}$	$^{**}0.41^{d}$	$^{*}0.39^{c}$
T_1 -(95 % AC+5% ODJ)	0.64^{b}	$^{NS}0.61^{b}$	$^{NS}0.59^{b}$	$^{**}0.58^{d}$	$^{**}0.56^{c}$	$^{**}0.52^{c}$	$^{*}0.49^{b}$
T_0 (100% AC	0.81^{a}	$^{NS}0.79^{a}$	$^{NS}0.77^{a}$	$^{**}0.73^{b}$	$^{**}0.70^{ab}$	$^{*}0.65^{ab}$	$^{*}0.59^{a}$
T_3 (95 % AC+5% WPP)	0.78^{a}	$^{NS}0.76^{a}$	$^{NS}0.74^{a}$	$^{*}0.68^{c}$	$^{*}0.67^{b}$	$^{*}0.62^{b}$	$^{*}0.58^{a}$
T_0 (100% NAC)	0.57^{c}	$^{NS}0.52^{c}$	$^{NS}0.49^{c}$	$^{**}0.47^{e}$	$^{**}0.44^{d}$	$^{**}0.42^{d}$	$^{*}0.39^{c}$
T_4 -(95 % NAC+5% ODJ)	0.65^{b}	$^{NS}0.62^{b}$	$^{NS}0.59^{b}$	$^{**}0.59^{d}$	$^{**}0.55^{c}$	$^{**}0.53^{c}$	$^{*}0.51^{b}$
T_5 (95 % NAC+5% B)	0.81^{a}	$^{NS}0.80^{a}$	$^{NS}0.79^{a}$	$^{*}0.77^{a}$	$^{*}0.73^{a}$	$^{*}0.69^{a}$	$^{**}0.63^{a}$
T_6 (95 % AC+5% WPP)	0.79^{a}	$^{NS}0.76^{a}$	$^{NS}0.75^{a}$	$^{*}0.69^{c}$	$^{*}0.68^{b}$	$^{*}0.63^{b}$	$^{*}0.58^{a}$
CV (%)	5.256	5.046	5.083	3.147	4.241	4.650	7.125
CD (0.05)	0.064	0.063	0.057	0.034	0.044	0.045	0.064

AC- Alkalised Chocolates, NAC – Non alkalised chocolates, ODJ – Osmodehydrated Jackfruit, A-Almond, WPP- White Pepper Powder

DMRT column wise comparison

Figures with same superscripts no significant difference

T test row wise comparison

*- Significant at1% level **- Significant at 5% level, NS-Not significant

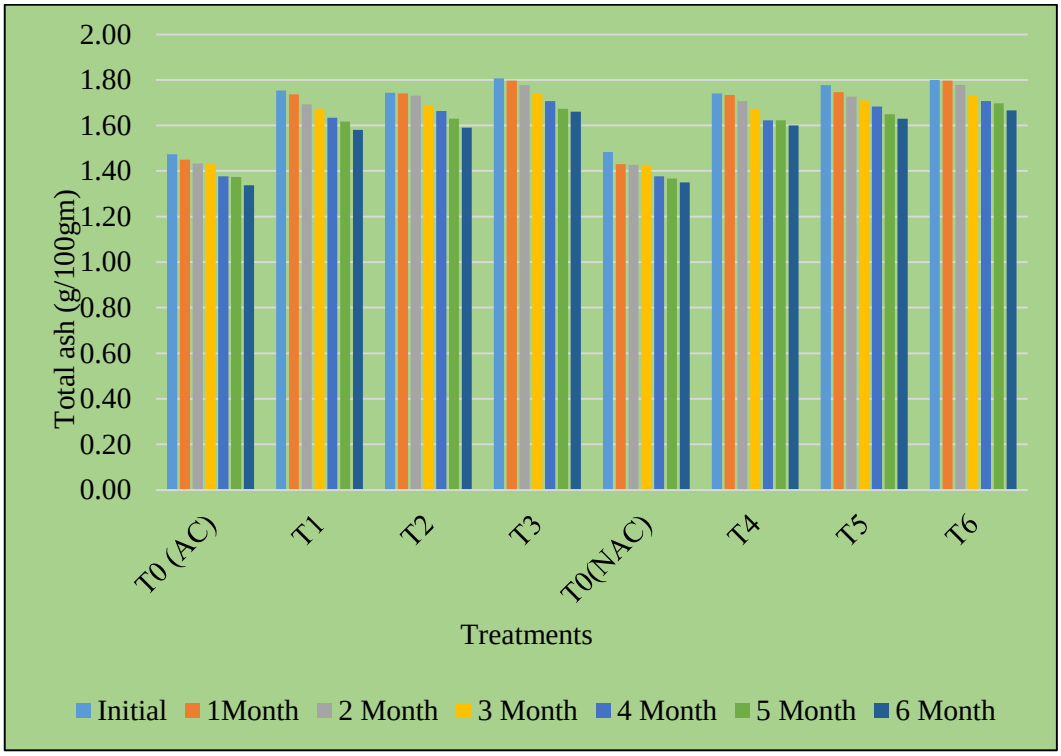


Fig.28.Total ash content of blended chocolates during storage

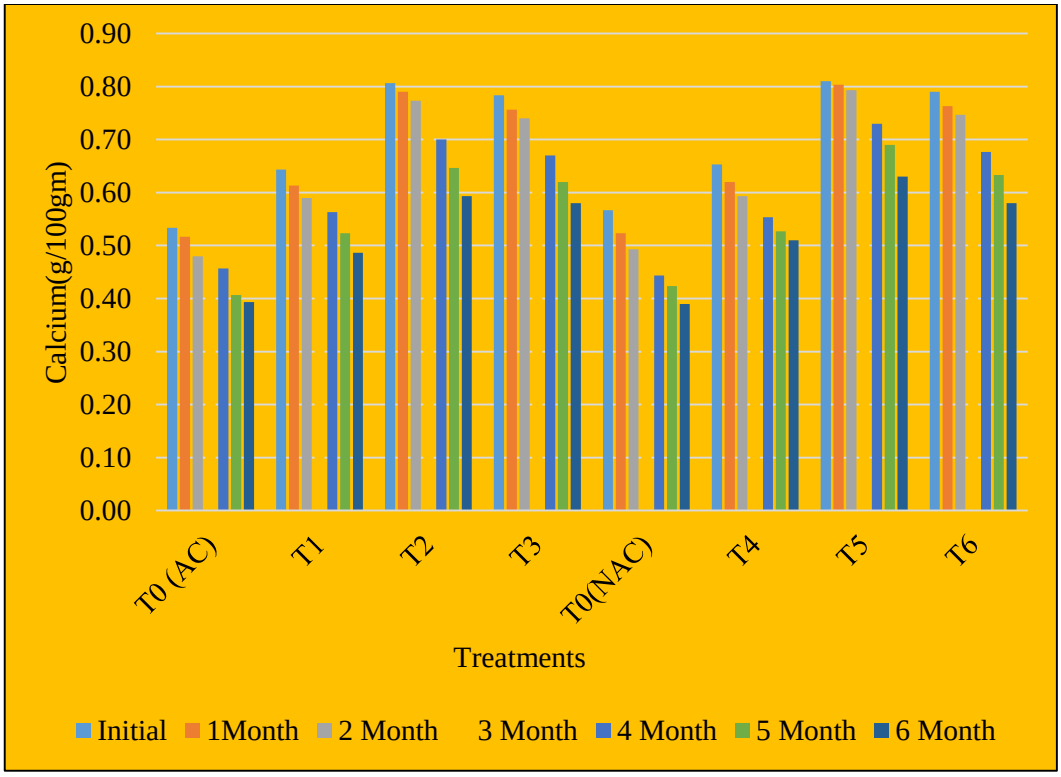


Fig.29.Calcium content of blended chocolates during storage

4.4.2.1.12. Phosphorus

The initial phosphorus content of different blended chocolates ranged from 0.19g/100g to 0.23g/ 100g with the lowest phosphorus content in T_0 (100% AC), T_1 (95 % AC+5% ODJ), T_0 (100% NAC) and highest phosphorus content in T_5 (95 % NAC+5% A) respectively. Based on one way ANOVA statistically significant difference was observed in phosphorus content of blended chocolates in all treatments initially and during each month of storage. There was a reduction in phosphorus content of blended chocolates was recorded in all treatments during storage (Figure 29). Based on one way ANOVA the treatments are non significant in phosphorus content at fifth and sixth month of storage. At the end of sixth month of storage the treatments T_5 and T_6 was with maximum phosphorus content (0.16g/100g) and T_0 (AC), T_1, T_2, T_3, T_0 (NAC) and T_4 was with minimum phosphorus content of 0.15g/100g (Table 49).

During storage periods, based on t test, no significant difference was observed up to second month of storage in all treatments. In the third month of storage statistically no significant difference was noticed in T_0 (AC), T_1, T_0 (NAC) and T_4 and at fourth month significant difference was observed in all treatments except T_1. During fifth and sixth month of storage all treatments are significantly varied in phosphorus content.

4.4.2.1.13. Iron

As revealed in Table 50, initially the iron content in blended chocolates ranged from 17.78mg/100g to 19.96mg/100g with the lowest iron content in T_0 (100% NAC) and highest in T_2 (95 % AC+5% A). Based on one way ANOVA, the treatments are significantly varied during storage periods, but the treatments T_2 (95 % AC+5% B) and T_5 (95 % NAC+5% A) are significantly on par and all other treatments T_0 (AC), T_1, T_3, T_0 (NAC), T_4 and T_6 are also on par. A reduction in iron content during storage of blended chocolates was revealed from Figure 30. Significant difference in iron content was recorded in fifth and sixth month also. The maximum iron content (18.23mg/100g) at sixth month of storage was recorded

in T_5 (95 % NAC+5% B) and lowest (16.20mg/100g) in T_3 (95 % AC+5% WP) followed by controls.

With respect to storage periods, t test shows no significant difference in iron content from first month to fourth month of storage in all treatments. But during fifth and sixth month, the treatments T_2 (95 % AC+5% A) and T_3 (95 % AC+5% WP) was significantly different. The treatments T_0 (100% AC), T_1, T_0 (100% NAC), T_5 and T_6 are non-significant in iron content throughout the storage periods. The treatments T_2 (95 % AC+5% A) and T_5 (95 % NAC+5% A) was superior in iron content throughout the storage periods.

4.4.2.1.14. Lipase

The lipase activity of different blended chocolates during storage was represented in Table 51 and Figure 31. Based on one way ANOVA, initially and up to forth month of storage no significant difference was observed in lipase activity. Initially and during first month of storage the lipase activity was recorded 0.0010µ eq in all treatments. But at fifth and sixth month of storage statistically significant difference was recorded in all treatments. At the end of sixth month of storage the lowest lipase activity (0.0013 µ eq) was in T_2 (95 % AC+5% A) and T_5 (95 % NAC+5% A) and highest ((0.0015 µ eq) in T_3 (95 % AC+5% WPP).There was a slight increase in lipase activity was observed in all treatments during storage. Based on t test, the lipase activity was statistically non significant throughout the storage periods for each treatments.

4.4.2.1.15. Free fatty acid

The free fatty acid content of blended chocolates are presented in Table 52 and Figure 32. Initially among the treatments the lowest (1.52%) free fatty acid content was observed in T_1 (95 % AC+5% ODJ), T_2 (95 % AC+5% A) and T_5 (95 % NAC+5% A) and highest (1.52%) in T_6 (95 % AC+5% WP). Based on one way ANOVA, there was no significant difference was observed between the treatments initially and during storage periods. There was an increase in trends in free fatty acid content of blended chocolates was recorded during storage and it was within

1.75% up to fourth month of storage. The treatment T_6 shows the maximum free fatty acid content throughout the storage periods and it was 2.80% at sixth month of storage. Regarding storage periods, based on t test, the free fatty acid content of blended chocolates was non significant throughout the storage periods

Table. 49. Phosphorus content of blended chocolates during storage

Treatments	Phosphorus (mg/100gm))						
	Initial	**1st month**	**2nd month**	**3rd month**	**4th month**	**5th month**	**6th month**
T_0 (100% AC)	0.19^{b}	$^{NS}0.18^{b}$	$^{NS}0.18^{d}$	$^{NS}0.17^{cd}$	$^{**}0.16^{c}$	*0.16	*0.15
T_1 -(95 % AC+5% ODJ)	0.19^{b}	$^{NS}0.19^{b}$	$^{NS}0.18^{d}$	$^{NS}0.17^{bcd}$	$^{NS}0.17^{bc}$	*0.16	*0.15
T_2 (95 % AC+5% A)	0.22^{a}	$^{NS}0.21^{a}$	$^{NS}0.20^{ab}$	$^{**}0.19^{a}$	$^{**}0.18^{a}$	*0.17	*0.15
T_3 (95 % AC+5% WPP)	0.21^{a}	$^{NS}0.21^{a}$	$^{NS}0.19^{abc}$	$^{**}0.18^{ab}$	$^{**}0.17^{abc}$	*0.16	*0.15
T_0 (100% NAC)	0.19^{b}	$^{NS}0.19^{b}$	$^{NS}0.18^{cd}$	$^{NS}0.17^{cd}$	$^{**}0.16^{bc}$	*0.16	*0.15
T_4 -(95 % NAC+5% ODJ)	0.19^{b}	$^{NS}0.18^{b}$	$^{NS}0.17^{d}$	$^{NS}0.17^{d}$	**0.16c	*0.15	*0.15
T_5 (95 % NAC+5% A)	0.23^{a}	$^{NS}0.21^{a}$	$^{NS}0.20^{a}$	$^{**}0.19^{a}$	$^{**}0.18^{a}$	*0.17	*0.16
T_6 (95 % AC+5% WPP)	0.22^{a}	$^{NS}0.20^{a}$	$^{NS}0.19^{bcd}$	$^{**}0.18^{ab}$	$^{*}0.17^{bc}$	*0.16	*0.16
CV (%)	3.991	3.296	4.394	4.293	4.838	4.590	4.995
CD (0.05)	0.014	0.011	0.014	0.013	0.014	NS	NS

AC- Alkalised Chocolates, NAC – Non alkalised chocolates, ODJ – Osmodehydrated Jack fruit,
A-Almond, WPP- White Pepper Powder DMRT column wise comparison
Figures with same superscripts no significant difference
t test row wise comparison
*- Significant at1% level
**- Significant at 5% level, NS-Not significant

Table. 50. Iron content of blended chocolates during storage

Treatments	Iron (mg/100gm))						
	Initial	1st month	2nd month	3rd month	4th month	5th month	6th month
T_0 (100% AC)	18.00^{b}	$^{NS}17.86^{b}$	$^{NS}17.77^{b}$	$^{NS}17.63^{b}$	$^{NS}17.36^{b}$	$^{NS}16.95^{bc}$	$^{NS}16.78^{bc}$
T_1 (95 % AC+5% ODJ)	18.49^{b}	$^{NS}18.35^{b}$	$^{NS}18.13^{b}$	$^{NS}18.01^{b}$	$^{NS}17.69^{b}$	$^{NS}17.44^{b}$	$^{NS}17.20^{b}$
T_2 (95 % AC+5% B)	19.96^{a}	$^{NS}19.70^{a}$	$^{NS}19.55^{a}$	$^{NS}19.36^{a}$	$^{NS}18.97^{a}$	$^{**}18.82^{a}$	$^{**}18.18^{a}$
T_3 (95 % AC+5% WPP)	17.98^{b}	$^{NS}17.78^{b}$	$^{NS}17.54^{b}$	$^{NS}17.39^{b}$	$^{NS}16.80^{b}$	$^{**}16.45^{c}$	$^{*}16.20^{c}$
T_0 (100% NAC)	17.78^{b}	$^{NS}17.65^{b}$	$^{NS}17.51^{b}$	$^{NS}17.34^{b}$	$^{NS}17.06^{b}$	$^{NS}16.69^{bc}$	$^{NS}16.48^{bc}$
T_4 (95 % NAC+5% ODJ)	18.29^{b}	$^{NS}18.05^{b}$	$^{NS}17.85^{b}$	$^{NS}17.69^{b}$	$^{NS}17.45^{b}$	$^{NS}17.26^{bc}$	$^{**}16.99^{bc}$
T_5 (95 % NAC+5% A)	19.95^{a}	$^{NS}19.66^{a}$	$^{NS}19.51^{a}$	$^{NS}19.29^{a}$	$^{NS}18.91^{a}$	$^{NS}18.83^{a}$	$^{NS}18.23^{a}$
T_6 (95 % AC+5% WPP)	17.79^{b}	$^{NS}17.67^{b}$	$^{NS}17.51^{b}$	$^{NS}17.36^{b}$	$^{NS}17.05^{b}$	$^{NS}16.74^{bc}$	$^{NS}16.54^{bc}$
CV (%)	3.276	3.258	3.355	3.564	3.451	2.833	3.294
CD (0.05)	1.051	1.034	1.055	1.111	1.055	0.853	0.973

AC- Alkalised Chocolates, NAC Non alkalised chocolates, ODJ Osmodehydrated Jack fruit,
A-Almond, WPP- White Pepper Powder DMRT column wise comparison
Figures with same superscripts no significant difference
t test row wise comparison
*- Significant at1% level
**- Significant at 5% level, NS-Not significant

Table. 51. Lipase activity of blended chocolates during storage

Treatments	Lipase (µ eq)						
	Initial	1st month	2nd month	3rd month	4th month	5th month	6th month
T_0 (100% AC)	0.0010	NS 0.0010	NS 0.0011	NS 0.0012	NS 0.0012	NS 0.0013^{b}	NS 0.0014^{b}
T_1 -(95 % AC+5% ODJ)	0.0010	NS 0.0010	NS 0.0010	NS 0.0011	NS 0.0012	NS 0.0013^{b}	NS 0.0014^{c}
T_2 (95 % AC+5% A)	0.0010	NS 0.0010	NS 0.0010	NS 0.0011	NS 0.0011	NS 0.0012^{d}	NS 0.0013^{d}
T_3 (95 % AC+5% WPP)	0.0010	NS 0.0010	NS 0.0011	NS 0.0011	NS 0.0013	NS 0.0014^{a}	NS NS0.0015^{a}
T_0 (100% NAC)	0.0010	NS 0.0010	NS 0.0011	NS 0.0011	NS 0.0012	NS 0.0013^{c}	NS 0.0014^{c}
T_4 -(95 % NAC+5% ODJ)	0.0010	NS0.0010	NS0.0011	NS0.0011	NS 0.0012	NS0.0013 c	NS 0.0014^{c}
T_5 (95 % NAC+5% A)	0.0010	NS 0.0010	NS 0.0010	NS 0.0010	NS 0.0011	NS 0.0012^{d}	NS 0.0013^{d}
T_6 (95 % AC+5% WPP)	0.0010	NS 0.0010	NS 0.0010	NS 0.0011	NS 0.0012	NS 0.0013^{b}	NS 0.0014^{b}
CV (%)	5.587	5.565	5.499	6.216	8.081	5.222	5.313
CD (0.05)	NS	NS	NS	NS	NS	0.000	0.000

AC- Alkalised Chocolates, NAC – Non alkalised chocolates,
ODJ – Osmodehydrated Jack fruit, A-Almond, WPP- White Pepper Powder
DMRT column wise comparison
Figures with same superscripts no significant difference t test row wise comparison
*- Significant at1% level **- Significant at 5% level, NS-Not significant

Table. 52. Free fatty acid of blended chocolates during storage

Treatments	Free fatty acids (%))						
	Initial	**1st month**	**2nd month**	**3 rd month**	**4th month**	**5th month**	**6th month**
T_0 (100% AC)	1.57 (7.188)	NS 1.58 (7.219)	NS 1.63 (7.327)	NS 1.65 (7.387)	NS 1.71 (7.520)	NS 1.79 (7.682)	NS 2.27 (8.645)
T_1 (95 % AC+5% ODJ)	1.52 (7.090)	NS 1.59 (7.250)	NS 1.65 (7.387)	NS 1.65 (7.387)	NS 1.69 (7.520)	NS 1.90 (7.916)	NS 2.10 (8.645)
T_2 (95 % AC+5% A)	1.52 (7.090)	NS 1.59 (7.250)	NS 1.64 (7.350)	NS 1.65 (7.387)	NS 1.72 (7.429)	NS 1.90 (7.916)	NS 2.09 (8.329)
T_3 (95 % AC+5% WPP)	1.58 (7.212)	NS 1.59 (7.206)	NS 1.64 (7.349)	NS 1.68 (7.440)	NS 1.72 (7.528)	NS 1.94 (7.997)	NS 2.54 (9.139)
T_0 (100% NAC)	1.57 (7.188)	NS 1.63 (7.341)	NS 1.65 (7.380)	NS 1.65 (7.387)	NS 1.71 (7.520)	NS 1.79 (7.682)	NS 2.20 (8.491)
T_4 (95 % NAC+5% ODJ)	1.54 (7.128)	NS 1.59 (7.250)	NS 1.65 (7.387)	NS 1.69 (7.468)	NS 1.72 (7.520)	NS 1.90 (7.916)	NS 2.20 (8.491)
T_5 (95 % NAC+5% A)	1.52 (7.090)	NS 1.57 (7.190)	NS 1.64 (7.349)	NS 1.65 (7.387)	NS 1.69 (7.468)	NS 1.90 (7.916)	NS 2.09 (8.256)
T_6 (95 % AC+5% WPP)	1.58 (7.212)	NS 1.61 (7.289)	NS 1.65 (7.387)	NS 1.68 (7.387)	NS1.75 (7.608)	**1.94 (7.997)	*2.80 (9.633)
CV (%)	2.193	2.095	1.675	1.504	2.302	4.773	9.775
CD (0.05)	NS	NS	NS	NS	NS	NS	NS

AC- Alkalised Chocolates, NAC – Non alkalised chocolates, ODJ – Osmodehydrated Jack fruitA-Almond, WPP- White Pepper Powder

DMRT column wise comparison

Figures with same superscripts no significant difference

t test raw wise comparison

*- Significant at1% level

**- Significant at 5% level, NS-Not significant

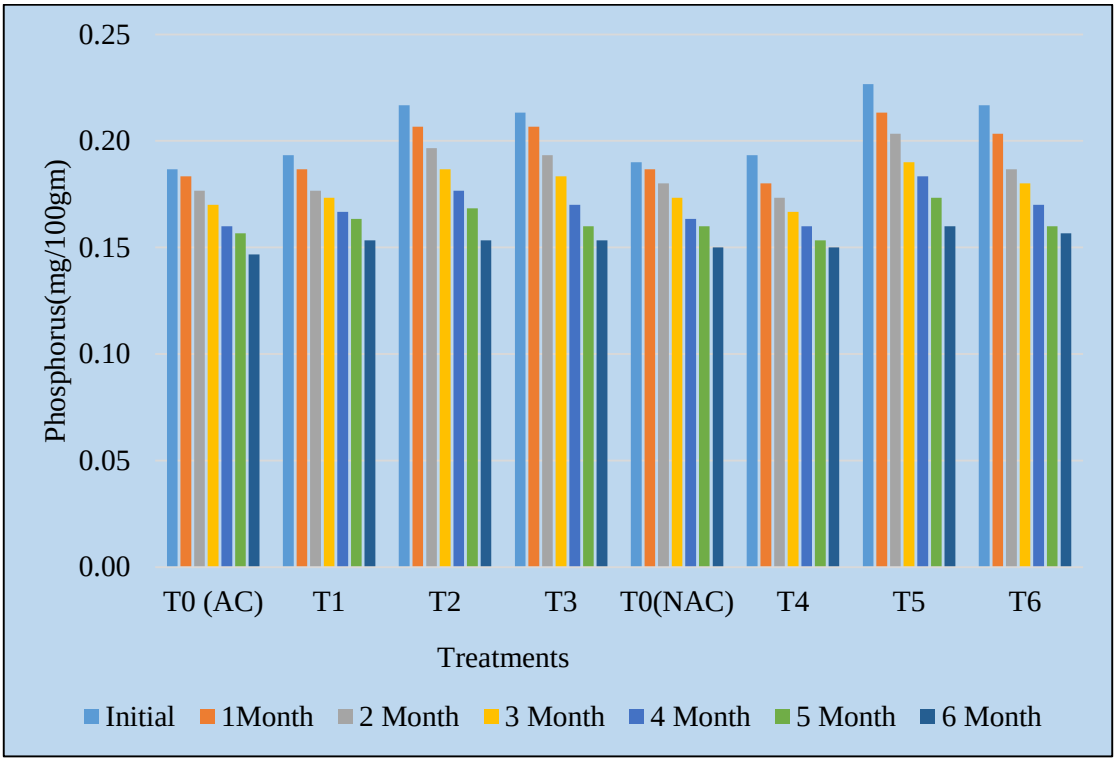


Fig.30. Phosphorus content of blended chocolates during storage

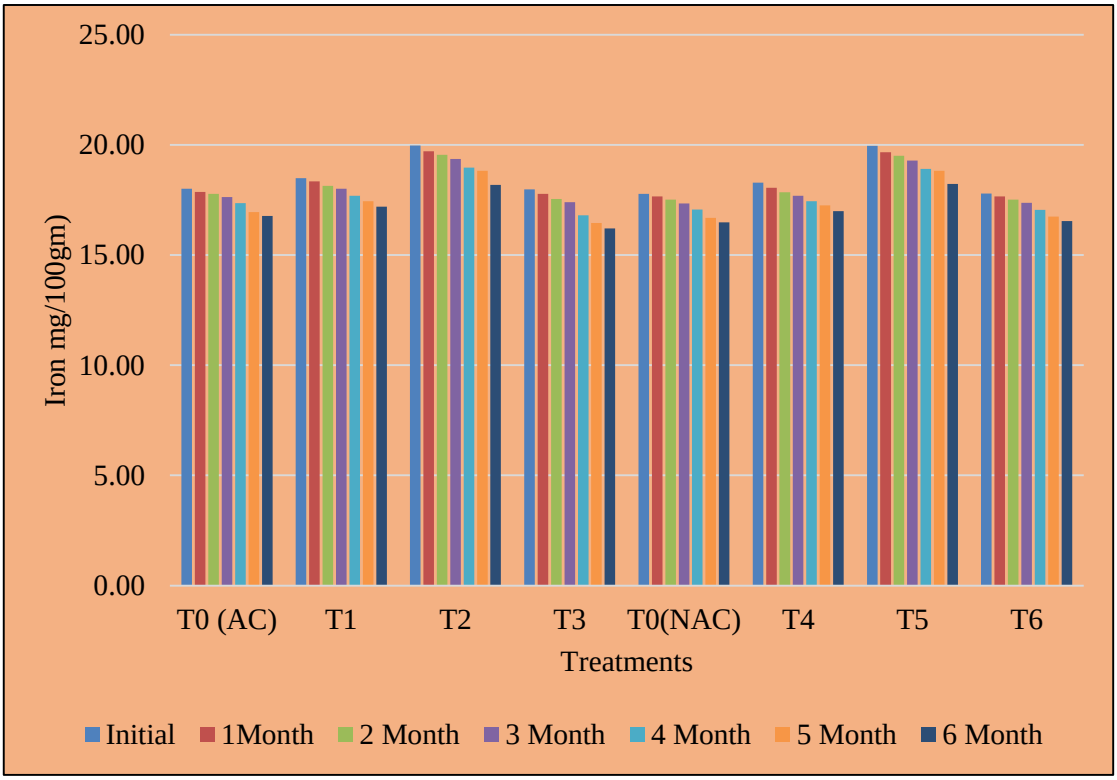


Fig.31. Iron content of blended chocolates during storage

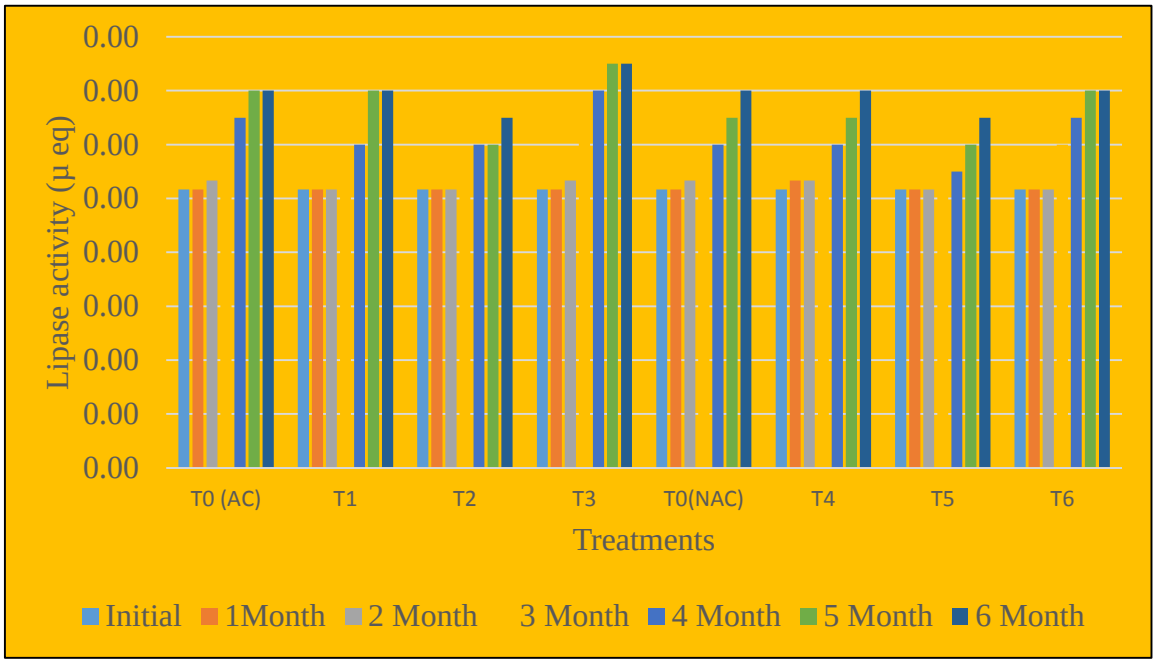


Fig.32. Lipase activity of blended chocolates during storage

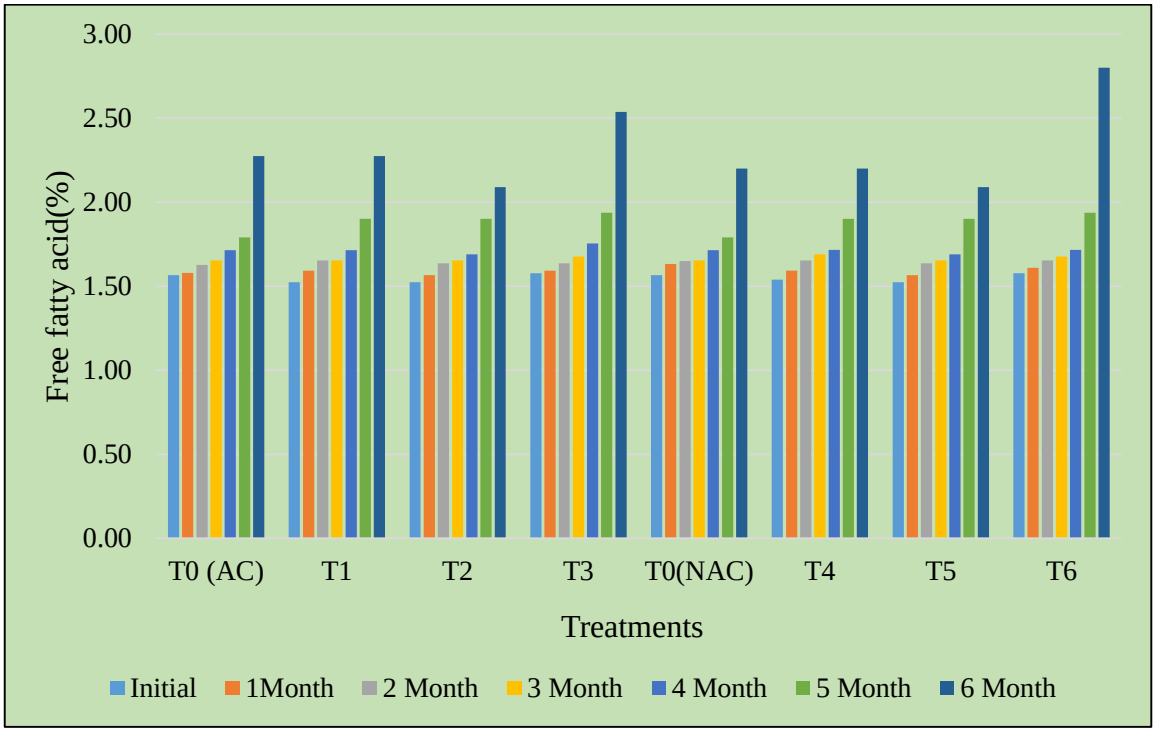


Fig.33. Free fatty acid content of blended chocolates during storage

4.4.2.2. Organoleptic evaluation of selected blended chocolates during storage

The organoleptic qualities of the selected chocolates during storage were tabulated. The mean score and mean rank score obtained for different quality attributes for appearance, colour, flavour, texture, taste and overall acceptability of alkalised and non alkalised blended chocolates during storage including control (chocolates prepared with alkalised cocoa nibs only) are presented in Table 53, 54, 55, 56, 57 and 58.

The initial score for appearance of blended alkalised and non alkalised chocolates varied from 8.89 to 9.00 (Table 53). The highest score of 9.00 was observed in blended chocolates ie, in treatments T_1 (95 % AC+5% ODJ), T_2 (95 % AC+5% A), T_3 (95 % AC+5% WPP), T_4 (95 % NAC+5% ODJ), T_5 (95 % NAC+5% A), and T_6 (95 % NAC+5% WPP) and the lowest score of 8.89 was noticed in control T_0 (100% alkalised chocolates) followed by 8.91 in T_0 (control-non alkalised chocolates). A gradual decrease was observed in the mean score for appearance in all the treatments during storage. The highest mean score and rank score during all the six months of storage period was recorded for chocolates blended with almond ie for the treatments T_2 (95 % AC+5% B) and T_5 (95 % NAC+5% B). At the end of storage the mean score of 8.60 and mean rank score of 5.00 and 5.10 was recorded in treatment T_2 and T_5. Kendall's value shows that there was significant agreement between the judges at 1% level.

With regards to the colour of alkalised and non alkalised blended chocolates (Table 54), before storage, the highest score of 9.00 was for the treatments T_1 (95 % AC+5% ODJ), T_2 (95 % AC+5% A) in alkalised chocolates, T_4 (95 % NAC+5% ODJ), and T_5 (95 % NAC+5% A) among the treatments which reduced to the score 8.96 at the fifteenth day of storage. The lowest mean score was recorded in controls (alkalised and non alkalised chocolates). A gradual decrease was observed in the mean score for colour in all treatments up to sixth month of storage. But there was a slight increase in mean score for colour in treatment T_1 (95 % AC+5% ODJ), T_4

(95 % NAC+5% ODJ), T_5 (95 % NAC+5% A) and T_6 (95 % NAC+5% WPP) in sixth month of storage at end of fifteenth day. And again there was a reduction in colour at last fifteenth day at the end of sixth month of storage in treatment T1, T_4 and T_5. The lowest score of 8.67 was for the treatments T_0 (control alkalised) and T_6 at the end of storage (Table 54).

Table 55 shows that, initially, the score for flavour of blended chocolates varied from 8.87 to 9.00, with the highest score for T_1 (95 % AC+5% ODJ), T_2 (95 % AC+5% A), T_3 (95 % AC+5% WPP), T_4 (95 % NAC+5% ODJ), T_5 (95 % NAC+5% B), and T_6 (95 % NAC+5% WPP) and the lowest score of 8.87 was noticed in control T_0 (100% non alkalised chocolates) followed by 8.93 in T_0 (control- alkalised chocolates). No change was observed in mean score for flavour at the fifteenth day and end of first month of storage. A decrease in mean score from the fifteenth day of storage in the second month up to end of sixth month of storage was recorded. The highest mean score and rank score during all the six months of storage period was recorded for the alkalised chocolate blended with almond ie, T_2 (95 % AC+5% A). Kendall's value shows that there was significant agreement between the judges.

As revealed in table 56, the mean score of texture of blended chocolates showed a reduction with the advancement of storage periods. Before storage and fifteenth day of storage, the highest mean score of 9.00 for texture was for the treatments T_0 (controls – alkalised and non alkalised), T_3 (95 % AC+5% WPP) and T_6 (95 % NAC+5% WP). The lowest mean score for texture was recorded initially for T_4 (95 % NAC+5% ODJ). After fifteenth day of storage there was a reduction in mean score for texture among all treatments. At end of storage the highest mean score was recorded in treatments T_2 (95 % AC+5% B), T_3 (95 % AC+5% WP), T_5 (95 % NAC+5% B), and T_6 (95 % NAC+5% WPP). T1 (95 % AC+5% ODJ) showed the least score for texture at the end of storage.

As revealed in Table 57, the taste of the chocolates varied with the storage periods of the bended alkalised and non alkalised chocolates. Among treatments T_0 (control –non alkalised) had the least score before storage (8.80) and on first (8.73),

second (8.71) and third month (8.69) month of storage. T_1 (95 % AC+5% ODJ), T_2 (95 % AC+5% A), T_3 (95 % AC+5% WPP), T_4 (95 % NAC+5% ODJ), T_5 (95 % NAC+5% A), and T_6 (95 % NAC+5% WPP), had the maximum score (9.00) before storage and fifteen days after storage and also showed maximum score in each storage periods. The chocolates (alkalised and non alkalised) blended with almond (T_2 -95 % AC+5% A and T_5 - 95 % NAC+5% A) had the highest score before storage (9.00) and at the end of the storage (8.82).

The overall acceptability score of blended chocolates throughout the storage period of six months are furnished in Table 58. The initial score for overall acceptability of blended chocolates varied from 8.76 to 9.00, with the highest score for T_1 (95 % AC+5% ODJ), T_2 (95 % AC+5% A), T_3 (95 % AC+5% WPP), T_4 (95 % NAC+5% ODJ), T_5 (95 % NAC+5% B), and T_6 (95 % NAC+5% WPP), and the lowest score of 8.76 was noticed in control T_0 (100% non alkalised chocolates) followed by 8.84 in T_0 (control- alkalised chocolates). A gradual decrease was observed in the mean score for overall acceptability in all treatments. The highest mean score and rank score during all the six months of storage period was recorded for the chocolates blended with almond (T_2 -95 % AC+5% A and T_5 -95 % NAC+5% A. The mean score (8.85) and mean rank score (5.33) was highest in T_2 followed by T_5 with the mean score and mean rank score of 8.80 and 5.03 respectively. Kendall's value shows that there was significant agreement between the judges at 1% level.

Table 53. Effect of storage on the mean score for appearance of blended alkalised and non alkalised chocolates

Treatments	Initial	1st Month		2nd Month		3rd Month		4th Month		5th Month		6th Month	
		15 days	30 days	15 days	30 Days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days
T_0 (100% AC)	8.91 (3.73)	8.91 (3.73)	8.89 (3.73)	8.89 (3.73)	8.87 (3.73)	8.87 (3.73)	8.78 (3.80)	8.78 (3.80)	8.69 (4.37)	8.60 (4.40)	8.60 (4.40)	8.49 (3.93)	8.49 (3.93)
T_1 -(95 % AC+5% ODJ)	9.00 (4.80)	9.00 (4.80)	8.98 (4.80)	8.98 (4.80)	8.96 (4.80)	8.96 (4.80)	8.89 (4.93)	8.89 (4.93)	8.69 (4.33)	8.62 (4.67)	8.62 (4.67)	8.58 (4.93)	8.58 (4.93)
T_2 (95 % AC+5% A)	9.00 (4.80)	9.00 (4.80)	8.98 (4.80)	8.98 (4.80)	8.96 (4.80)	8.96 (4.80)	8.89 (4.93)	8.89 (4.93)	8.76 (4.97)	8.65 (4.83)	8.65 (4.83)	8.60 (5.00)	8.60 (5.00)
T_3 (95 % AC+5% WP)	9.00 (4.80)	9.00 (4.80)	8.98 (4.80)	8.98 (4.80)	8.96 (4.80)	8.96 (4.80)	8.82 (4.20)	8.82 (4.20)	8.69 (4.33)	8.58 (4.37)	8.58 (4.37)	8.51 (4.27)	8.51 (4.27)
T_0 (100% NAC)	8.89 (3.47)	8.89 (3.47)	8.87 (3.47)	8.87 (3.47)	8.85 (3.47)	8.85 (3.47)	8.80 (3.87)	8.80 (3.87)	8.67 (4.10)	8.56 (4.03)	8.56 (4.03)	8.49 (3.83)	8.49 (3.83)
T_4 -(95 % NAC+5% ODJ)	9.00 (4.80)	9.00 (4.80)	8.98 (4.80)	8.98 (4.80)	8.96 (4.80)	8.96 (4.80)	8.87 (4.70)	8.87 (4.70)	8.73 (4.87)	8.62 (4.77)	8.62 (4.77)	8.58 (4.93)	8.58 (4.93)
T_5 (95 % NAC+5% A)	9.00 (4.80)	9.00 (4.80)	8.98 (4.80)	8.98 (4.80)	8.96 (4.80)	8.96 (4.80)	8.89 (4.90)	8.89 (4.90)	8.78 (5.00)	8.65 (4.87)	8.65 (4.87)	8.60 (5.10)	8.60 (5.10)
T_6 (95 % AC+5% WPP)	9.00 (4.80)	9.00 (4.80)	8.98 (4.80)	8.98 (4.80)	8.96 (4.80)	8.96 (4.80)	8.87 (4.67)	8.87 (4.67)	8.67 (4.03)	8.58 (4.07)	8.58 (4.07)	8.49 (4.00)	8.49 (4.00)
Kendall's value	**0.279****	**0.279****	**0.189****	**0.189****	**0.143****	**0.143****	**0.072****	**0.072****	**0.038****	**0.026****	**0.026****	**0.071****	**0.071****

Values in parenthesis is mean rank score based on Kendall's W which was significant

**Significant at 1% level, AC- Alkalised Chocolates, NAC – Non alkalised chocolates, ODJ – Osmodehydrated Jack fruit, A-Almond, WPP- White Pepper Powder

Table 54. Effect of storage on the mean score for colour of blended alkalised and non alkalised chocolates

Treatments	Initial	1st Month		2nd Month		3rd Month		4th Month		5th Month		6th Month	
		15 days	30 Days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days
T_0 (100% AC)	8.98 (4.47)	8.93 (4.47)	8.91 (4.70)	8.87 (4.50)	8.87 (4.50)	8.87 (4.57)	8.87 (4.57)	8.82 (4.60)	8.80 (4.90)	8.80 (4.90)	8.80 (4.90)	8.71 (4.90)	8.67 (4.90)
T_1 (95 % AC+5% ODJ)	9.00 (4.73)	8.96 (4.73)	8.93 (5.00)	8.89 (4.77)	8.89 (4.77)	8.89 (4.87)	8.82 (4.81)	8.82 (4.67)	8.76 (4.43)	8.76 (4.43)	8.76 (4.43)	8.82 (4.43)	8.78 (4.43)
T_2 (95 % AC+5% A)	9.00 (4.73)	8.96 (4.73)	8.82 (4.10)	8.89 (4.77)	8.89 (4.77)	8.89 (4.87)	8.82 (4.81)	8.82 (4.81)	8.80 (4.90)	8.80 (4.90)	8.80 (4.90)	8.71 (4.90)	8.71 (4.90)
T_3 (95 % AC+5% WPP)	8.96 (4.20)	8.93 (4.47)	8.91 (4.70)	8.89 (4.77)	8.89 (4.77)	8.87 (4.60)	8.85 (4.58)	8.82 (4.47)	8.78 (4.53)	8.78 (4.53)	8.78 (4.53)	8.76 (4.53)	8.73 (4.53)
T_0 (100% NAC)	8.98 (4.47)	8.87 (3.67)	8.84 (3.97)	8.87 (4.43)	8.87 (4.43)	8.87 (4.47)	8.82 (4.42)	8.82 (4.42)	8.80 (4.73)	8.80 (4.73)	8.80 (4.73)	8.80 (4.73)	8.80 (4.73)
T_4 (95 % NAC+5% ODJ)	9.00 (4.73)	8.96 (4.73)	8.89 (4.63)	8.82 (4.30)	8.82 (4.30)	8.82 (4.37)	8.82 (4.42)	8.78 (4.37)	8.73 (4.33)	8.73 (4.33)	8.73 (4.33)	8.76 (4.33)	8.73 (4.33)
T_5 (95 % NAC+5% A)	9.00 (4.73)	8.96 (4.73)	8.91 (4.70)	8.85 (4.30)	8.85 (4.30)	8.85 (4.40)	8.82 (4.36)	8.78 (4.67)	8.76 (4.47)	8.76 (4.47)	8.76 (4.47)	8.78 (4.47)	8.76 (4.47)
T_6 (95 % AC+5% WPP)	8.93 (3.93)	8.90 (4.47)	8.87 (4.20)	8.85 (4.23)	8.85 (4.23)	8.80 (3.87)	8.80 (3.87)	8.73 (3.67)	8.73 (3.67)	8.69 (3.70)	8.69 (3.70)	8.67 (3.70)	8.67 (3.70)
Kendall's value	**0.970****	**0.593****	**0.047****	**0.016****	**0.016****	**0.033****	**0.033****	**0.044****	**0.039****	**0.037****	**0.037****	**0.036****	**0.036****

Values in parenthesis is mean rank score based on Kendall's W which was significant, ** Significant at 1% level,

AC- Alkalised Chocolates, NAC Non alkalized chocolates, ODJ Osmodehydrated Jack fruit, A-Almond, WPP- White Pepper Powder

Table 55. Effect of storage on the mean score for flavour of blended alkalised and non alkaliseed chocolates

Treatments	Initial	1st Month		2nd Month		3rd Month		4th Month		5th Month		6th Month	
		15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days
T_0 (100% AC)	8.93 (4.00)	8.93 (4.07)	8.93 (4.07)	8.89 (3.80)	8.89 (3.80)	8.85 (3.80)	8.85 (3.80)	8.82 (4.03)	8.82 (4.03)	8.76 (4.27)	8.76 (4.27)	8.76 (4.57)	8.76 (4.57)
T_1 (95 % AC+5% ODJ)	9.00 (4.80)	9.00 (4.87)	9.00 (4.87)	8.96 (4.60)	8.96 (4.60)	8.93 (4.87)	8.93 (4.87)	8.89 (4.83)	8.89 (4.83)	8.82 (4.97)	8.82 (4.97)	8.76 (4.97)	8.76 (4.97)
T_2 (95 % AC+5% A)	9.00 (4.80)	9.00 (4.60)	9.00 (4.60)	8.98 (4.87)	8.98 (4.87)	8.96 (5.13)	8.96 (5.13)	8.89 (4.80)	8.89 (4.80)	8.82 (5.00)	8.82 (5.00)	8.76 (5.23)	8.76 (5.23)
T_3 (95 % AC+5% WPP)	9.00 (4.80)	9.00 (4.87)	9.00 (4.87)	8.98 (4.87)	8.98 (4.87)	8.89 (4.33)	8.89 (4.33)	8.85 (4.43)	8.85 (4.43)	8.76 (4.30)	8.76 (4.30)	8.67 (3.53)	8.67 (3.53)
T_0 (100% NAC)	8.87 (3.20)	8.87 (3.53)	8.87 (3.53)	8.84 (3.30)	8.84 (3.30)	8.84 (3.80)	8.84 (3.80)	8.80 (3.80)	8.80 (3.80)	8.76 (4.27)	8.76 (4.27)	8.76 (4.27)	8.76 (4.27)
T_4 (95 % NAC+5% ODJ)	9.00 (4.80)	9.00 (4.60)	9.00 (4.60)	8.98 (4.77)	8.98 (4.77)	8.91 (4.60)	8.91 (4.60)	8.80 (4.43)	8.80 (4.43)	8.80 (4.87)	8.80 (4.87)	8.76 (4.60)	8.76 (4.60)
T_5 (95 % NAC+5% A)	9.00 (4.80)	9.00 (4.87)	9.00 (4.87)	8.98 (4.90)	8.98 (4.90)	8.93 (4.87)	8.93 (4.87)	8.89 (4.87)	8.89 (4.87)	8.73 (4.20)	8.73 (4.20)	8.73 (4.60)	8.73 (4.60)
T_6 (95 % AC+5% WPP)	9.00 (4.80)	9.00 (4.60)	9.00 (4.60)	8.98 (4.90)	8.98 (4.90)	8.91 (4.60)	8.91 (4.60)	8.89 (4.80)	8.89 (4.80)	8.78 (4.43)	8.78 (4.43)	8.71 (4.23)	8.71 (4.23)
Kendall's value	**0.326****	**0.064****	**0.064****	**0.010****	**0.010****	**0.081****	**0.081****	**0.043****	**0.043****	**0.754****	**0.754****	**0.079****	**0.079****

Values in parenthesis is mean rank score based on Kendall's W which was significant

** Significant at 1% level, AC- Alkalised Chocolates, NAC Non alkalised chocolates, ODJ Osmodehydrated Jack fruit, A- Almond, WPP- White Pepper Powder

Table 56. Effect of storage on the mean score for texture of blended alkalised and non alkaliseed chocolates

Treatments	Initial	1st Month		2nd Month		3rd Month		4th Month		5th Month		6th Month	
		15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days
T_0 (100% AC)	9.00 (4.77)	9.00 (4.77)	8.98 (4.77)	8.98 (4.77)	8.98 (4.77)	8.89 (4.77)	8.89 (4.77)	8.85 (4.77)	8.85 (4.77)	8.80 (4.70)	8.80 (4.70)	8.67 (4.40)	8.67 (4.40)
T_1 -(95 % AC+5% ODJ)	8.96 (4.23)	8.96 (4.23)	8.93 (4.23)	8.93 (4.23)	8.93 (4.23)	8.89 (4.50)	8.89 (4.50)	8.82 (4.40)	8.82 (4.40)	8.78 (4.43)	8.78 (4.43)	8.62 (3.97)	8.62 (3.97)
T_2 (95 % AC+5% A)	8.96 (4.23)	8.96 (4.23)	8.93 (4.23)	8.93 (4.23)	8.93 (4.23)	8.85 (3.97)	8.85 (3.97)	8.80 (4.03)	8.80 (4.03)	8.76 (4.17)	8.76 (4.17)	8.71 (4.80)	8.71 (4.80)
T_3 (95 % AC+5% WPP)	9.00 (4.77)	9.00 (4.77)	8.98 (4.77)	8.98 (4.77)	8.98 (4.77)	8.89 (4.77)	8.89 (4.77)	8.85 (4.60)	8.85 (4.60)	8.78 (4.37)	8.78 (4.37)	8.71 (4.93)	8.71 (4.93)
T_0 (100% NAC)	9.00 (4.77)	9.00 (4.77)	8.98 (4.77)	8.98 (4.77)	8.98 (4.77)	8.89 (4.77)	8.89 (4.77)	8.87 (4.83)	8.87 (4.83)	8.82 (4.87)	8.82 (4.87)	8.67 (4.43)	8.67 (4.43)
T_4 -(95 % NAC+5% ODJ)	8.93 (3.97)	8.93 (3.97)	8.91 (3.97)	8.91 (3.97)	8.91 (3.97)	8.85 (3.97)	8.85 (3.97)	8.80 (4.00)	8.80 (4.00)	8.76 (4.07)	8.76 (4.07)	8.69 (4.60)	8.69 (4.60)
T_5 (95 % NAC+5% A)	8.98 (4.50)	8.98 (4.50)	8.96 (4.50)	8.96 (4.50)	8.96 (4.50)	8.89 (4.50)	8.89 (4.50)	8.85 (4.53)	8.85 (4.53)	8.80 (4.60)	8.80 (4.60)	8.71 (4.80)	8.71 (4.80)
T_6 (95 % AC+5% WPP)	9.00 (4.77)	9.00 (4.77)	8.98 (4.77)	8.98 (4.77)	8.98 (4.77)	8.91 (4.77)	8.91 (4.77)	8.87 (4.83)	8.87 (4.83)	8.82 (4.80)	8.82 (4.80)	8.71 (4.93)	8.71 (4.93)
Kendall's value	**0.111**	**0.111**	**0.072**	**0.051**	**0.051**	**0.060**	**0.060**	**0.038**	**0.038**	**0.030**	**0.030**	**0.033**	**0.033**

Values in parenthesis is mean rank score based on Kendall's W which was significant, **Significant at 1% level,

AC- Alkalised Chocolates, NAC – Non alkalized chocolates, ODJ – Osmodehydrated Jack fruit, A-Almond, WPP- White Pepper Powder

Table 57. Effect of storage on the mean score for taste of blended alkalised and non alkaliseed chocolates

Treatments	Initial	1st Month		2nd Month		3rd Month		4th Month		5th Month		6th Month	
		15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days
T_0 (100% AC)	8.82 (3.72)	8.82 (3.72)	8.80 (4.97)	8.80 (3.71)	8.80 (3.71)	8.71 (3.63)	8.71 (3.63)	8.69 (3.77)	8.64 (3.70)	8.60 (3.53)	8.60 (3.53)	8.60 (3.73)	8.60 (3.73)
T_1 -(95 % AC+5% ODJ)	9.00 (4.80)	9.00 (4.80)	8.98 (4.97)	8.93 (4.82)	8.93 (4.82)	8.89 (4.77)	8.89 (4.77)	8.87 (4.67)	8.82 (4.67)	8.80 (4.70)	8.80 (4.70)	8.78 (4.67)	8.78 (4.67)
T_2 (95 % AC+5% A)	9.00 (4.80)	9.00 (4.80)	8.98 (4.50)	8.96 (5.07)	8.96 (5.07)	8.91 (5.03)	8.91 (5.03)	8.87 (4.87)	8.82 (4.77)	8.82 (5.03)	8.82 (5.03)	8.82 (5.00)	8.82 (5.00)
T_3 (95 % AC+5% WPP)	9.00 (4.80)	9.00 (4.80)	8.93 (3.30)	8.91 (4.61)	8.91 (4.61)	8.89 (4.80)	8.89 (4.80)	8.85 (4.63)	8.80 (4.57)	8.78 (4.57)	8.78 (4.57)	8.76 (4.53)	8.76 (4.53)
T_0 (100% NAC)	8.80 (3.47)	8.80 (3.47)	8.73 (4.70)	8.71 (3.32)	8.71 (3.32)	8.67 (3.27)	8.67 (3.27)	8.67 (3.60)	8.64 (3.70)	8.62 (3.70)	8.62 (3.70)	8.62 (3.93)	8.60 (3.73)
T_4 -(95 % NAC+5% ODJ)	9.00 (4.80)	9.00 (4.80)	8.96 (4.97)	8.93 (4.82)	8.93 (4.82)	8.91 (5.00)	8.91 (5.00)	8.87 (4.83)	8.85 (5.03)	8.82 (5.07)	8.82 (5.07)	8.80 (5.00)	8.80 (5.00)
T_5 (95 % NAC+5% A)	9.00 (4.80)	9.00 (4.80)	8.98 (4.97)	8.96 (5.11)	8.96 (5.11)	8.91 (5.00)	8.91 (5.00)	8.89 (5.03)	8.85 (4.97)	8.82 (5.00)	8.82 (5.00)	8.82 (5.17)	8.82 (5.17)
T_6 (95 % AC+5% WPP)	9.00 (4.80)	9.00 (4.80)	8.93 (4.43)	8.91 (4.54)	8.91 (4.54)	8.87 (4.50)	8.87 (4.50)	8.85 (4.60)	8.80 (4.60)	8.76 (4.40)	8.76 (4.40)	8.69 (3.97)	8.69 (3.97)
Kendall's value	**0.299**	**0.299**	**0.137**	**0.150**	**0.150**	**0.121**	**0.121**	**0.071**	**0.066**	**0.085**	**0.085**	**0.078**	**0.078**

Values in parenthesis is mean rank score based on Kendall's W which was significant

**Significant at 1% level

AC- Alkalised Chocolates, NAC – Non alkalized chocolates, ODJ – Osmodehydrated Jack fruit, A-Almond, WPP- White Pepper Powder

Table 58. Effect of storage on the mean score for over all acceptability of blended alkalised and non alkaliseed chocolates

Treatments	Initial	1st Month		2nd Month		3rd Month		4th Month		5th Month		6th Month	
		15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days
T_0 (100% AC)	8.84 (3.23)	8.84 (3.23)	8.84 (3.25)	8.82 (3.25)	8.82 (3.25)	8.80 (3.37)	8.76 (3.30)	8.73 (3.50)	8.73 (3.50)	8.71 (3.33)	8.69 (3.67)	8.67 (3.77)	8.67 (3.77)
T_1 -(95 % AC+5% ODJ)	9.00 (5.10)	9.00 (5.10)	8.98 (4.97)	8.93 (4.88)	8.93 (4.88)	8.91 (4.93)	8.89 (4.90)	8.87 (4.87)	8.87 (4.87)	8.84 (4.87)	8.82 (4.93)	8.80 (4.97)	8.80 (4.97)
T_2 (95 % AC+5% A)	9.00 (5.10)	9.00 (5.10)	9.00 (5.31)	8.98 (5.44)	8.98 (5.44)	8.96 (5.37)	8.93 (5.33)	8.91 (5.30)	8.91 (5.30)	8.89 (5.30)	8.87 (5.33)	8.85 (5.33)	8.85 (5.33)
T_3 (95 % AC+5% WP)	9.00 (5.10)	9.00 (5.10)	9.00 (5.31)	8.96 (5.22)	8.96 (5.22)	8.93 (5.13)	8.91 (5.10)	8.89 (5.10)	8.89 (5.10)	8.87 (5.13)	8.80 (4.70)	8.76 (4.50)	8.76 (4.50)
T_0 (100% NAC)	8.76 (2.17)	8.76 (2.17)	8.73 (2.13)	8.71 (2.25)	8.71 (2.25)	8.69 (2.37)	8.67 (2.37)	8.64 (2.40)	8.64 (2.40)	8.62 (2.43)	8.60 (2.60)	8.60 (2.60)	8.60 (2.60)
T_4 -(95 % NAC+5% ODJ)	9.00 (5.10)	9.00 (5.10)	8.98 (4.97)	8.93 (4.88)	8.93 (4.88)	8.91 (4.93)	8.89 (4.90)	8.87 (4.93)	8.87 (4.93)	8.84 (4.97)	8.82 (5.03)	8.80 (5.03)	8.80 (5.03)
T_5 (95 % NAC+5% A)	9.00 (5.10)	9.00 (5.10)	9.00 (5.31)	8.96 (5.22)	8.96 (5.22)	8.93 (5.13)	8.93 (5.37)	8.91 (5.37)	8.91 (5.37)	8.89 (5.40)	8.84 (5.27)	8.82 (5.23)	8.82 (5.23)
T_6 (95 % AC+5% WPP)	9.00 (5.10)	9.00 (5.10)	8.96 (4.75)	8.93 (4.88)	8.93 (4.88)	8.89 (4.77)	8.87 (4.73)	8.82 (4.53)	8.82 (4.53)	8.80 (4.57)	8.75 (4.47)	8.73 (4.50)	8.73 (4.50)
Kendall's value	**0.617**	**0.617**	**0.543**	**0.401**	**0.401**	**0.370**	**0.329**	**0.276**	**0.276**	**0.309**	**0.229**	**0.213**	**0.213**

Values in parenthesis is mean rank score based on Kendall's W which was significant

**Significant at 1% level

AC- Alkalised Chocolates, NAC – Non alkalized chocolates, ODJ – Osmodehydrated Jack fruit, A-Almond, WPP- White Pepper Powder

4.4.1.3. Enumeration of total micro flora of blended chocolates during storage

The blended chocolates were evaluated for the presence of bacteria, yeast and mould initially and monthly intervals for a period of six months at refrigerated conditions and are presented in Table 59 to 61 and Figure 33 to 35.

4.4.1.3.1. Total bacterial count of bended chocolates during storage

As revealed in Table 59, the bacterial count was not detected from initial to fourth month of storage in all treatments during storage. But in fifth and sixth month of storage the bacterial count was detected. Based on one way ANOVA, statistical difference was observed between the treatments during fifth and sixth month of storage. During fifth month of storage the lowest bacterial count of $0.33x\ 10^4$ cfu g-1was in treatment T_1, T_4, T_5, and T_6 and highest (0.66×10^4 cfu g-1) in T_0 (100% AC), T_0 (100% AC), T_2 , T_3 and T_0 (100% NAC). At the end of sixth month of storage the highest bacterial count of 2.0×10^4 cfu/g was found in treatments T_1, T_2,T_3,T_4,T_5 and T_6 and lowest (1.66×10^4 cfu g-1) in controls (T_0 -100% AC and T_0 -100% NAC). Regarding storage periods, based on t test, statistically significant difference was recorded during fifth and sixth month of storage.

4.4.1.3.2. Total fungal count of bended chocolates during storage

The fungal count was not detected from initial to fifth month of storage in all treatments during storage. During sixth month of storage, the fungal count was detected and based on one way ANOVA, the lowest count (1.33×10^3 cfu g-1) was in treatment T_0 (100% AC), T_2 (95 % AC+5% A),T_3 (95 % AC+5% WPP), T_0 (100% NAC) and T_4 (95 % NAC+5% ODJ) and highest (1.66×10^3 cfu/g) in T_1(95 % AC+5% ODJ), T_5 (95 % NAC+5% A) and T_6 (95 % AC+5% WPP) (Table 60). Regarding to storage periods t test shows statistically significant difference at sixth month of storage.

4.4.1.3.3. Total yeast count of bended chocolates during storage

From the Table 61, it is clear that the yeast count of blended chocolates during storage was not detected initially and up to fifth month of storage. During sixth month of storage, the presence of yeast was detected and based on one way ANOVA, statistically significant difference was observed between the treatments. The lowest (2.0 x 10^3 cfu g-1) yeast count in blended chocolates was in treatment T_1 (95 % AC+5% ODJ), T_0 (100% NAC), T_4 (95 % NAC+5% ODJ) and T_5 (95 % NAC+5% A) and highest (2.66 x 10^3 cfu g-1) in T_6 (95 % AC+5% WPP). Regarding storage periods, t test shows significant difference at sixth month of storage.

Table. 59. Total bacterial count of blended chocolates during storage

Treatments	Total bacterial count (x 10^4 cfu g-1)						
	Initial	1st month	2nd month	3rd month	4th month	5th month	6th month
T_0 (100% AC)	ND	ND	ND	ND	ND	*0.66^a (4.660)	*1.66^b (8.722)
T_1 -(95 % AC+5%ODJ)	ND	ND	ND	ND	ND	*0.33^b (4.552)	*2.0^a (8.145)
T_2 (95 % AC+5% A)	ND	ND	ND	ND	ND	*0.66^a (4.660)	*2.0^a (8.722)
T_3 (95 % AC+5%WPP)	ND	ND	ND	ND	ND	*0.66^a (4.588)	*2.0^a (9.096)
T_0 (100% NAC)	ND	ND	ND	ND	ND	*0.66^a (4.624)	*1.66^b (8.864)
T_4 -(95 % NAC+5%ODJ)	ND	ND	ND	ND	ND	*0.33^a (4.552)	*2.0^a (8.722)
T_5 (95 % NAC+5% A)	ND	ND	ND	ND	ND	*0.33^a (4.660)	*2.0^b (8.528)
T_6 (95 % AC+5% WPP)	ND	ND	ND	ND	ND	*0.33^b (4.660)	*2.0^{bb} (8.722)
CV	-	-	-	-	-	0.076	2.207
CD	NS	NS	NS	NS	NS	0.062	0.322

AC- Alkalised Chocolates, NAC – Non alkalised chocolates, ODJ – Osmodehydrated Jack fruit, A- Almond, WPP- White Pepper Powder
DMRT column wise comparison
Values with same superscripts do not vary significantly
T test row wise comparison *- Significant aT1% level, ND-Not detected

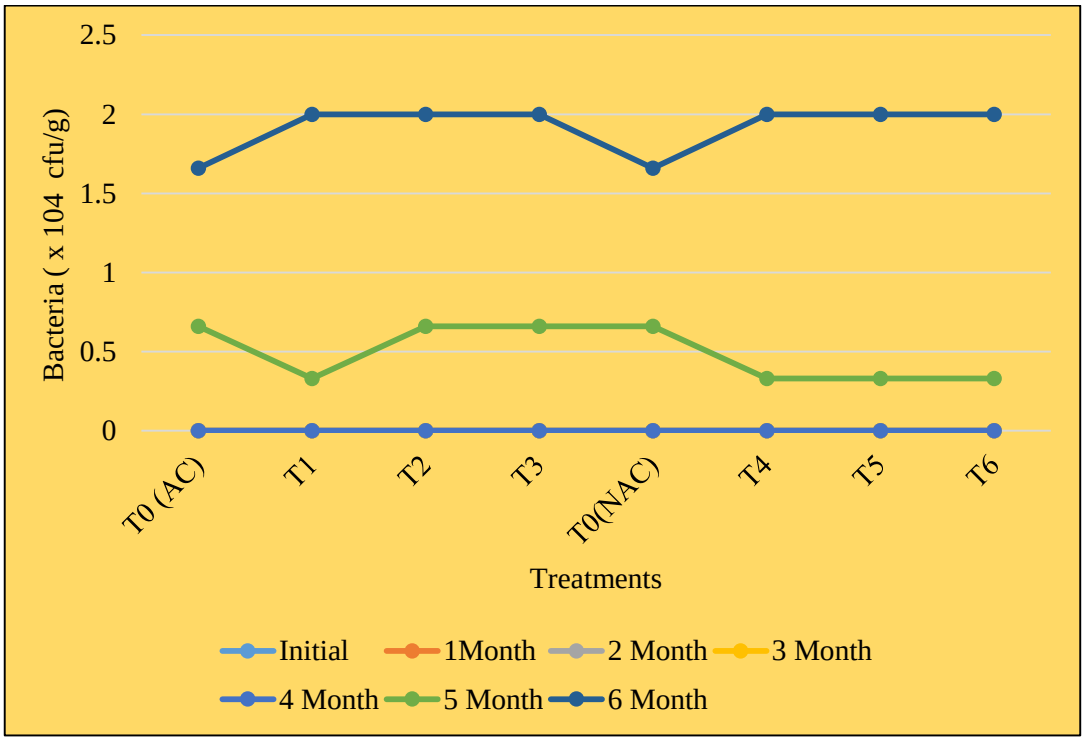


Fig.34. Total bacterial count of blended chocolates during storage

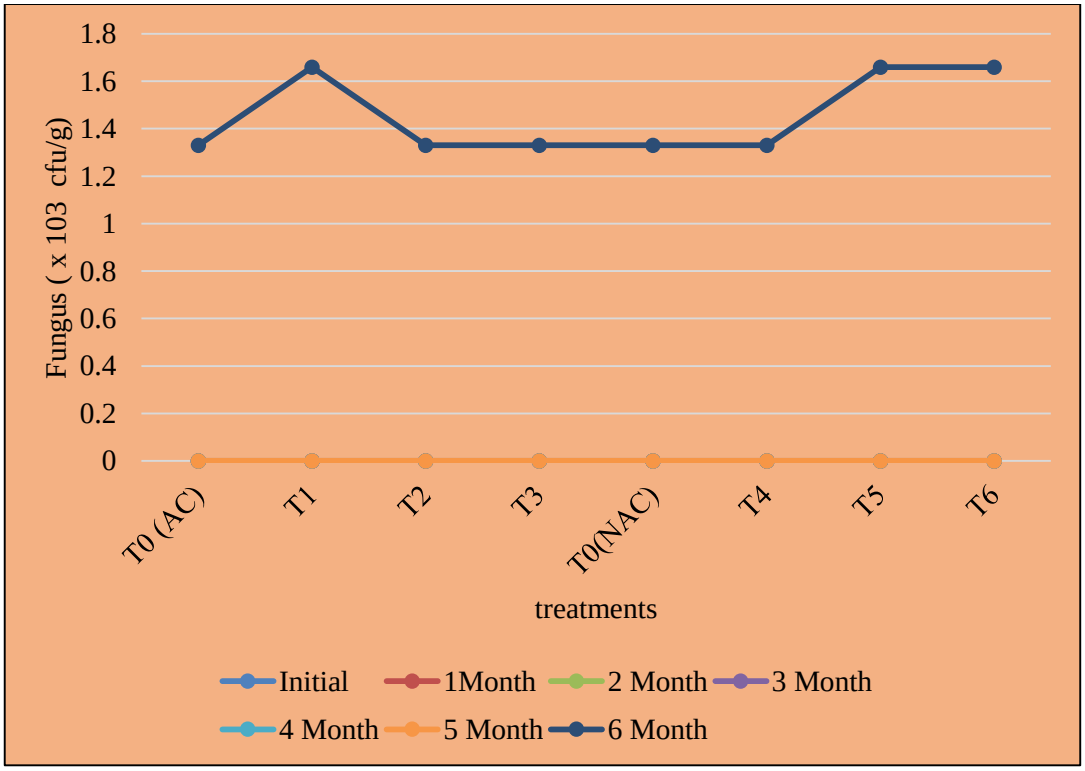


Fig.35. Total fungal count of blended chocolates during storage

Table. 60. Total fungal count of blended chocolates during storage

Treatments	Total fungal count (x 10^3 cfu g-1)						
	Initial	**1st month**	**2nd month**	**3rd month**	**4th month**	**5th month**	**6th month**
T_0 (100% AC)	ND	ND	ND	ND	ND	ND	*1.33[b] (6.012)
T_1 -(95 % AC+5% ODJ)	ND	ND	ND	ND	ND	ND	*1.66[a] (6.732)
T_2 (95 % AC+5% A)	ND	ND	ND	ND	ND	ND	*1.33[ab] (6.213)
T_3 (95 % AC+5% WPP)	ND	ND	ND	ND	ND	ND	*1.33[ab] (6.012)
T_0 (100% NAC)	ND	ND	ND	ND	ND	ND	*1.33[ab] (6.213)
T_4 (95 % NAC+5% ODJ)	ND	ND	ND	ND	ND	ND	*1.33[ab] (6.544)
T_5 (95 % NAC+5% A)	ND	ND	ND	ND	ND	ND	*1.66[a] (6.544)
T_6 (95 % AC+5% WPP)	ND	ND	ND	ND	ND	ND	*1.66[a] (6.012)
CV (%)	-	-	--	-	-		4.166
CD (0.05)	-	-	-	-	-		0.455

AC- Alkalised Chocolates, NAC – Non alkalised chocolates, ODJ – Osmodehydrated Jack fruit, A-Almond, WPP- White Pepper Powder
DMRT column wise comparison
Values with same superscripts do not vary significantly
T test row wise comparison
*- Significant aT_1% level
**- Significant at 5% level, ND-Not detected

Table. 61. Total yeast count of blended chocolates during storage

Treatments	Total yeast count (x 10^3 cfu g-1)						
	Initial	**1st month**	**2nd month**	**3rd month**	**4th month**	**5th month**	**6th month**
T_0 (100% AC)	ND	ND	ND	ND	ND	ND	*2.33^{bc} (8.912)
T_1 (95 % AC+5% ODJ)	ND	ND	ND	ND	ND	ND	*2.0d^{e} (8.158)
T_2 (95 % AC+5% A)	ND	ND	ND	ND	ND	ND	*2.33^{bc} (8.532)
T_3 (95 % AC+5% WPP)	ND	ND	ND	ND	ND	ND	*2.33^{bc} (8.722)
T_0 (100% NAC)	ND	ND	ND	ND	ND	ND	*2.0^{de} (8.532)
T_4 (95 % NAC+5% ODJ)	ND	ND	ND	ND	ND	ND	*2.0^{de} (8.331)
T_5 (95 % NAC+5% A)	ND	ND	ND	ND	ND	ND	*2.0^{de} (8.187)
T_6 (95 % AC+5% WPP)	ND	ND	ND	ND	ND	ND	*2.66^{a} (9.096)
CV (%)	-	-	-	-	-	-	2.724
CD (0.05)	-	-	-	-	-	-	0.337

AC- Alkalised Chocolates, NAC Non alkalised chocolates, ODJ Osmodehydrated Jack fruit, A-Almond, WPP- White Pepper Powder
DMRT column wise comparison
Values with same superscripts do not vary significantly
t test row wise comparison
*- Significant at1% level
**- Significant at 5% level, NS-Not significant
ND-Not detected

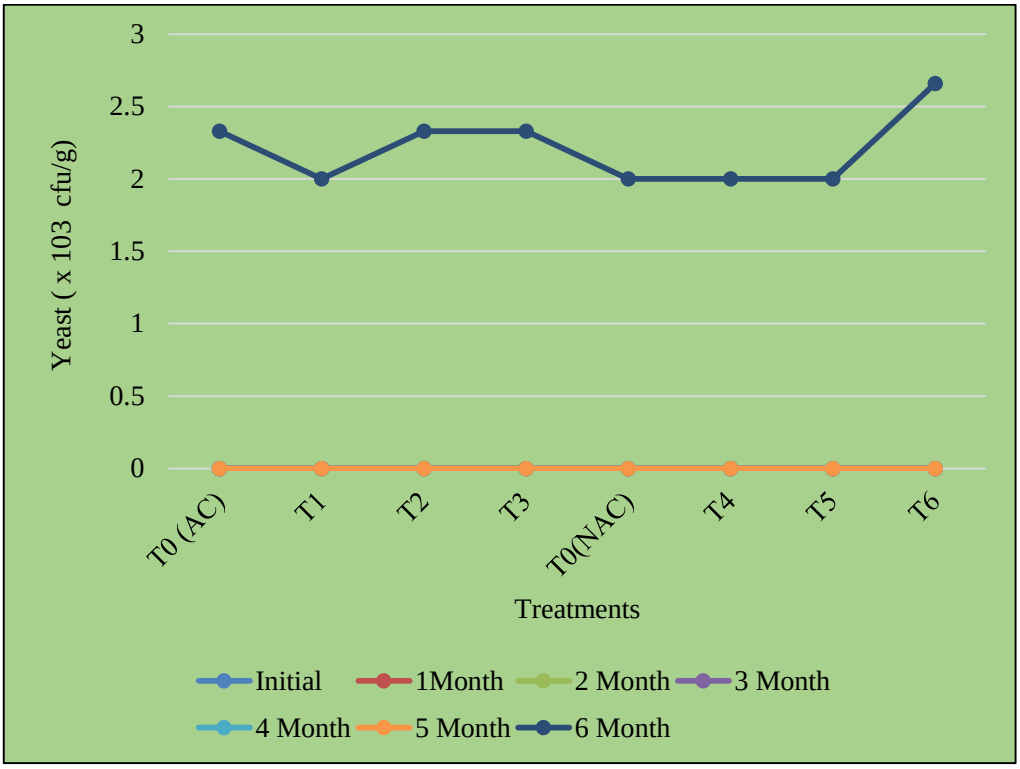


Fig.36. Total yeast count of blended chocolates during storage

4.5. Cost of production of the selected chocolates

The cost of production of blended chocolates is presented in table 62 to 65. For the calculation of unit cost, the total cost of production is divided with the number of chocolate bar units (40gm) produced. The cost varied from Rs.46.54 to Rs.50.62 / 40 g of chocolate bar. The lowest cost was found to be in the chocolates(Controls- T_0 (AC)/ T_0 (NAC) prepared from alkalised and non alkalised cocoa nibs and the highest cost (Rs.50.62) was for the chocolates blended with 5% almond (T_2 95 % AC+5% A and T_5 -95 % NAC+5% A),which was followed by Rs. 49.40/40gm in chocolates blended with osmodehydrated jack fruit T_1 (95 % AC+5% ODJ) and T_4 (95 % NAC+5% ODJ). The cost of chocolates blended with 5% white pepper powder was found to be Rs. 48.26/ 40 gm.

Table 62. Cost of production of chocolate, T_0 (AC)/ T_0 (NAC)

No	Items	Quantity Used	Cost (Rs/-)
1.	**Raw materials**		
	Cocoa nibs	800gm	750
	Cocoa butter	1 kg	750
	Sugar	2 kg	72
	Milk powder	1kg	245
	Vanilla powder	25gm	5.00
2.	**Other items**		
	Electricity charges	0.75 units/h	23.10
	Labour@ Rs. 400 for 8 hours	2 hours	100
	Aluminium foil	42	10
			1955

Cost of 42 bar of chocolates : Rs. 1955
Cost of unit (40gm) bar of chocolate : Rs. 46.54

Table 63. Cost of production of chocolate, T_1 and T_4 (Chocolates blended with osmodehydrated jack fruit)

No	Items	Quantity Used	Cost (Rs/-)
1.	**Raw materials**		
	Cocoa nibs	800gm	750
	Cocoa butter	1 kg	750
	Sugar	2 kg	72
	Milk powder	1kg	245
	Vanilla powder	25gm	5.00
	Osmodehydrated jack fruit	240gm	120
2.	**Other items**		
	Electricity charges	0.75units/h	23.10
	Labour@ Rs. 400 for 8 hours	2 hours	100
	Aluminium foil	42	10
			2075

Cost of 42 bar of blended chocolates: Rs. 2075
Cost of unit (40gm) bar of chocolate: Rs. 49.40

Table 64. Cost of production of chocolate, T_2 and T_5 (Chocolates blended with almonds)

No	Items	Quantity Used	Cost (Rs/-)
1.	**Raw materials**		
	Cocoa nibs	800gm	750
	Cocoa butter	1 kg	750
	Sugar	2 kg	72
	Milk powder	1kg	245
	Almond	240gm	171
	Vanilla powder	25gm	5.00
2.	**Other items**		
	Electricity charges	0.75 units/h	23.10
	Labour@ Rs. 400 for 8 hours	2 hours	100
	Aluminium foil	42	10
			2126

Cost of 42 bar of blended chocolates : Rs. 2126
Cost of unit (40gm) bar of chocolate : Rs. 50.62

Table 65. Cost of production of chocolate, T_3 and T_6 (Chocolates blended with white pepper powder)

No	Items	Quantity Used	Cost (Rs/-)
1.	**Raw materials**		
	Cocoa nibs	800gm	750
	Cocoa butter	1 kg	750
	Sugar	2 kg	72
	Milk powder	1kg	245
	Pepper	240	72
	Vanilla powder	25gm	5.00
2.	**Other items**		
	Electricity charges	0.75 units/h	23.10
	Labour@ Rs. 400 for 8 hours	2 hours	100
	Aluminium foil	42	10
			2027

Cost of 42 bar of blended chocolates : Rs. 2027
Cost of unit (40gm) bar of chocolate : Rs. 48.26

Discussion

DISCUSSION

Cocoa (*Theobroma cacao L.*) is a perinial crop of enormous economic importance in the world and the key raw material for chocolate production. Chocolate production consists of mainly two stages, primary processing and secondary processing. Primary processing includes harvesting of cocoa beans, fermentation and drying and the secondary processing include roasting, nib grinding, refining, conching and tempering (Magi *et al*., 2012). Fermentation, drying and roasting are critical to the development of flavour precursors that generate the distinctive chocolate flavour during chocolate manufacture. These processes also lead to reduction in acidity and free fatty acids of nibs, which dictates the levels of bitterness and colour development in chocolates (Afoakwa *et al*., 2013). High free fatty acid content is a serious quality defect and reduces the economic value of the cocoa beans. So a reduction in the free fatty acids level during processing and product development will have a great impact.

The cocoa beans are rich in hundreds of compounds, this volatile fraction give the characteristic flavours of cocoa beans. Flavour is significant to the acceptability of cocoa beans and cocoa products such as chocolate. Quality and flavour of cocoa products strongly depend on the various stages of cocoa processing (Saltini *et al.*, 2013).

Hence, in the present study, a protocol were standardized for primary processing of cocoa beans and for chocolate production based on free fatty acid content (<1.75%). Products such as chocolates were developed and evaluated for their quality attributes. The results of the study entitled 'Process optimisation and quality evaluation of cocoa based chocolates' are discussed under the following headings.

5.1. Development of protocol for primary processing of cocoa

5.1.1. Standardisation of fermentation based on free fatty acid content

5.1.2. Physico-chemical analysis of fermented beans

5.1.3. Standardisation of drying methods based on free fatty acid

5.1.4. Physico-chemical analysis of dried beans

5.1.5. Standardisation of method of storage based on free fatty acid content

5.1.6. Physico-chemical analysis of stored beans

5.1.7. Microbial load on stored cocoa beans

5.2. Alkalisation of stored beans

5.3. Standardisation of time and temperature for chocolate making using machine

5.3.1. Physico-chemical analysis of chocolates

5.3.2. Organoleptic evaluation of chocolates

5.3.3. Enumeration of total micro flora

5.4. Blending of chocolates with other ingredients

5.4.1. Quality evaluation of selected blended chocolates

5.4.1.1. Physico- chemical analysis

5.4.1.2. Organoleptic evaluation

5.4.1.3. Enumeration of total micro flora

5.5. Cost of production of the selected chocolates

5.1. Development of protocol for primary processing of cocoa

Fermentation and drying are the two major steps of the primary processing of cocoa beans (*Theobroma cacao* L.) (Hii *et al.,* 2009). According to Nielson *et al*. (2007) heap and box methods are the two widely used methods of fermentation. Guehi *et al*. (2010) also reported that different fermentation methods are used for fermenting cocoa beans depending on farmers, areas and countries. Camu *et al.* (2008) reported that platforms, heaps, baskets, and boxes are the most used fermentation methods.

5.1.1. Standardisation of fermentation based on free fatty acid content

In the present study the cocoa beans were subjected to three different types of fermentation like basket method, heap method and sack method for the periods of 5^{th}, 6^{th} and 7^{th} day of fermentation. The physico- chemical qualities of fermented beans using basket, heap and sack methods were carried out initially and on fifth, sixth and seventh day of fermentation. Thompson *et al*. (2001) reported that six to seven days are taken for completing the fermentation of cocoa beans. The cocoa beans are mixed thouroughly in alternate days to ensure even fermentation. Schwan and Wheals (2004) opined that the longer fermentation causes the growth of unwanted moulds and resulted information off-flavours.

5.1.2. Physico-chemical analysis of fermented beans

Cut test or fermentation index was used to determine the extent of fermentation of fermented cocoa beans. Based on the colour, beans were characterized into fully fermented, partially fermented and not fermented. White colour at center or full dark brown colour at the periphery indicated as fully fermented, partly pink colour or brown colour across and along margin indicated as partially fermented beans and fully purple colour indicated as not fermented (Amma *et al*., 2002).

Fermentation index or cut test score was observed highest in heap method at seventh day of fermentation with 84.99 per cent of fully fermented beans. The treatment T_3 (basket at seventh day of fermentation), T_5 (heap at sixth day of fermentation), T_6 (heap at seventh day of fermentation) and T_9 (sack at seventh day of fermentation) showed greater than or equal to 70per cent fermentation index (FI). Sunilkumar *et al*. (2008) reported that highest mean fermentation index through cut test score was 63.76 per cent at seventh day of fermentation in heap method. Ajmal (2016) also reported a highest fermentation index of 78 per cent in fully fermented beans at the end of basket method of fermentation.

Optimum fermentation index aid in the improvement of quality of cocoa products and it results in the development of flavour and reduction in sourness, astringency and bitterness through biochemical reactions (Meyer *et al*., 1989; Biehl *et al*., 1990). Misnawi *et al*. (2002) reported that the purple beans indicate the unfermented cocoa beans and the percentage of purple beans was high in cocoa beans fermented for 3, 4 and 5 days. Guehi *et al*. (2010) also reported that a higher per cent of purple beans was high in cocoa beans fermented for 4 days than in cocoa beans fermented for 5 days. Under-fermented beans usually produce high per cent of purple beans, and cocoa with high percentage of purple beans gives a bitter and astringent chocolate. Peláez *et al*. (2016) reported that the highest percentage of fermented beans was 91.67 per cent with semi-mechanised way and 87.30 per cent in manually fermented cocoa beans with cocoa beans transfer between wooden fermentation boxes. Alvarez *et al.* (2010) found that the proportion of brown beans increases with decreasing cocoa pulp acidity. Cut test is a measure of the fermentation index. The oxidation of anthocyanins in cocoa beans resulted in colour change of fermented cocoa beans. The gray and purple beans are characteristic of poorly fermented beans and brown beans are typical of good fermentation (Hernandz, 2015). The findings of the present study was on par with the results of Hernandz (2015). In his study, when the fermentation was stopped (day seven), 68 per cent brown beans were obtained in boxes, 85.33 per cent in 300 kg boxes, 79.33 per cent in 100kg boxes, and 82.66 per cent in the rotary drum.

The good quality beans show high fermentation index because it require less time for maximum fermentation and high fermentation index is the characteristics of heap method fermentation. In the present study, the high fermentation index was observed in heap method at seventh day of fermentation. It can be explained by the fact, that the heap method contain high amount of cocoa beans for fermentation compared to basket and heap method and thus increased the fermentation index with highest per cent of fully fermented beans at seventh day of fermentation.

In the present study, highest fermentation bean recovery was recorded in treatments T_1 (Basket), T_4 (Heap) and T_7 (Sack) at fifth day of fermentation. But fermentation index was highest at seventh day of fermentation in all three methods of fermentation. Since the high fermentation index of fully fermented beans is the major criteria for assessing the quality of beans, the fermented bean recovery at seventh day of fermentation had taken in to account for further studies. Fermentation bean recovery was recorded for different methods of fermentation in a range from 52 to 76 per cent at seventh day of fermentation. Amma *et al*. (2009) reported that heap method is a large scale traditional standard method for fermentation of cocoa beans and have high fermentation bean recovery while compared to other small scale traditional standard methods. In the present study also the treatment T_6 (heap method at seventh day of fermentation) have the highest fermentation recovery of 76 per cent. The highest bean recovery in heap method may be due to the high quantity of cocoa bean in heap method (50 kg), but for sack and basket method it was 3 and 2 kg respectively. So this difference in quantity of beans is the reason for significant changes for all the three types of fermentation namely basket, heap and sack method fermentation.

The moisture content of all fermented cocoa beans in three different methods were found to decrease during various fermentation periods (Fig.4). But significant difference in moisture content was not observable between fermentation periods and methods. Minimum moisture content of 37 .83 per cent at seventh day of fermentation was observed in treatment T_6 (heap method at seventh day of fermentation).

The moisture content in the fermented cocoa beans reduced due to cell rupture and release of intracellular juices by microbial action in cocoa pulp. Earlier study conducted by Rodriguez *et al.* (2012) had reported a moisture content of 43.7, which is similar to the findings of present study. Pelaez *et al*., (2016) also reported that the mean moisture content of cocoa beans was statistically different ($p \leq 0.05$) throughout the fermentation process and may be due to the variety and maturity of the fruit. In his study the highest moisture content of 51.67 per cent was in fresh

beans and after 168 hours of fermentation, the moisture content reduced to 46.33 per cent.

The pH of the cocoa beans varied from 5.93 to 4.28 in basket method, 6.11 to 4.38 in heap method and 5.82 to 4.26 in sack method during fermentation. At the end of fermentation, pH decreased compared to initial pH in all methods of fermentation (Table.9). Schwan and Wheals (2004) opined that the changes in pH values during fermentation are very important for microbial activity. Rivera *et al.* (2012) indicated that acids produced by microorganisms during fermentation cause an increase in acidity and consequent decrease in pH. In the present study also a steady decline in pH with days of fermentation were noticed in all methods of fermentation. Unfermented cocoa beans contain a very low amount of free amino acids (Misnawi *et al.*, 2002). So the initial pH is high compared to fermented beans. Low pH at the beginning of fermentation favours proteolysis and the liberation of free amino acids, while too low pH reduces the flavour precursors and leads to over-acidification of the final fermented beans (Camu *et al.*, 2008). Hernandez (2015) also analysed the pH of four different cocoa (*Theobroma cacao* L.) fermentation conditions and he observed a decrease in pH from 6.31 ± 0.40 to 4.76 ± 0.03 and an increase in acidity due to the formation of organic acids. These results are similar to the findings of present study, in which pH decreased from 6.11 to 4.38.

According to Bonvehi (2005) the increased levels of organic acids breakdown the sugars from the pulp surrounding of the cocoa beans and reducing the pH during fermentation. Proteolysis in the seeds takes place within 24 h after destruction of the cells and acidification by acetic acid (Ziegleder, 2009). Aculey *et al*. (2010) also noted an increased level of organic acids such as propanoic acid, 2-methylpropanoic acid, 3-methylbutanoic acid and acetic acid after 72 h of cocoa fermentation. Wallace and Guehi *et al*. (2010) observed that the heterogeneity in the size of the cocoa beans, as well as the construction material of the baskets and method of fermentation significantly affects the pH value and the presence of purple beans.

Apriyanto (2016) also reported that the pH decreased during fermentation periods and thereby increasing acidity. The citric acid content in fermented cocoa beans, increased the acidity, and it was preferable for growth of yeast as fermentation was on the process. The dominant microbes was yeasts with good pectinolytic activity, and they degraded cocoa beans pulp to produce organic acids especially citric acid. Citric acid will change the fermentation conditions and makes it for the growth of bacteria.

The fat content of the fermented beans as observed in this study were slightly lower than the reported values. This findings was similar to the findings of Dand (2011). He also reported that smaller beans size results in lower fat yield. Initial fat content of fermented beans was 40.44 per cent in basket method, 42.66 per cent in heap method and 41 per cent in sack method and at the end of fermentation the fat content become 31 per cent, 32.89 per cent and 32.55 per cent in all three methods of fermentation (T_3, T_6 and T_9) respectively (Figure. 4. 5). But no significant difference was observed with respect to the fermentation methods and period of fermentation. Wood and Lass (1985) opined that generally, the initial fat content ranged from 52.27 – 55.21per cent in unfermented beans. Jonfia and Navarro (2016) found that the fat content was decreased with the increasing the fermentation periods, the fat content was decreased from 51.95 to 48.82 per cent with the increase in fermentation periods. It is on par with the findings of our study. The enzyme lipase is responsible for lipid breakdown in plants and produce the energy required for plant growth and this lipid breakdown increased the fat content in initial non-fermented beans (Imeson *et al*., 1993).

The fat content of the fermented cocoa beans decreased with fermentation and pod storage (Aremu *et al*.,1995). The author also found that the lipid content of the cocoa beans decreased from 62.9 per cent to 55.7 per cent by the sixth day of fermentation. This suggests that the reductions in fat content in cocoa beans could be avoided by reducing fermentation time. The consistent decrease in fat content

noted with increasing pod storage might have resulted from the action of lipase enzymes which breakdown the triglyceride in the beans into its separate groups of fatty acids, thereby increasing the free fatty acids levels leading to the production of rancid flavour in the beans from the prolonged stored pods.

In the present study a decrease in lipase activity with advancement of fermentation periods was observed in all three methods of fermentation (Fig. 4.6). Changes in the temperature of the beans during fermentation seem to affect the lipase activity (Samah *et al.,* 1998). A reduction in lipase activity due to decrease in pH of cotyledon was also reported by Abd *et al*. (1998). Hansen *et al*. (1998) opined that many enzymes are inactivated during fermentation, the actual period of enzyme action is short and several key enzymes are not completely inactivated during fermentation. Some enzyme reactions can continue throughout the fermentation process. The activity of enzymes increased in the early days of fermentation with the microorganisms growth especially fungi and yeast (Sousa *et al.,* 2016).

Wood and Lass (1985) opined that foodstuffs with high moisture content were easily attacked by moulds, these moulds could produce lipase. The enzyme lipase is responsible for lipid breakdown in plants to produce the energy required for plant growth (Imeson *et al*., 1993). During cocoa beans fermentation, the rise in temperature and diffusion of acid products occur through the cotyledons due to partial cell walls lysis, hence leading to death of germ and prevent germination (Barel, 1998).

The quality of final product depends upon the fermented dried cocoa beans. An increase in the percentage of free fatty acid is one of the clear indications of deterioration in cocoa quality (Dand, 2011). Free fatty acids are carboxylic acids released from triglycerides through the effect of a lipase (Guehi *et al*., 2008). Higher FFA content leads to quality reduction in fermented cocoa beans. Free fatty acid must be considered as a commercial value reducing factor of raw cocoa both for

producers and chocolate manufacturers (Pontillon, 1998). The directive 73/241/EEC (EEC, 1973) limits the maximum FFA content to 1.75 per cent oleic acid in cocoa butter.

In the present study the initial free fatty acid content was in the range of 2.39 to 2.51 per cent in three fermentation methods and it was found to decrease with increasing fermentation periods. Though at the end of fermentation the free fatty acid content was below the critical limits of 1.75 per cent in all three methods of fermentation (Table 12 and Figure 8). The free fatty acid content (%FFA) generally decreased with fermentation periods. The findingsof the present study confirm the results of Simplice *et al.* (2003), who revealed that the formation of free fatty acid in fermented cocoa beans have a crucial effect on duration of cocoa beans fermentation. Afoakwa *et al.* (2013) also observed that the free fatty acid content was 0.87 per cent after fermentation of cocoa beans and this result was similar to the results of present study, in which the free fatty acid content of fermented cocoa beans was 0.86 per cent after fermentation.

According to Guenot *et al*. (1996) the lower the quality of the beans due to the higher initial FFA content of the raw cocoa beans. Wood and Lass (1985) had reported that due to high moisture content of fresh beans the moulds were easily attacked and these moulds could produce lipase and leads to release of free fatty acid from triglycerides. Cros (2001) also opined that the FFA content of healthy cocoa beans was very low and fermentation process leads to fluctuations in FFA contents. Jonfia (2016) also recorded the FFA content increased in fermented cocoa beans with the duration of fermentation.

The temperature profile in all three fermentation methods inside the fermentation mass was increased with advancement of fermentation periods. The maximum temperature was observed at fourth day of fermentation in all three methods (Fig.9). During the the first two days of fermentation the temperature of fermented mass ranged between 27 and 34°C. By the fourth day the temperature

increased to 49°C and remaining constant until the end of the fermentation process without significant differences among treatments. The temperature rise during fermentation in this study is in line with the findings of Hernardz (2015), who reported that the temperature rise in the fermentation process of the cocoa bean mass is not affected by increasing the amount of cocoa or by the rotation.

Samah *et al*. (1998) had reported that during fermentation, simultaneous development of yeasts, lactic acid bacteria and acetic acid bacteria has been found and is the main reason for increase in acidity. According to Nielsen *et al*. (2007) the temperature rise is caused by the energy released in the exothermic reaction and the conversion of ethanol into acetic acid by acetic acid bacteria. Camu *et al*. (2008) also opined that temperature increases during fermentation causes the death of the embryo and changes in the tissue structure of the cotyledon.

Buamah (1997) had reported that the temperature during fermentation within 24 to 28 hours was 45 °C in fermented beans. Effendi and Panji (1994) also stated that the pod storage and subsequent fermentation of beans resulted in a tremendous increase in temperature. The rise in temperature of the fermenting mass could be taken as an indication of adequate favourable biochemical reaction during fermentation and the lack of temperature development as a symptom of inadequate fermentation (Amma *et aI.,* 2002). Sunilkumar (2005) had reported that the maximum temperature attained in fermented mass when the beans from pods stored for four days.

5.1.3. Standardisation of drying methods based on free fatty acid

The second step of primary processing of cocoa beans was drying. Drying is an excellent way to preserve cocoa and artificial dryers are appropriate food preservation technology for sustainable development. Natural cocoa bean drying is directly dependent on weather conditions. In the present study after fermentation, the heap method of fermentation at seventh day were subjected to sun drying and

artificial drying and below mentioned quality parameters were carried out in the study.

5.1.4. Physico-chemical qualities of dried beans

The recovery per cent of cocoa beans was more in sun dried beans compared to oven dried beans. This could be attributed to the higher moisture level retained in the sun dried samples (4.42 per cent) compared to oven dried beans (3.37 per cent). The results of this present study is on par with the observations recorded by Sunilkumar (2005). In his study he recorded a bean recovery of 39.09 per cent in sun dried beans and 33.23 per cent in oven dried beans and he opined that a higher recovery per cent in sun dried beans may be due to the higher moisture content in sun dried beans. The findings is tuned with the range (23-46%) of cocoa bean recovery by drying as reported by Amma *et al*. (2009) and a recovery of 37per cent have been reported by PHAMA (2018). Afoakwa (2015) reported that the bean recovery of dried beans varied from 30 to 46 per cent depending up on the season and variety of cocoa.

Drying is usually terminated when the dried beans moisture content is as low as 7 per cent (Oke and Omotayo, 2012). In the present study the moisture content was less (3.76 per cent) in oven dried beans than that of moisture content of 4.22 per cent in sun dried cocoa beans. Hii *et al*. (2000) reported that in oven drying at 60^0C the moisture content was 3.61per cent. Sunilkumar (2005) opined that there was rapid moisture removal in oven than in sun drying.

According to Fagunwa *et al*., (2009) an increase in the temperature gain of the drying air in artificial drying may cause more evaporation of water from cocoa beans than sun drying. Ajmal (2015) also 0 reported that moisture content among the hybrids and CCRP (Cadbury Cocoa Research Project) varieties of cocoa after sun drying was observed in the range from 3.79% to 7.45%. Deus *et al*. (2018) had observed that the cocoa beans show low moisture content when subjected to oven

drying compared to sun drying. Artificial drying of fermented cocoa beans has mostly concentrated on the removal of moisture from the beans at the shortest possible time (Dand, 2011and Hi *et al*., 2009).

In the present study, the pH value was significantly high in sun dried beans (5.29) compared to oven dried beans (4.89). This findings is on par with the findings of Sunilkumar (2005). In his study maximum pH was observed as 5.82 and minimum pH as 5.14. The pH of sun-dried beans is usually higher (less acidic) than artificially dried beans because of the slow and gentle drying process that enable the evaporation of more acetic acid (Hii *et al*., 2009). Too slow drying results in lower pH, the absence of the optimal color of cocoa beans and increased growth of molds (Zahouli *et al.*, 2010; Rodriguez-Campos *et al.*, 2012; Hurst *et al.*, 2011).

In the present study the total fat content of dried beans in sun dried and oven dried beans did not differ significantly. Fagunwa *et al*. (2009) also reported that the fat content in dried beans shows 41.6%. In another study reported by Jonffia *et al*. (2016) the fat content ranged from 47.99 to 51.72 per cent in the dried cocoa beans. PHAMA (2018) also had reported that the fat content in sun dried cocoa beans was 50 per cent, this value was almost on par with the value of total fat (48.33 per cent) of present study in sun dried beans.

The lipase activity was 0.0018µeq in sun dried cocoa beans and 0.0027 µeq in T_2 (oven dried beans), but there was no significant difference in lipase activity in sundried and oven dried beans. According to Hansen *et al*. (1999) the enzyme polyphenol oxidase was strongly inactivated during sun and artificial drying of the beans. The other enzymes were stable during the drying process. Enzymes like lipases, endoprotease and glycosidases are active in properly fermented and dried beans. Dias *et al*. (2018) opined that the reduced moisture content in dried cocoa beans, reduce the microbial activity of dried beans and the absence of microbes may reduce the lipase activity. In the present study also, the moisture content was within the permissible limit of 6 per cent in both the methods of drying.This reduced

moisture content may reduce the microbial activity and a consequent reduced lipase activity.

In the present study the free fatty acid content was 1.26 per cent in sun dried beans and 1.47 per cent in oven dried beans but they are significantly different. Drying could be attributed to the activity of lipase enzyme, this resulted in breakdown of the triglycerides into separate groups of the fatty acids and glycerol thereby release free fatyy acids (Dand, 2011). Guehi *et al*. (2010) also reported that oven-dried cocoa beans show higher free fatty acid content than solar dried beans. Microbial lipase activities hydrolysed the ester bonds between fatty acids and hydroxyl functions of glycerol in triglycerides of cocoa butter and liberated the free fatty acids. This may be the reason for more free fatty acid content in the dried cocoa beans. Oke and Omotayo (2012) reported that the free fatty acid content in oven dried beans was 0.67mg/g. Afoakwa (2015) opined that there was an increase in free fatty acid content during drying compared to fermented beans.

5.1.5. Standardisation of method of storage based on free fatty acid content

Good storage management is important to control the insect pests and growth of microbes. Storage management plays a vital role in maintaining the quality of cocoa beans in storage. The dry and cool conditions should be preferred for the storage of cocoa beans, because the cocoa beans absorb moisture from the surrounding atmosphere and this will cause the growth of microorganisms, which make it unsuitable for further processing (Amma *et al*., 2004). Lower temperatures would result in maintaining better quality cocoa beans by inhibiting FFA and insect development (Jonfia, 2010). Hence in the present study the sun dried cocoa beans were stored in gunny bags, polythene cover and plastic container in a dry and cool places for six months. The effect of storage methods studied in relation to bean recovery, increase in moisture content of beans, change in pH, total fat content, lipase activity and free fatty acids of stored beans during storage. The microbial

load on cocoa beans were also analysed initially and at the end of storage (after six month) with respect to the storage methods.

5.1.6. Physico-chemical analysis of stored beans

The bean recovery of stored cocoa beans during storage was more in cocoa beans stored in plastic container from initial to six months after storage and it was 98 per cent at the end of storage. A decrease in bean recovery was observed in all three treatments (Table.16). The lowest bean recovery was recorded in cocoa beans stored in gunny bags. The reduction in bean recovery may be due to the mould attack during storage reported by Jonfia (2010). Afoakwa *et al*. (2014) also reported that reduction in bean recovery may be due to the decrease in protein content during drying and storage might be caused by the continuous breakdown of cocoa bean proteins to oligopeptides and free amino acids. Dand *et al*. (2011) also reported that bean recovery per cent was lower than the initial bean recovery of stored cocoa beans.

In the present study the mean moisture content of stored cocoa beans was initially 4.22per cent in all three methods of storage like gunny bags, polythene cover and plastic container. Table.11 shows that moisture content of stored beans gradually increased during storage. A steady increase in moisture content was observed in all three treatments but did not cross the safe level of moisture content below 6 per cent. The maximum increase in moisture content was 5.21 per cent in cocoa beans stored in gunny bags, which was recorded at the end of six month after storage. Our results are in agreement with those of Mounjouenpou *et al*. (2011), they opined that beyond three months, mould growth often develops due to the hygroscopic properties of cocoa beans and this will cause increase in moisture content in stored cocoa beans. According to Villers *et al*. (2006), the oxidation effects the growth of moulds in cocoa beans after storage and in his study he observed that the hermitic storage of cocoa beans reduced the oxygen levels to 2 per cent and resulted in absence of insects.

Bopaiah (1992) opined that the cured cocoa beans could be store in jute bags up to 36 months under South Indian conditions without quality deterioration. Amma *et al.* (2004) also opined that the cocoa beans could be stored at acceptable moisture level (below 6 per cent) up to six months using jute bag with double lining of polythene. Sunilkumar (2005) also found that the moisture content (7.17 %) of cured beans increased during storage and it cross the safe limit after six month after storage. According to Villers *et al*. (2007) during storage, the moisture content of the cocoa beans increased due to insect infestation, mould contamination and moisture exchange between atmosphere and the beans, which are hygroscopic. Jonfia (2012) also reported that the moisture content of stored cocoa beans increased due to increase in free fatty acid content. Oke and Omotoyo (2012) also observed that there is a slight increase in moisture content in cocoa beans stored in polythene covers for three years and after three years the moisture content increased from 6.50 to 6.59 per cent. These observations were similar to the findings of present study.

In the present study, the pH of stored cocoa beans was slightly increased during storage (Figure.12). A slight increase in pH was observed among all three methods of storage beans. The cocoa beans stored in gunny bags have increased pH value compared to other cocoa beans stored in plastic and polythene cover. The pH of stored cocoa beans 5.31, 5.28 and 5.27 was in gunny bags, polythene cover and plastic container respectively at six month after storage. The same trend was observed in the study of Oke and Omotayo (2012) ,the results shows that the beans stored for 3 years inside polythene bags were less acidic (pH values of 4.77 to 5.32) and he opined that the increase in pH may have been due to increase in moisture content during storage periods. This is consistent with what was reported for rice, coffee and corn by Navarro *et al*. (2007). Sunilkumar (2005) opined that in his study the pH was increased during storage, this could be due to the enhanced hydrolytic reaction taking place inside the bean due to increased moisture content.

Initially the total fat content of stored cocoa beans was varied from 50.31 to 50.34 per cent in all three treatments. There was a reduction in fat content of stored cocoa beans during storage. But significant difference was observed in T_1 (cocoa beans in gunny bag) with the treatments T_2 (cocoa beans in polythene cover) and T_3 (cocoa beans in plastic container) during storage periods. The maximum fat content of 48.07 per cent was observed in T_3 (cocoa beans in plastic container) at the end of storage periods (Table.19). Jonfia (2016) also reported that the fat content significantly decreased (P<0.05) in stored cocoa beans with the increase in the storage periods. Selamat *et al*. (1996) opined that the action of lipase enzymes breakdown the triglyceride in the beans into its separate groups of fatty acids, thereby increasing the free fatty acids levels leading to the production of rancid flavour in the beans from the prolonged stored pods and there by decrease the fat content.

In the present study, the lipase activity was slightly increased in all three treatments during storage. Statistically, lipase activity was more in cocoa beans stored in gunny bags compared to other two treatments (Table.20). According to Abd *et al*. (1998) a steady increase in lipase activity due to increase in pH of stored beans during storage and this findings support the findings of present study, increasing the pH and lipase activity of stored cocoa beans during storage of cocoa beans. In the present study the cocoa beans stored in gunny bags had damaged due to fungal attack. The results of present study similar to the findings of Mounjouenpou *et al*. (2011), he reported mycotoxin (Ochratoxin A) is produced after 2 and 4 months of storage, this will cause the increase in lipase activity of stored cocoa beans. The present study results are in agreement with those observations of Wood and Lass (1989) for the storage of cocoa beans, he opined that the storage should not exceed 2 to 3 months, beyond three months, mould growth often develops due to the hygroscopic properties of cocoa beans in jute bags.

Oke and Omotayo (2012) reported that hermetic storage of artificially-dried cocoa beans inside hot-sealed plastic bag is appropriate for storage of cocoa beans

for a long period of 3 years and there was any adverse effects on its quality, this findings support the findings of present study, the lipase activity was less in cocoa beans stored in polythene cover and plastic container compared to beans stored in gunny bags.

As revealed in Table.21 the initial free fatty acid content in stored cocoa beans was 1.12 per cent in all three treatments. During storage, there was an increase in free fatty acid content (2.80%) with more in beans stored in gunny bags and 2.01 per cent in beans stored in polythene covers at six month after storage. The lowest free fatty acid of 1.68 per cent was in beans stored in plastic container at six month after storage. The mould infestation and increased moisture content may be responsible for the increases in FFA levels in stored cocoa beans (Hodges *et al*, 1999; Hodges *et al*., 2002). Jonfia (2010) also reported that the insect infestation caused the increased free fatty acid levels in stored cocoa beans. This is in line with the result of the present study, the high free fatty acid content was recorded in cocoa beans stored in gunny bags.

According to Afoakwa (2010), the enzyme lipase, which are naturally present in raw cocoa beans attributed to the increase in FFA during storage of cocoa beans. The moisture content of the beans and temperatures of storage environment make the enzymes active and this may be the reason for increasing free fatty acid content in cocoa beans stored in polythene covers and plastic containers. Free fatty acids are carboxylic acids released from triglycerides by the action of lipase or oxidation and the low moisture content can reduce theaction of lipase and thre by decreasing the free fatty acid content (Selamat *et al*., 1996). The cocoa butter contained low unsaturated fatty acid, high polyphenols and natural antioxidants, the risks of oxidation are negligible in cocoa beans (Nickless, 1996 and Whitefield, 2005). According to Cros (2001), FFA formation in cocoa does not arise from enzyme autolysis, but involves lipolytic activity of microbial origin combined with other factors such as bean quality, physical integrity and storage conditions.

5.1.7. Microbial load on stored cocoa beans

In the present study, the maximum bacterial count was increased from initial of 1.6x 10^{-4} cfu g-1 to 10.4 x 10^{-4} cfu g-1 at the end of storage in gunny bags. The maximum fungal growth was also in treatment T_1 (cocoa beans stored in gunny bags). Wood and Lass (1985) opinened that the deterioration of beans can prevent by storing the beans in jute bags with double lining of polythene and it will reduce the mould and insect attack as well as moisture uptake. Bopaiah (1992) also reported that the cured beans can be stored in jute bags upto 36 months under South Indian conditions without quality deterioration. Amma *et al.* (2004) also reported that at acceptable moisture level (below 6 %) the cured cocoa beans can be stored upto six months using jute bag with double lining of polythene. Sunilkumar (2005) analysed the microbial load on cocoa beans at bimonthly intervals showed that there was significant variation in microbial load with respect to the packaging methods.

In the present study the microbial load was more in cocoa beans stored in gunny bags (without polythene lining), it may be due to moisture exchange between atmosphere and the beans, which are hygroscopic (Villers *et al*., 2006). The microbial load was less in cocoa beans stored in polythene cover and plastic container. Although the observed microbial growth in three methods of storage was within the safe limits of FSSAI specification (10000 cfu g-1).

5.2. Alkalisation of stored beans

According to Shafi *et al*. (2018), alkalisation is the process by which the cocoa beans, nibs or liquor are treated with an alkali is described as 'alkalised' or'ducted' beans. This consists of treating the cocoa nibs with an alkali solution such as potassium or sodium bi carbonate. Amma *et al.* (2004) reported that the fermented dried beans have high acidity (low pH) and to neutralise this acidity the cocoa beans are alkalised. The alkalisation will raise the pH of the beans or nibs from 5.2 to 5.6 to near neutrality at 6.8–7.5, and the there by modify the colour and

flavour of cocoa powder or cocoa liquor, and also improve dispersibility or suspension of the cocoa solids in water. In the present study, sodium bicarbonate was used for the alkalisation of cocoa beans.

Roasting is an important step in the secondary processing of cocoa. Fermentation and drying of cocoa beans generate precursors of flavours and during roasting develop the original cocoa flavour. According to Krysiak (2006) the cocoa beans are roasted at the temperature of 120–150°C for the duration of 50–120 minutes. During roasting of the dried fermented beans, several physical and chemical changes take place, which include, loosening of the shells, moisture loss from the beans to about 20 per cent, the nibs (cotyledons) become more friable and generally darken in colour, degradation of amino acids takes place and proteins are partly denatured. The natural reducing sugars are almost destroyed during degradation of amino acids. Volatile acids and other substances are also lost during this procedures (Becket, 2000).

5.3. Standardisation of time and temperature for chocolate making using machine

The chocolate production basically consists of five stages: mixting of ingredients, refining, conching, tempering and final crystallisation. For production of high quality chocolate, not only the quality of the ingredients defines the final product, but also there is a great influence of the productive process (Cidell and Alberts, 2006). In the conching process the undesirable flavours are suppressed and the pleasant ones are produced, generating the typical flavour of the chocolate (Lucisano *et al*., 2006). Conching influences the development of flavour and the flow properties. The time of conching influences in the flavour development and the majority of milk and dark chocolates are conched during 5–12 h (Bolenz *et al*., 2003). Imperfections in the conching process result in inadequate distribution of the fat on solid particles generating a heterogeneous chocolate, migration of fat and sugar, acid flavour and absence of desirable flavours.

Tempering is a very important step during chocolate making. It is necessary and the last step before molding. By adjusting the temperature, cocoa butter could generate many little crystal seeds, which serve as nuclei to create small crystals in the chocolate. These small crystals generate harder chocolate with high viscosity (Briggs and Wang, 2004).

In the present study the chocolates were prepared in tempering and conching machine at different temperatures of 60^0C and 70^0C for 7, 9 and 11 hours. Two groups of chocolates were standardised using alkalised and non alkalised cocoa beans by incorporating sugar, milk powder and cocoa butter in accordance with the specifications suggested by Cocoa Research Centre Kerala Agriculture University. Physico chemical analysis, organoleptic evaluation and enumeration of total microflora of the freshly prepared chocolates were carried out.

5.3.1. Physico-chemical analysis of chocolates

The textural properties such as hardness, cohesiveness, adhesiveness and gumminess of alkalised and non alkalised chocolates were analysed. The hardness of chocolates is a quality parameter, the fat content, the particle volume fraction, particle size distribution and the tempering and conching process affect the hardness of chocolates. In the present study, the hardness of the alkalised chocolates were varied between 72.87N and 97.70N and in non alkalised chocolates the hardness varied from 72.51 N to 99.11N. Afoakwa *et al*. (2008) reported that chocolates with fine particles (18–25 μm) have more particle–particle interactions resulting in harder chocolate than chocolate with coarser particles (35–50 μm). According to Prawira and Barringer (2008), longer conching time results in smoother chocolate with smaller particle size and hardness is reduced and the hardness of milk chocolates decreased with conched for at least 12 hours. During conching of chocolates the solid particles are ground well due to the friction disassociated, become rounded and hardness of chocolate reduced (Mentink and Serpelloni, 1994). During conching the particle size of the solid particles in the

chocolate is steadily reduced and become soft (El-deep *et al*., 2000 and Liang and Hartel, 2004).

The ingredients of chocolates also affect its hardness, Full *et al.*(1996) opined that the addition of milk reduces the hardness of chocolate since it dilutes the cocoa butter and thus increases the amount of the liquid phase. Subramaniam and Murphy. (2001) also opined that milk chocolate with higher milk fat content was softer than milk chocolate with lower milk fat content.

Cohesiveness is a determination of intermolecular strength. Cohesiveness of chocolates varied in alkalised chocolates from 0.008N to 0.021N and in non alkalised chocolates it varied from 0.009N to 0.022N. Glibowski *et al.* (2008) found that the fat partial replacement with oil content caused significant decrease in the spread cohesiveness. Mousazadeh *et al*. (2014) opined that the cohesiveness of chocolate spread varied with different formulations. Vereecken *et al*.(2009) reported that the cohesiveness of semi solid particles like spread and chocolate high because of the smaller size of particles.

Adhesiveness or stickiness is described as the force required to overcome the attractive forces between the food surface and the surface in which the food sample comes in contact (Glibowski *et al.*,2008). In the present study the adhesiveness of 0.0027N in alkalised chocolates was found to be highest in treatment T_6 (chocolate prepared at 60^0C for 11 hours) and lowest in treatment T1 (chocolate prepared at 60^0C for 7 hours) and in non alkalised chocolates, the adhesiveness varied from 0.0007N to 0.0021N. The adhesiveness of chocolates varied, it depends on the formulations and process of chocolate preparations. In the present study the adhesiveness increased with duration of tempering and conching. It may be attributed to smaller particle size of chocolates and the consequent increase in the attractive force between the molecules of chocolates. Mousazadeh *et al*. (2014) reported that the adhesiveness of chocolate spread increased when the contents of xanthan gum and distilled monoglyceride increased. Radocaj *et al.*

(2011) also found that the spreads adhesiveness had lower values at higher levels of hemp oil content.

Gumminess is the force required to disintegrate a semi - solid food before it is ready for swallowing (Rosenthal, 2010). In the present study the gumminess varied in alkalised chocolates from 0.9482N to 1.3782N and in non alkalised chocolates from 1.0620N to 1.3201 N. The presence of suspended particles and polymers may affect the stability and the rheology of the suspensions as a result of interactions or gumminess between them (Shakerardekani *et al*., 2013). In the present study the presence of cocoa butter give the stability to the product, so with increasing the duration of tempering and conching will increase the gumminess.

The attributes of texture like hardness, cohesiveness, adhesiveness and gumminess was most affected by conching, and tempering. Longer conching produced smoother, more mouthcoating chocolate with no change in flavor. The 11 hours period of conching and tempering produced smoother chocolate than 7 hours of conching and tempering.

In the present study, the moisture content of chocolates prepared in tempering and conching machine generally decreased with increasing duration of tempering and conching with in a temperature range. The moisture content also decreased in chocolates with increasing temperature from 60^0C to 70^0C. The lowest moisture content of 1.18per cent was found in chocolates prepared at 70^0C for 11 hours. The moisture content in the present study was found to be higher than the moisture content of 0.7per cent in chocolates prepared at laboratory level at 70^0C for 12 hours as reported by Schumacher *et al*. (2010). Bolenz *et al*. (2003) describe an acceptable moisture content was 0.4–0.6 per cent in chocolate at the end of conching. According to Ghosh *et al*. (2005), the moisture in the chocolates enters through the empty spaces of fat crystals, this moisture absorb the sugar and cocoa solids. Increased moisture content cause migration of these ingredients to the chocolate surface. For this reason the moisture control is very important in the

chocolate processing. In the study of Moreno *et al*. (2015) the moisture content was slightly decreased from 1.49 to 1.40 per cent in Echoador chocolates and from 1.47 to 1.39 per cent in chocolates from Ghana with increasing conching time.

In the present study, the energy value in both alkalised and non alkalised chocolates varied significantly with increasing duration of tempering and conching. The alkalised chocolates provide the highest calorific value of 579.04 K.cal and 579.82 K.cal in chocolates prepared at 60^0c and 70^0C for 11 hours in treatment T_3 and T_6. In non alkalised chocolates also the highest calorific value of 579.62 K.cal was noticed in treatment T_{12} (chocolate prepared at 70^0C for 11 hours) followed by treatment T_9 (chocolate prepared at 60^0C for 11 hours) with 575.04 K.cal. This findings are similar to the findings of Pandey and Singh (2011), in their study the calorific content of chocolates vary from 574.5 K.cal/100 g to 583.1 K.cal/100 g. Mehta. (2017) had also reported that the energy/ calorific value of different branded chocolates was 520 K.cal/100 g, 500 K.cal/100 g, 562 K.cal/100 g, 552 K.cal/100 g and 546 K.cal/100g in Perk ,Munch, Amul (Fruits and Nut), Cadbury (Fruits and Nut) and Nestle (Fruits) respectively.

In alkalised and non alkalised chocolates the highest TSS of 68^0 Bx was recorded in treatment T_1 (Chocolate prepared at 60^0C for 7 hours). All fruit based chocolates had the brix values higher than 65^0B at room temperature and having a good shelf life to the product (Miquelim *et al*., 2011. Spreads are prepared using high concentrations of sugar, which functions as a preservative (Vaclavik and Christian, 2008). Therefore, the high level of sugar present in confectionery products makes them less prone to microbial spoilage. However, the TSS obtained in the chocolates of present study were similar compared with over 68°Brix in Jack-Passion spread produced by Chakraborty *et al*. (2011) and higher compared to 38.65°Brix in a red flesh dragon fruit spread produced by Barcelon *et al.* (2015).

In the present study, the total sugar content in alkalised chocolates ranged from 46.7 to 48.01g/100gm and in non alkalised chocolates it varied from 46.6 to 48.02 g/100gm. Zupanic *et al*. (2018) also reported that the total sugar content in

chocolates was 44.6 g/100 g, this value is similar to the findings of present study. Arentz (2018) also found a total sugar of 48.27 g/100g in fructose sweetened chocolates and 51.80 g/100g in sucrose sweetened chocolates.

The reducing sugar content in alkalised chocolates varied between 4.9 and 5.3 g/100gm and was significantly high in T_9 (60^0C for 11 hours). In non alkalised chocolates it varied from 4.8 to 5.0g/ 100gm and the highest reducing sugar content of 5.3g/100gm was recorded in treatment T_9 (60^0C for 11 hours). Afoakwa *et al*. (2012), also reported that the reducing sugar content of 1.13 to 4.10 g/100gm in chocolates.

In the present study the protein availability of alkalised chocolates varied between 7.30 and 10.45g/100g with the lowest protein in T_6 (70^0C for 11 hours) and highest in T_1 (60^0C for 7 hours). In non alkalised chocolates the highest protein content of 9.45g/100g was noticed in treatment T_7 (60^0C for 7 hours) and the lowest protein content of 7.58g/100g was observed in T_{12} (70^0C for 11 hours). Schumacher *et al.* (2010) reported that the protein content in industrial and laboratory based chocolates varied from 3.45 to 4.48g/100gm. The protein value of different branded chocolates was 7.21g/100 g, 6.56/100 g, 8.50g/100 g, 8.74g/100 g and 7.08 g/100g in Perk, Munch, Amul (Fruits and Nut), Cadbury (Fruits and Nut) and Nestle (Fruits) respectively (Mehta, 2017). In the study of Amevor *et al*. (2018) the protein in chocolate spread was 10.13 g/100gm. Moreno *et al*. (2015) also reported that the protein content in chocolates reduced from 6.30 to 6.50g/100gm with increasing duration of conching time.

The total fat content of alkalised chocolates ranged from 41.1 to 44.5 per cent. In non alkalised chocolates, the total fat content was found to be highest in T_{12} (46.50 per cent) and lowest in treatment T_7 (42 per cent) (Table.24). This results obtained are in line with the findings of Amevor *et al*. (2018), who reported the fat content in chocolate based spread as 41.42g/100gm. The fat content in industrial and laboratory based chocolates was 26.06 and 27.82g/100gm (Schumacher *et al.*,

2010). These were lower than the findings of the present study. Mehta. (2017) also reported that the fat content of different branded chocolates was 26.52g/100g, 22.52/100g, 34.60g/100g, 32.97g/100g and 32.40g/100g in Perk, Munch, Amul (Fruits and Nut), Cadbury (Fruits and Nut) and Nestle (Fruits) respectively. Moreno *et al*. (2015) also found that the fat content in chocolates slightly increased (from 31.20 to 32.30g/100gm) with increasing duration of conching time.

In alkalised chocolates the highest total polyphenol of 0.21g/100g was in treatment T_1 (Chocolates prepared at 60^0C for 7houres) and lowest of 0.16g/100g of polyphenol in chocolate prepared at 70^0C for 11 hours (T_6). In non alkalised chocolates statistically the treatment T_7 had the maximum polyphenol content (0.23g/100g) and T_{11} and T_{12} had the lowest polyphenol content (0.20g/100g). The results obtained were in agreement with the study done by Meng *et al*. (2008), they reported that the poly phenol content in dark chocolate was 0.56g/100g, in milk chocolate it is 0.16g/100g and in white chocolate it is 0.12g/100g. (Miller *et al*. (2009) reported that the total polyphenol content in chocolates was 1.20-6.67mg/g. Grassi *et al*. (2004) reported that where 100 g of chocolate contained approximately 500 mg of polyphenols.

In the present study the poly phenol content in alkalised chocolates was less compared to non alkalised chocolates. Similar results was observed by Miller *et al*.(2008), that non alkalised cocoa beans are high in polyphenols, but when the cocoa is processed with alkali the polyphenol content are substantially reduced. Alkalisation (or dutching) of cocoa beans will influence the polyphenol contents (Gu *et al*., 2006 and Cooper *et al*., 2007).

In the present study the conching process does not impair the phenolic content and pattern, since small significant variations were found regardless of the time/temperature combination applied. The earlier research works also found such a reduction in phenolic content during tempering and conching (Konar, 2013; Owsu *et al.,* 2013; Mattia *et al.,* 2015)

In alkalised chocolates the total ash content varied significantly between the treatments. The maximum total ash content of 1.45g/100g was recorded in treatment T_6 (70^0C for 11 hours) followed by chocolates prepared at 70^0C for 9 hours (1.44g/100g). The minimum total ash content of 1.41g/100g was in alkliased chocolates in treatment T_1 (70^0C for 11 hours). The total ash content in non alkalised chocolates ranged from 1.43g/100g to 1.40g/100g. The minimum total ash content was in treatment T_7 (60^0C for 7 hours). The results were in tune with Moreno *et al*. (2015), where the total ash content in chocolates varied from 1.50 to 1.58mg/g. Mehta. (2017) also reported that the total ash content of different branded chocolates was 1.29 mg/100g, 22.52 mg/100g, 34.60 mg/100g, 32.97mg/100g and 32.40 mg/100g in Perk, Munch, Amul (Fruits and Nut), Cadbury (Fruits and Nut) and Nestle (Fruits) respectively.

In alkalised chocolates the calcium content was significant between the treatments. The longer duration of tempering and conching in treatments T_3 and T_6 resulted in significantly superior calcium content (0.04mg/100g) than other treatments (0.03mg/100g). In non alkalised chocolates the calcium content was more in treatment T_8, T_9 and T_{12} (0.04mg/100g). The lowest calcium content of 0.03mg/100g was recorded in treatment T_7, T_{10} and $T_{11.}$ The calcium content of different branded chocolates was 0.35mg/g, 0.79mg/100g, 0.47mg/100g, 0.50mg/100g and 0.40 mg/100g in Perk, Munch, Amul (Fruits and Nut), Cadbury (Fruits and Nut) and Nestle (Fruits) respectively (Mehta, 2017). Muller *et al*. (2012) also recorded that the calcium content in chocolate was in the range of 0.46 -0.49 mg/100g.

The phosphorus content in alkalised chocolates had slight difference between the treatments. The maximum phosphorus content of (0.17mg/100g) was recorded in treatment T_3 and T_6 in alkalised chocolates. In non alkalised chocolates the phosphorus content was maximum in treatment T_{12} (0.17mg/100g). However higher phosphorus content of 0.31mg/ 100gm was reported by Muller *et al*. (2012).

The iron content in alkalised chocolates differed significantly between the treatments. The maximum iron content of 18.45 mg/100g was observed in the treatment T_3 followed by T_2 (17.93mg/100g). The lowest iron content of 15.09 mg/100g was found to be in chocolate prepared at 70^0C for 7 hours. In non alkalised chocolates, slight difference was observed in iron content between the treatments. But statistically significant variation was observed in iron content between the treatments. Ciguanta *et al*. (2016) also reported that the iron content in white chocolates was 10.9 mg/100g. The mineral content of cocoa and cocoa products reflects the mineral characteristics of the soil in which they have grown and is also influenced by the ingredients in the products. (Borchers *et al*., 2000).

In alkalised chocolates the lipase activity was decreased with increasing the temperature and duration of tempering and conching. The lipase activity of alkalized chocolates ranged from 0.0010 to 0.0015 µ eq with the lowest and highest lipase activity in treatment T_5 (70^0C for 9 hours) and T_6 (70^0C for 11 hours) and the highest in T_1 (60^0C for 7 hours) respectively. In non alkalised chocolates the highest lipase activity was recorded in T_7 (0.0017 µ eq) and lowest in treatment T_{12} (0.0010 µ eq), ie. Chocolate prepared at 70^0C for 11 hours. The combination of mechanical energy from the mixing elements and external heating leads to evaporation of moisture. In addition, some acids and enzymes particularly acetic acid, enzymes and small quantities of aldehydes are also distilled off with the water (Zeigleder *et al*., 2004). This may be the reason for reducing lipase activity by increasing tempering and conching time.

The free fatty acid content in the alkalised and non alkalised chocolates differed significantly between the treatments during conching and tempering of chocolates. In alkalised chocolates it vary from 2.80per cent to 1.67per cent and in non alkalised chocolates it vary from 2.61 to 1.68. The free fatty acid content generally decreased with increasing duration of tempering and conching with in a temperature range. According to Sulistyowati and Misnawi (2008), tempering and conching will improve the flavor characteristics and reduce the concentration of

free acids and other volatile by products from the cocoa bean. Cordelia *et al*. (2017) also observed that the free fatty acid content in chocolates was in the range of 0.04 per cent to 2.25 per cent. The difference in free fatty acid content in chocolates confirm that the geographical origin had an influence on the fatty acid composition of cocoa butters, as pointed out by Lipp and Anklam (1998).

In the present study the initial concentration of FFA in chocolate prepared at 7 and 9 hours conching was above 1.75 per cent cut off value. It may be due to the high FFA content in added cocoa butter in chocolates. But increasing the tempering and conching time the FFA value decreased to 1.67 and 1.68per cent in alkalised and non alkalised chocolates due to the volatile nature of FFA. However the free fatty acid content was below the 1.75per cent cut off, clearly indicates that fatty acid composition should be considered when using FFA concentration as a proxy for chocolate quality.

5.3.2. Organoleptic evaluation of chocolates

Chocolate is generally considered as a luxury product and perfect food. Chocolate is solid at room temperature, but melts in the mouth releasing a smooth, delicate taste makes it a unique product. The aroma, flavour and texture are also sensory characteristics used to define chocolate, and these depend on the manufacturing process. Sensory evaluation is used to measure, analyse and interpret how the attributes of a product are perceived by peoples. These sensory attributes are the combination of characteristics that together produce a sensory experience (texture, aroma, colour, flavour) and the human senses like sight, hearing, taste, smell and touch are used to measure the attributes.

The human senses are used to evaluate the attributes of sensory evaluation that describe the behaviour of chocolates and permits to differentiate the high quality products. The sensory evaluation is a key process in the chocolate industry as it ensures quality and permanence of the brand (Gutierrez, 2015)

Nancy (2007) opined, the conching of chocolate for longer conching time (10–14 hr) results in smoother chocolate with smaller particle size. During conching, the solid particles such as sugar, non fat cocoa and milk powder are coated with fat and it take time all of the solid particles to covered with fat and so longer conching time give smother chocolates (Mentink and Serpelloni,1994).

According to Afoakwa *et al.* (2007) if the particle size of the chocolate solids was not reduced to the ideal size of less than 20 mm, the tongue can not detect them. Mentink and Serpelloni (1994) and El- deep *et al*. (2000) also reported that during conching, solid particles are ground, disassociated by friction and become rounded, thus the particle size of the solid particles in the chocolate is steadily reduced. As particle size is reduced, the texture of the chocolate becomes smoother (Liang and Hartel 2004).

In the present study, the chocolates prepared in tempering and conching machine for different time and temperature were evaluated organoleptically using score cards for different quality attributes like appearance, colour, flavour, texture, taste and overall acceptability (Table 25 and 26). In the present study among different treatments tried for chocolates with alkalised cocoa beans, the sensory profile of the chocolates revealed an increased liking and higher mean rank for treatment T_6 (chocolates prepared at 70^0C for 11 hours). The treatment T_6, secured a maximum total score of 53.13, followed by treatment T_3 (52.95) for all quality attributes like appearance, colour, flavor, texture, taste and overall acceptability. Among the different chocolates prepared with alkalised beans, the consumers rated highest mean score for colour (8.84), flavour (8.71), texture (8.89), taste (9.00) and overall acceptability (8.84) for treatment T_6 ie. the chocolate prepared at 70^0C for 11 hours.

In non alkalised chocolates the highest total score of 52.63 was observed in treatment T_{12} (chocolate prepared at 70^0C for 11 hours) followed by treatment T_9

(chocolates prepared at 60^0C for 11 hours) with mean score of 52.30 for all quality attributes like appearance, colour, flavor, texture, taste and overall acceptability.

Ramli *et al*. (2006) also found that the tempered and conched chocolate prepared with goat milk, improved the texture, sweetness and colour. Polyphenols in chocolates also contribute the sensory characteristics of the cocoa, these substances are involved in the chocolate's flavour and in the primary sensory characteristics of bitter and astringency intensity of the chocolate (Misnawi *et al*., 2005). (Zeigleder *et al.* (2004) suggested that conching improve the flavour development using high temperatures (>75°C).

According to Afoakwa *et al.* (2009) the texture, appearance and flvour are important factors used to evaluate the quality of the products by the consumers. (Leite *et al*. (2013) also reported that there was high positive correlation between brown colour with the descriptors chocolate odour, bitterness and roasted flavour. Thus, the chocolate sample that had a more intense brown colour also yielded a higher intensity in descriptors such as chocolate odour, chocolate flavour, bitterness, firmness, toasted odour and toasted flavour. In the present study the mean score of texture in alkalised chocolates was 8.87 and in non alkalised chocolates the mean score was 8.62. The chocolates with the longer conching time makes the product smoother (Mentink and Serpelloni, 1994). During conching, solid particles are ground, disassociated by friction and become rounded thus the particle size of the solid particles in the chocolate is steadily reduced (El-deep *et al.* 2000). As particle size is reduced, the texture of the chocolate becomes smoother (Liang and Hartel, 2004).

Even though the ingredients in each samples or treatments are same, the difference in the tempering and conching time gave differences in appearance, colour, flavour, texture, taste and overall acceptability of chocolates.

5.3.3. Enumeration of total micro flora

The microbial count in a product determines shelf life of the food products. High microbial count indicates the poor shelf life product which cannot be recommended for the safe consumption. In the present study the bacterial, fungal and yeast population was not detected in all treatments or chocolates prepared with alkalised and non alkalised cocoa beans. Mishra *et al*. (2017) also reported that initial total plate count, yeast and mould count was 0.21×10^{2}, $0.3\times10^{2}$and 0.1×10^{2}respectively in guava milk chocolate. Mittal and Bajwa. (2014) also reported that microbial count was not detected in freshly prepared chocolate milk drink packaged in HDPE bottles. Mehta. (2017) also reported that the initial microbial count of different branded chocolates was $6x10^{4}$, $31x10^{4}$, $2x10^{4}$, $13x10^{4}$and $40x10^{4}$in Perk, Munch, Amul (Fruits and Nut), Cadbury (Fruits and Nut) and Nestle (Fruits) respectively. But in the present study the microbial count was not detected, it may be due to the high sugar content in the chocolates and also temperature (70^{0}C) during tempering and conching.

5.4. Blending of chocolates with other ingredients

Today the consumers are looking for products that provide more health benefits. The health-enhancing roles of specific foods or food components has been observed due to the increase in consumer awareness and interest about health benifits (Jnawali *et al*., 2016). Among the confectionary products, milk chocolate is much more popular than either white or dark chocolate in most countries (Beckett, 2008). During chocolate manufacturing, refining and conching determine the particle size and suspension consistency and viscosity for specific textural and sensory qualities (Konar, 2013). To improve the quality and functionality of the chocolates, the addition of milk powder and blending of chocolates with ingredients affect the physical and sensory properties of milk chocolate (Liang and Hartel, 2004). Dried fruit enrichment influence the consumer acceptability of milk chocolate (Komes *et al*., 2013).

Two groups of blended chocolates with alkalised and non alkalised chocolates were prepared using different dehydrated fruits (grapes and dates), osmodehydrated fruits (jackfruit and pineapple), nuts (almond and cashew nut) and powdered dehydrated mint leaves and white pepper powder. The amount of mint and white pepper powder to be added were standardised using sensory evaluation. The main intention of development of the blended chocolates was utilisation of important locally available fruits like jackfruit and pineapple which are readily available in abundance and at a reasonable cost and also blending of protein sources like cashew nut and almond. Blending of chocolates with white pepper powder and dehydrated mint leaves added a nice and interesting twist to flavours.

Among different treatments tried for blended alkalised chocolates, three blended chocolates, T_3 (95 % alkalised chocolates +5% osmodehydrated jack fruit), T_6 (95 % alkalised chocolates +5% Almond) and T_8 (95 % alkalised chocolates +5% white pepper) secured a total score of 53.24, 53.70 and 53.19 respectively for all quality attributes like appearance, colour, flavor, texture, taste and overall acceptability. The blended chocolates prepared with non alkalised chocolates, T_{11} (95 % non alkalised chocolates +5% osmodehydrated jack fruit), T_{14} (chocolate blended with 95 % non alkalised chocolates and 5% almond) and T_{16} (95 % non alkalised chocolates and 5% white pepper) attained the highest total score of 53.77, 53.80 and 53.78 respectively for all quality attributes investigated like appearance, colour, flavour, texture, taste and overall acceptability.

The sensory profile of blended chocolates revealed an increased liking and higher mean rank. However, blending with dehydrated jack fruit, almond and white pepper powder attained the highest mean ranks in alkalised and non alkalised chocolates. Amevor *et al* (2018) also reported a good sensory acceptability for the cashew nut blended chocolate spread. The fundamental fat crystal network in nut butters influences many of the sensory attributes such as spreadability, mouthfeel and texture (Matsiko *et al*., 2014). Shakerardekani *et al*. (2013) also reported that the smooth and soft texture of chocolates, facilitates its sensory characters and it is

preferable to the children. Aidoo *et al.* (2010) and Glicerina *et al*. (2015) reported that the effect of raw materials substitution and the effect of manufacturing process influence on the quality attributes of milk chocolate.

The findings of sensory evaluation of the present study was found to be in agreement with the study conducted by Divya *et al*. (2017) which reported that the blending of coconut milk and cream in chocolates at 10 per cent, 20 per cent, 30 per cent and 40 per cent levels enhances its appearance, colour, flavour, texture and over all acceptability. El-kalyoubi (2011) indicated that the lower replacement rate of fat affected the rheological properties of chocolate, this findings were found to be in accordance with the research findings of present study ie, replacement of 5per cent chocolates and blending with dehydrated fruits, nuts, mint and white pepper affected or enhanced its sensory qualities. Rasuluntari *et al* (2016) also reported that the blending of cinnamon powder in chocolates at different concentration 5per cent, 10 per cent and 15 per cent also enhances the sensory attributes like appearance, taste, flavour and overall acceptability.

5.4.1. Quality evaluation of selected blended chocolates

From the, organoleptic evaluation of different treatments, based on appearance, colour, flavor, texture, taste, overall acceptability and total score the best rated treatments from alkalised chocolates were T_3 (95 % alkalised chocolates +5% osmodehydrated jack fruit), T_6 (95 % alkalised chocolates +5% almond) and T_8 (95 % alkalised chocolates +5% white pepper) and from non alkalised chocolates the treatments T_{11} (95 % non alkalised chocolates +5% osmodehydrated jack fruit), T_{14} (chocolate blended with 95 % non alkalised chocolates and 5% almond) and T_{16} (95 % non alkalised chocolates and 5% white pepper) were selected for storage studies.

5.4.1.1. Physico- chemical qualities of selected blended chocolates during storage

The textural properties were analysed initially and at the end of storage and all other constituents like moisture, energy, TSS, total sugar, reducing sugar, protein, fat, polyphenol, total ash, minerals like calcium, phosphorus, iron, lipase and free fatty acids were analysed initially and at monthly intervals for a duration of six months.

5.4.1.1. Textural properties of blended chocolates

Milk chocolate contains solid particles (cocoa, milk powder and sugar) dispersed in cocoa butter that construct a complex rheological system (Pajin *et al.*, 2013; Glicerina *et al.*, 2015). The particle size, suspension consistency and viscosity for specific textural and sensory qualities were considered during chocolate manufacturing, refining and conching (Konar, 2013). Storage temperature and humidity of chocolate greatly impact the texture of chocolates (Nightingale, 2009). Nishinari *et al*. (2013) opined that the hardness, adhesiveness and cohesiveness have been widely used for comparison of the textural properties of various foods.

In the present study, the textural quality parameters such as hardness, cohesiveness, adhesiveness and gumminess of blended chocolates (alkalised and non alklised) were observed initially and at the end of six month of storage and compared with the textural properties of commercial chocolates (Dairy milk, Dairy milk (silk and For U). In the present study the hardness of all blended chocolates and controls (alkalised and non alkalised chocolates) increased at the sixth month of storage compared to initial hardness. This finding was similar to the findings of Nightingale, (2009) in his study also the hardness of chocolates increased in refrigerator and freezer conditions. The initial hardness of 74.41N and 76.71N in controls (non alklised and alkalised chocolates) increased to 77.16N and 79.37N respectively. Cocoa butter is the major factor for the stability of chocolates at room temperature and is responsible for the characteristics of melting and hardness of chocolates. The crystalline structure of fat determines the macroscopic properties of chocolate and sensory perception (Efraim *et al*., 2013). Ali *et al*. (2001) also

reported that filled chocolates stored at 30°C were significantly softer. In the present study also the lowest hardness of control chocolates may be due to the migration of fat and also the hardness of blended chocolates increased during storage. This findings was confirmed with the findings of Machalkova *et al*. (2014), in his study also the hardness of chocolates slightly increased during storage. According to Mishra *et al*. (2017) the hardness of guava –milk chocolates stored at 2°C had an increase in hardness during storage. Pandy and Singh (2011) also found that the hardness of soy blended chocolates increased from 36.7N to 38.4N during storage.

The hardness of commercial chocolates (29.34N and 37.46N) was less or smooth compared to hardness of blended chocolates 84.33 N (T_1 -95 % AC+5% ODJ), 99.11N (T_2 -95 % AC+5% B), 81.75N (T_3 -95 % AC+5% WP). The less hardness of commercial chocolates may be due to the more tempering and conching time of commercial chocolates. Full *et al.* (1996) determined that instrumental and sensory hardness of milk chocolate decreased with an increase in milk fat. According to Afoakwa *et al.* (2008); Afoakwa *et al.* (2009) an increase in instrumental hardness may be due to the fat bloom formation. But in the present study the fat bloom formation was less.

In the present study the highest cohesiveness of 0.028N was observed in chocolates prepared with 95% non alkalised chocolates and 5% almond (T_5) and lowest (0.022N) in treatment T_0 (100% AC) and T_3 (95 % AC+5% WP). The maximum cohesiveness of 0.031N was recorded at the sixth month of storage in treatment T_5 (95 % NAC+5% A). The cohesiveness of blended chocolates and commercial chocolates increased with storage (Figure17). This findings was analogous to the findings of Nightingale, (2009), who reported that the cohesiveness of milk and dark chocolate increased on storage in different temperatures. Glibowski *et al.* (2008) opined that the partial fat replacement caused significant decrease in cohesiveness of spread. In the present study the fat content of stored chocolates slightly decreased. This may be the reason for increasing cohesiveness in stored chocolates. The cohesiveness of blended chocolates was

higher than the cohesiveness of commercial chocolates it may be due the longer conching and tempering time of commercial chocolates and also due to the difference in the quantity of fat, milk and sugar content of the chocolates (Miquel and Hall, 2002).

Initially the maximum adhesiveness of 0.0028N was recorded in chocolates prepared with 100% alkalised chocolates ie; T_0 (100% AC) and the lowest adhesiveness of 0.0017N was recorded in treatment T_5 (95 % NAC+5% B) followed by 0.0018N in treatment T_2 (95 % AC+5% A). At the end of sixth month of storage the highest adhesiveness was found to be 0.0026N in treatment T_0 (100% AC) and lowest adhesiveness of 0.0014N in treatment T_2 (95 % AC+5% B) and T_5 (95 % NAC+5% B). The adhesiveness of blended stored chocolates decreased at the end of six month of storage. This is in line with the findings of Nightingale, (2009). In the present study, the moisture content of stored chocolates increased, this will cause the decrease in adhesiveness of stored chocolates.The adhesiveness of chocolates varied, it depends on the formulations and process of chocolate preparations. This may be the reason for significant differences in adhesiveness between the treatments (Kumagai *et al*., 2011). The adhesiveness of commercial chocolates was higher than the adhesiveness of blended chocolates initially and at the end of storage.

` In the case of gumminess the highest gumminess of 1.8813N was recorded in treatment T_4 (95 % NAC+5% ODJ) and lowest in treatment T_6 (95 % AC+5% WPP). At the end of storage the maximum gumminess of 1.9841 N was recorded in T_4 (95 % NAC+5% ODJ) and the lowest gumminess of 1.3798 N was recorded in T_3 (95 % AC+5% WP). From the figure 19 it is clear that the gumminess of stored blended chocolates slightly increased. This results obtained in this study was in accordance with the findings of Nightingale, (2009), who also reported that the gumminess of milk and dark chocolates increased when stored for 5 weeks at various temperatures and relative humidity (RH). Gumminess is the force required to disintegrate a semi -solid food (Rosenthal, 2010). Hardness, cohesiveness and gumminess are directly proportional to each other. In the present study the hardness

and cohesiveness of stored chocolates were increased and caused an increase in the gumminess of stored chocolates. The moisture content of stored chocolates increased, this also added the gumminess of stored chocolates.

The gumminess of commercial chocolates was less compared to blended chocolates initially and at the end of storage, but much difference was not observed. The effect of storage temperature and method of manufacture of chocolate leads to changes in the product quality (Machalkova *et al*., 2014). Eventhough the hardness was increased during storage under refrigerated condition there was no fat bloom.

5.4.1.2. Moisture

The initial moisture content of blended chocolates with eight treatments ranged between 1.16 to 1.24 per cent. The results are in accordance with the findings of Moreno *et al*. (2015), who reported that the moisture content of 1.3 per cent to 1.4 per cent in chocolates prepared from different geographical origin, but Mehta. (2017) also reported a moisture content of 1.1 per cent to 1.5 per cent in different brands of chocolates. He and Hoseney (1990) stated that temperature, humidity and permeability of the material is a very important parameter to extend the moisture absorption capacity of the product. Significant increase in moisture content was recorded in blended chocolates during storage. At the end of storage the highest moisture content of 1.52 per cent was recorded in T1 (95 % AC+5% ODJ) and T_4 (95 % NAC+5% ODJ) and lowest (1.43%) in T_2 (95 % AC+5% B). Molu (2018) stated that the moisture content in enriched nutri spread increased during storage. Mirkovic *et al*. (2018) also reported that the moisture content in different dark chocolates increased from 0.80 per cent to 0.82 per cent during refrigerated storage. Dias *et al*. (2017) also reported that the moisture content of optimised chocolates increased during storage.

5.4.1.3. Energy

Initially the energy content of blended chocolates varied significantly in all treatments with the highest energy content (582.98K.cal) in treatment T_5 (95 %

NAC+5% A) and lowest energy content (579.67kcal) in treatment T_0 (100% AC). This findings are in accordance with the findings of Pandey and Singh (2011), they reported that the calorific content of chocolates vary from 574.5 K.cal/100 g to 583.1 K.cal/100 g. Mehta. (2017) had also reported that the energy/ calorific value of different branded chocolates also varied from was 500 K.cal/100 g to 562 K.cal/100 g. A significant decrease in energy content was recorded in all treatments in blended chocolates during storage. At the end of sixth month of storage the maximum energy content of 580.15 K.cal was recorded in treatment T_5 (95 % NAC+5% A). Gutiérrez (2015) also reported that the energy content of dark, white and milk chocolate was 534 K.cal, 542 K.cal and 529 K.cal respectively. (Pandey and Gurmukh) 2011 also found that the calorific value of chocolates (control) and soy blended chocolates was 574.5 K.cal and 583.1K.cal respectively.

5.4.1.4. TSS

Initially the highest TSS content of 68^0B was observed in treatments T_1 (95 % AC+5% ODJ) and T_4 (95 % NAC+5% ODJ) and based on DMRT, statistically no significant difference was observed in TSS content of blended chocolates in all treatments. In the present study, the TSS remained stable without much difference throughout the storage period in controls and blended chocolates (Fig. 22). According to Miquelim *et al*. (2011) the fruit based chocolates had the TSS higher than 65^0Bx indicating a good shelf life to the product at room temperature. However, the TSS obtained in the chocolates of present study were similar to Jack-Passion spread produced by Chakraborty *et al*. (2011) and higher compared to 38.65^0Bx in a red flesh dragon fruit spread produced by Barcelon *et al.* (2015). In the present study, at the end of storage a slight increase in TSS was observed might be due to the hydrolysis of polysaccharides in to mono and soluble disaccharides as reported by Sarkar *et al*. (2002).A change in moisture content affects the amount of soluble sugars and thus, the proportions of crystalline sugar versus sugar dissolved in solution (Hartel, 2001). The increase in TSS in all stored chocolates might be due to formation of monosaccharides from disaccharides i.e. degradation of sucrose into glucose and fructose (Shah *et al*., 2010).

5.4.1.5. Total sugar

The total sugar content of blended chocolates initially varied from from 40.23 g/100gm to 41.59 g/100gm with the lowest in treatment T_6 (95 % AC+5% WPP) and highest in treatment T_1 (95 % AC+5% ODJ). Statistically significant increase was observed in total sugar content of blended chocolates during storage periods. At the end of sixth month of storage the maximum total sugar content of 47.81g/100gm was recorded in T_1 (95 % AC+5% ODJ). The study by Zupanic *et al*. (2018) revealed that, the total sugar content in chocolates was 44.6 g/100 g and Arentz (2000) also found the total sugar content of 48.27 g/100g was in fructose sweetened chocolates and 51.80 g/100g in sucrose sweetened chocolates. The total sugar content generally increased during the storage (Fig. 23). Molu (2016) also reported that the total sugar of nutri spreads were increased gradually during storage. Thilagavathi *et al*. (2015) stated that increased level of air and moisture content in formulated products hasten the breakdown of total sugars to reducing sugars. They observed increase in total sugars content on storage period due to the formation of simpler sugar like sucrose, glucose and fructose on starch degradation. According to Borchers *et al*. (2000) sugar is hygroscopic and readily absorbs water making the grains stick together forming clumps. Thus, it makes up approximately half the chocolate mass, it is important to prevent water absorption as this affects the quality of the final product.

5.4.1.6. Reducing sugar

Initially the reducing sugar content of blended chocolates varied from 5.23g/100g to 6.35 g/100g. The highest reducing sugar content among all treatment from initial to the end of six months of storage was recorded in T_1 which is reduced from 6.35g/100g to 5.60g/100g. Afoakwa *et al*. (2013), also reported that the reducing sugar content of 1.13 to 4.10 g/100gm was in chocolates. In the present study a reduction in reducing sugar content of blended chocolates in all treatments throughout the storage was observed.

The main reducing sugar in white chocolate is lactose, a disaccharide formed by glucose and galactose found in milk (Messia *et al.* 2007). Chocolate is protected from the external water by its fatty surface, which strongly affects moisture uptake. Although the moisture content have increased, the highest value was 1.52 percent in the present study, thus prohibiting microbial growth. It contribute the non enzymatic browning reactions. According to Friedman (1996) and Van Boekel (1998), in white chocolate, the initial stage of the Maillard reaction involves interaction between the NH_2 of protein-bound lysine with lactose to form lactuloselysine. According to Miao and Roos (2005) the increase in moisture observed during storage of blended chocolate may favor the development of non enzymatic browning. In the present study this may be the reason for decreasing in reducing sugars in blended chocolates during storage.

5.4.1.7. Protein

Initially the protein content of blended chocolates varied but it was not significant, the maximum protein content (7.74g/100g) was noticed in treatment T_2 (95 % AC+5% A) and T_5 (95 % NAC+5% A) and the minimum (7.31g/100g) in T_0 (100% AC). This results are in accordance with Mehta (2017) who reported the protein content of different branded chocolates was in the range of 6.56 to 8.573g/100g. In the study of Amevor *et al*. (2018) the protein in chocolate spread was 10.13 g/100g. Moreno *et al*. (2015) also reported that the protein content in chocolates was from 6.30 to 6.50g/100gm. Mirkovic (2018) also reported that the protein content in control and probiotic chocolates was 9.88 and 9.90 g/100g respectively. Pandey and Gurmukh (2011) also found that the protein content of chocolates (control) and soy blended chocolates was 8.4g/100g and 10.3g/100g respectively.

In the present study the reduction in protein content was noticed in all treatments during storage (Figure 24). Murugkar and Jha (2011) observed that, the total protein content decreased on storage which might be because of the raise in the moisture absorption and production of free amino acids. Rahman *et al*. (2015)

also reported that the initial protein content in soymilk fat spread was 25.1 g/100 g, which decreased to 24.9 g/100 g after 20 days of storage.

5.4.1.8. Total fat

Significant difference was recorded in the fat content of various blended chocolates initially and during each month of storage. The treatment T_5 (95 % NAC+5% A) had significantly high fat (48.35 %) content and the lowest (46.40%) fat content was recorded in T_4 (95 % NAC+5% ODJ). Mirkovic *et al*. (2018) reported that the fat content of different chocolates was 39.92 to 40.02 per cent this value was similar with the fat content of present study. Mehta. (2017) also reported that the fat content of different branded chocolates was in the range of 22.52/100g and 34.60g/100g. Moreno *et al*. (2015) also found that the fat content in chocolates was fom 31.20 to 32.30g/100gm. Pandey and Gurmukh (2011) also found that the total fat content of chocolates (control) and soy blended chocolates was 41g/100g and 42.6g/100g respectively.

In the present study a decrease in fat content during storage was observed among all treatments (Figure 25). At the end of the storage the maximum fat content of 47.50 was in T_5 (95 % NAC+5% A). Molu (2018) also reported that the fat content of enriched nutri spread decreased from 35.02 to 33.98 per cent during storage. Shahanas (2014) also reported that a steady decrease in fat content was observed in blended tender coconut jam and spread during six month of storage.

5.4.1.9. Total polyphenol

The initial polyphenol content of different blended chocolates varied from 0.18g/100g to 0.23g/100g with highest in treatment T_0 (100% NAC) and lowest in treatment T_3 (95 % AC+5% WPP). Meng *et al*. (2008), reported that the poly phenol content in dark chocolate was 0.56g/100g, in milk chocolate it is 0.16g/100g and in white chocolate it is 0.12g/100g. Miller *et al*. (2009) reported that the total polyphenol content in chocolates was 1.20-6.67mg/g. In the present study there was a reduction in poly phenol content with advancement of storage periods was observed in all treatments, but the reduction was not significant up to fourth month

of storage. The poly phenols are stable at 20^0C. Very low and high temperature will cause the degradation of polyphenol content (Sanchez *et al*., 2005). This may be the reason for reduction in polyphenol content at refrigerated storage. But the reduction was not significant in controls throughout the storage periods.

5.4.1.10. Total ash

The total ash content of blended chocolates are varied from 1.47mg/100g to 1.81mg/100g with highest in treatment T_3 (95 % AC+5% WPP) and lowest in T_0 (100% AC). Mirkovic *et al*. (2018) reported that the total ash content of chocolates was 0.23 to 0.24g/100g in different chocolates. Mehta (2017) also reported that the total ash content of different branded chocolates was between 1.27 to 1.86mg/100g .The total ash content reduced slightly during storage. At end of storage total ash content of blended chocolates varied from 1.34 g /100 g to 1.67 g /100 g. Statistically significant decrease was observed in total ash content of blended chocolates during storage periods, this may be due to the increase in moisture content which will reduce the concentration of total ash in blended chocolates during storage. Molu (2018) also reported that the total ash content of enriched nutri spread decreased during storage.

5.4.1.11. Calcium

The calcium content of blended chocolates varied from 0.53g/100g to 0.80g/100g with lowest in T_0 (100% AC) and the highest in T_0 (100% AC) and T_5 (95 % NAC+5% A) and showed statistically significant difference among the treatments. Mehta (2017) also reported that the calcium content in branded chocolates ranged from 0.35 to 0.79 g/100g. These results were in accordance with the results of present study. In the present study a reduction in calcium content was observed in all treatments and the calcium content of blended chocolates at the end of storage was 0.39 to 0.6g/100g. Gutiérrez (2015) also reported that the calcium content in white chocolate was 0.19g/100g and 0.27g/100g in dark chocolate. Rangaswami and Bagyaraj (2000) stated that mineral content of the product reduced on storage because of the use of available nutrients by the microbes present within the products. Similar results were observed by Reshma (2017), who also observed

that mineral content of food products decreased on storage. Bouaziz *et al*. (2017) reported that the calcium content in Tunisian date seed fibers enriched chocolate was 158 mg/100 g of dry matter.

5.4.1.12. Phosphorus

Statistically significant difference was observed in phosphorus content of blended chocolates in all treatments initially and during each month of storage. The initial phosphorus content of different blended chocolates ranged from 0.19g/100g to 0.23g/ 100g with the lowest phosphorus content in T_0 (100% AC), T1 (95 % AC+5% ODJ), T_0 (100% NAC) and highest phosphorus content in T_5 (95 % NAC+5% B) respectively. Gutiérrez (2015) reported that the phosphorus content in dark, white and milk chocolate was 0.16g/100g, 0.19g/100g and 0.23g/100g respectively. Pandey and Gurmukh (2011) also recorded that the phosphorus content of chocolates (control) and soy blended chocolates was 185mg/100g and 276 mg/100g respectively. The higher phosphorus content of 0.31g/ 100gm was reported by Muller *et al*. (2012). Molu (2018) also reported that the phosphorus content in enriched nutri spread was 3.15 mg /100 g. The difference in phosphorous content in different chocolates reflects the mineral characteristics of the soil in which they have grown and is also influenced by the ingredients in the products (Borchers *et al*., 2000).

5.4.1.13. Iron

Initially the iron content in blended chocolates ranged from 17.78mg/100g to 19.96mg/100g with the lowest iron content in T_0 (100% NAC) and highest in T_2 (95 % AC+5% A). Ciguanta *et al*. (2016) also reported that the iron content in white chocolates was 10.9 mg/100g. Molu (2018) also reported that the iron content in enriched nutri spread was 2.59mg/100g. Gutiérrez (2015) also reported that the iron content in dark, white and milk chocolate was 2.2mg/100g, 0.8mg/100g and 0.20mg/100g respectively. Pandey and Gurmukh (2011) also found that the iron content of chocolates (control) and soy blended chocolates was 0.83mg/100g and 0.75mg/100g respectively. The maximum iron content (18.23mg/100g) at sixth

month of storage was recorded in T_5 (95 % NAC+5% B) and lowest (16.20mg/100g) in T_3 (95 % AC+5% WP). From the Figure 30, it is revealed that the iron content of blended chocolates reduced during storage, but the reduction was non significant in all treatments except in T_2 and T_3 at fifth and sixth month of storage.

5.4.1.14. Lipase activity

Initially and during first month of storage the lipase activity was recorded 0.0010(µ eq) in all treatments. At the end of sixth month of storage, the lowest lipase activity (0.0013 µ eq) was in T_2 (95 % AC+5% A) and T_5 (95 % NAC+5% A) and highest ((0.0015 µ eq) in T_3 (95 % AC+5% WP). Some acids and enzymes particularly acetic acid, enzymes and small quantities of aldehydes are also distilled off with the water (Zeigleder *et al*., 2004). This may be the reason for initial reduced lipase activity by increasing tempering and conching time. According to Singh and Soghi (2015) the peroxide value did not inhibit the activity lipase activity but promoted oil degradation in butter. In the present study the peroxide value was not detected, this may be also the reason for initially reduced lipase activity in blended chocolates. But in the present study there was a slight increase in lipase activity in all treatments during storage. According to Braun and Baulzerh (2001) the increase in lipase activity in chocolate was due to microbial action, they reported that the low storage temperature was associated with a significant reduction of enzyme activity, but no complete inactivation even at −4°C. According to Vercet (2003) storage temperature of −20°C was found to be effective in controlling the lipase activity. So during storage, lipolytic activity was can occur that enzyme synthesis has taken place before chilling has started.

5.4.1.13. Free fatty acid

Initially the free fatty acid content in blended chocolates varied from 1.52 per cent to 1.58 per cent. Among the treatments the lowest (1.52%) free fatty acid content was observed in T_1 (95 % AC+5% ODJ), T_2 (95 % AC+5% A) and T_5 (95

% NAC+5% A) and the highest (1.58%) in T_6 (95 % AC+5% WP). The free fatty acid (FFA) content together with the lipase activity control can be considered as useful indice of good quality and correct storage of food, especially for milk products (Antonelli,2002). Jonfia and Navarro (2016) reported that the free fatty acids (FFA) content must be less than 1.0% to meet the acceptable level of 1.75% in cocoa butter extracted from the dry cocoa beans. Cordelia *et al*. (2017) also observed that the free fatty acid content in chocolates was in the range of 0.04 per cent to 2.25 per cent. The difference in free fatty acid content in chocolates confirm that the the difference in geographical origin had an influence on the fatty acid composition of cocoa butters, as pointed out by Lipp and Anklam (1998).

In the present study, the increase in free fatty acid content was observed in blended chocolates during storage and it was within 1.75%. This findings was in accordance with the findings of Yadav *et al*. (2009) and Ali *et al*. (2001), who reported that increase in FFA content during storage of butter paper packaged chocolate was observed. In the present study the increase in free fatty acid content may be due to the increase in lipase activity due to microbial action. This was apparently due to the fact that cocoa butter is sufficiently saturated to exhibit excellent resistance to hydrolytic rancidity as a result of the activity of fat splitting enzymes (Johnston 1992). Pandey and Singh (2011) also reported that the free fatty acid content in soy blended chocolates was also increased from 0.21 per cent to 0.56 percent during sixth month of storage.

5.4.1.2. Organoleptic evaluation of selected blended chocolates during storage

The selected blended chocolates were evaluated organoleptically using score cards for different quality attributes like appearance, colour, flavour, texture, taste and overall acceptability. The evaluation was done initially and at fifteen days interval, for a period of six months of storage under refrigerated condition.

The organoleptic evaluation shows gradual reduction in the mean score for appearance for six months of storage. The initial score for appearance of blended alkalised and non alkalised chocolates varied from 8.89 to 9.00.The highest score

of 9.00 was observed in blended chocolates ie, in treatments T_1 (95 % AC+5% ODJ), T_2 (95 % AC+5% A), T_3 (95 % AC+5% WPP), T_4 (95 % NAC+5% ODJ), T_5 (95 % NAC+5% A), and T_6 (95 % NAC+5% WP). A gradual decrease was observed in the mean score for appearance in all the treatments during storage. The highest mean score and rank score during all the six months of storage period was recorded for chocolates blended with almond ie for the treatments T_2 (95 % AC+5% A) and T_5 (95 % NAC+5% A). Appearance, according to Briones and Aguilera (2005), depends on complex interaction of the incident light, its optical properties and visual sensory perception. Dubost *et al*. (2003) reported that attribute appearance of dietary chocolates changes statistically very significantly in all periods of storage during one year of time. Leite *et al*. (2013) also reported that the three chocolate samples produced from resistant and conventional cocoa cultivar showed good sensory acceptance related to all studied attributes: appearance, odour, flavour, texture and overall acceptability. Rasuluntari *et al* (2016) reported that the blending of cinnamon enriched milk chocolates improve the appearance of milk chocolates.

The initial scores for colour of blended chocolates varied from 8.93 to 9.00 at refrigerated conditions (Table 38). A gradual decrease was observed in the mean score for colour in all treatments up to sixth month of storage. A slight increase in mean score was observed for colour in treatment T_1 (95 % AC+5% ODJ), T_4 (95 % NAC+5% ODJ), T_5 (95 % NAC+5% A) and T_6 (95 % NAC+5% WPP) in sixth month of storage. This results was agreement with the study of Mexis *et al*. (2010) observed that significant changes were observed in color (whitening of dark chocolate with hazelnuts) as a result of fat bloom. Color changes were the least in samples packaged under vacuum. Ali *et al*. (2001) who state that the migration of fat under these temperatures adversely affects the product integrity and appearance.

The flavour of blended chocolates varied from 8.87 to 9.00, with the highest score for T_1 (95 % AC+5% ODJ), T_2 (95 % AC+5% A), T_3 (95 % AC+5% WPP), T_4 (95 % NAC+5% ODJ), T_5 (95 % NAC+5% A), and T_6 (95 % NAC+5% WPP)

and the lowest score of 8.87 was noticed in control T_0 (100% non alkalised chocolates) followed by 8.93 in T_0 (control- alkalised chocolates). The highest mean score and rank score during all the six months of storage period was recorded for the alkalised chocolate blended with almond ie, T_2 (95 % AC+5% A). A decrease in mean score of flavour from the fifteenth day of storage in the second month up to the end of sixth month of storage was recorded, This is in general agreement with the volatile compounds decreased with storage time while secondary oxidation compounds increased significantly resulting to loss of natural aroma of chocolate as recorded by Mexis *et al*. (2010). Ali *et al*. (2001) also found that chocolate stored at 30 °C scored higher in flavour.

The mean score of texture of blended chocolates showed a reduction with the advancement of storage periods. At end of storage the highest mean score was recorded in treatments T_2 (95 % AC+5% A), T_3 (95 % AC+5% WPP), T_5 (95 % NAC+5% A), and T_6 (95 % NAC+5% WPP). T_1 (95 % AC+5% ODJ) showed the least score for texture at the end of storage. Jovanka *et al.* (2016) reported that the firmness and chewiness of dietary chocolates was not statistically significantly changed during first 90 days of storage, and after that period differences were statistically highly significant. Ali *et al*. (2001) also reported that colour and texture of filled dark chocolate stored at 30°C were significantly less preferred than the control and chocolate stored at 18°C.

The taste of the chocolates varied with the storage periods of the bended alkalised and non alkalised chocolates. Among treatments. T_0 (control-non alkalised) had the least score before storage (8.80) and on first (8.73), second (8.71) and third month (8.69) month of storage. T_1 (95 % AC+5% ODJ), T_2 (95 % AC+5% A), T_3 (95 % AC+5% WPP), T_4 (95 % NAC+5% ODJ), T_5 (95 % NAC+5% A), and T_6 (95 % NAC+5% WP), had the maximum score (9.00) before storage and fifteen days after storage. The chocolates (alkalised and non alkalised) bended with almond (T_2 -95 % AC+5% A and T_5 - 95 % NAC+5% A) had the highest score before storage (9.00) and reduced to 8.82. Mexis *et al*. (2010) also reported that smaller changes was observed in the taste profile of hazel nut blended chocolates

after 12 month of storage and the products packaged with the oxygen absorber stored at 4 °C (5.9) had the highest score while the lowest scores were recorded for the commercially packaged product stored at 20 °C (3.8). In cinnomon blended milk chocolates taste is the most affecting attributes that influences panellist (Rasuluntari *et al*., 2016)

The initial score for overall acceptability of blended chocolates varied from 8.76 to 9.0. A gradual decrease was observed in the mean score for overall acceptability in all treatments. The highest mean score and rank score during all the six months of storage period was recorded for the chocolates blended with almond (T_2 -95 % AC+5% A and T_5 -95 % NAC+5% A). The mean score (8.85) and mean rank score (5.33) was highest in T_2 followed by T_5 with the mean score and mean rank score 8.80 and 5.03 respectively. Ali *et al*. (2001) also found that chocolate stored at 30 °C scored higher in overall acceptability. Leite *et al*. (2013) also reported that the three chocolate samples produced from resistant and conventional cocoa cultivar showed good sensory acceptance for overall acceptability. Rasuluntari *et al*. (2016) opined that milk chocolate with the addition of 5per cent of cinnamon powder has the highest level of acceptance.

5.4.1.3. Enumeration of total micro flora

The microbial safety is one of the main criteria for the acceptability of the food products. Numerous changes can take place in foods during processing and storage and shelf-life issues in confections can be challenging.

Lees (1990), reported that major causes of deterioration of chocolate and confectionery products are fermentation, rancidity and moulds. The shelf life of chocolate depends on several parameters like storage temperature, humidity, availability of oxygen in the environment, packaging material used and the addition of other ingredients such as fats and nuts (Nattress *et al*., 2004). The composition of the chocolate can play an important role on its shelf life. Microbial data can play an important role in the verification of implemented controls, but their validity and limitations need to be understood (Swanson and Anderson, 2000).

In the present study, the total bacterial count was not detected initially and up to fourth month of storage. Fungal and yeast count was also not detected up to fifth month of storage. The total bacterial count was varied from 0.33 x 10^4 cfu g-1 to 0.66 x 10^4 cfu g-1 during fifth month of storage and 1.66 10^4 cfu g-1 to 2.0 10^4 cfu g-1 during sixth month of storage between the treatments. The total fungal count was varied between 1.33 x 10^3 cfu g-1 and 1.66x 10^3 cfu g-1 and total yeast count was varied from 2.0 x 10^3 cfu g-1 to 2.66 x 10^3 cfu g-1. This results is in accordance with the results of Pandey and Singh (2011), who found that the total mould and yeast count increased from 2.4 to 3.1 log10 cfu g-1 and 2.3 to 3.0 log10 cfu g-1 respectively during storage. The presence of microbial count can be attributed to the increase in moisture content and moisture absorption capacity of the blended chocolates on storage. According to Mossel *et al*. (1995) water activity of >0.6 and <0.85 would potentially allow for xerophilic yeasts / moulds growth that are of importance in spoilage of low moisture foods.

Baylis *et al*. (2004) in his study observed that the microbiological changes in chocolate samples during storage of optimised chocolates with additives have lesser standard plate count as compared to control chocolate throughout the storage period. Nebesney *et al*. (2007) also reported that lactic acid bacteria had very good survival in dark chocolate during storage at 4^0C for 12 months. Dias *et al*. (2017) also reported that the moulds and yeast count in white chocolates was below 2.0 x 10^3 cfu/g and 1.3 x10^3cfu/g respectively during storage. De Clerq *et al.* (2015) observed that xerophilic fungi are amongst the most potential spoilage organisms present in chocolate fillings, especially the genera *Penicillium, Aspergillus, Eurotium* and *Zygosaccharomyces*. These organisms were observed in the environment of chocolate factories but mostly in some ingredients used to produce chocolate fillings, like nuts and sugar syrup (Marvig *et al.*, 2014). In the present study the results of bacteria, moulds and yeast was detected at the end of storage. Mehta. (2017) also reported that the initial microbial count of different branded chocolates was 6x10^4, 31x10^4, 2x10^4, 13x$10^4$and 40x10^4in Perk, Munch, Amul (Fruits and Nut), Cadbury (Fruits and Nut) and Nestle (Fruits) respectively. But in

the present study the microbial count was not detected up to fourth month of storage, it may be due to the high sugar content in the chocolates and also temperature (70^0C) during tempering and conching.

The observed bacterial count was within the safe limits of FSSAI (2015) specification (Not more than 50,000 cfu g-1 up to sixth month of storage of blended chocolates. But the observed fungal and yeast count was not in the limits of FDA (2010) specification less than 100 cfu g-1. So the shelf life of the bended chocolates was up to fifth month of storage at refrigerated conditions.

5.5. Cost of production of the selected chocolates

The cost of blended chocolates varied from Rs.46.54 to Rs.50.62 / 40 g of chocolate bar. The lowest cost was found in the chocolates(Controls T_0 (AC) and T_0 (NAC) prepared from alkalised and nonalkalised cocoa nibs and the highest cost (Rs.50.62) was for the chocolates blended with 5% almond (T_2 -95 % AC+5% A and T_5 -95 % NAC+5% A). The cost of commercial chocolate, dairy milk silky was Rs.70/40gm. The cost of the chocolates prepared in the present study was lesser as compared to the commercial chocolates.

Summary

SUMMARY

The study entitled "Process optimisation and quality evaluation of cocoa based chocolates was under taken with the objectives to develop a protocol for primary processing of cocoa beans based on free fatty acid content (<1.75 %), to standardise the time and temperature of chocolate making using machine. The study also aims to evaluate the quality attributes and shelf life of the products.

The study was conducted for the development of protocol for primary processing of cocoa beans based on free fatty acid content. Cocoa fermentation was carried out with three different types of fermentation methods like basket, heap and sack method for the periods of 5, 6 and 7 days of fermentation with nine different treatments [(T_1 - 5 th day-Basket), (T_2- 6th day-Basket), (T_3- 7 th day-Basket), (T_4- 5 th day-Heap), (T_5- 6 th day-Heap), (T_6 - 7 th day-Heap), (T_7- 5 th day-Sack), (T_8 - 6 th day-Sack), (T_9 - 7 th day-Sack)]**.** The physico-chemical qualities of fermented cocoa beans such as fermentation bean recovery, fermentation index/cut test, moisture, pH, peroxide value, lipase activity, total fat and free fatty acid content were evaluated. The highest (84.99%) fermentation index or cut test score was recorded in heap method at seventh day of fermentation (T_6), the basket method (T_3) exhibited 72.22per cent fully fermented beans and the sack method (T_9) was observed with least fully fermented beans at seventh day of fermentation (69.10%).

The highest fermentation bean recovery of 84.33per cent was noticed in treatment T_4 in heap method at fifth day of fermentation, followed by 82 per cent of bean recovery were noticed in treatment T_5 at sixth day of fermentation in heap method and 78.33 per cent of bean recovery were in treatment T_7 at fifth day of fermentation in sack method.

A decrease in moisture content of fermented beans was observed with increasing duration in fermentation periods for all methods of fermentation. The moisture content vary from 50.40 per cent, 53.67 per cent and 54.40 per cent to 39.80 per cent, 37.83 per cent and 39.43 per cent from initial to seventh day of

fermentation in sack, heap and basket method respectively. The pH of fermented beans decreased during fermentation periods, it varied from 6.11 to 4.26 in all methods. Among the fermentation methods the maximum fat content was in heap method from initial 42.66 to 32.89 per cent. In the case of lipase activity, it decreased with fermentation periods in all three fermentation methods. In basket method the activity varied from initial of 0.0027 to 0.0011 at the end of fermentation, in heap method it varied from 0.0022 to 0.0005 and for sack it decreased from 0.0026 to 0.0012 respectively. A mean free fatty acid content of 2.51 percent was recorded on the first day of fermentation and was reduced in to 1.05 per cent in basket method. Similarly in heap and sack method the free fatty acid content was reduced from 2.39 per cent 0.80 per cent and from 2.52 per cent to 1.18 per cent respectively. The selected heap method, based on the lowest free fatty acid content (0.80%) with seven days of fermentation were subjected to sun drying and oven drying.

The sun dried and oven dried beans were analysed for the physico-chemical qualities, such as bean recovery, moisture, pH, peroxide value, lipase activity, total fat, free fatty acid content and peroxide value. The bean recovery of fermented dried beans was high (41.00 %) in treatment T_1 (sun dried cocoa beans) as compared to treatment T_2 (oven dried beans) (40.12 %). The moisture content of 3.76 per cent was less in treatment T_2 (oven dried beans) than the moisture content of 4.22 per cent in treatment T_1 (sun dried cocoa beans). The pH value of treatment T_1 (sun dried cocoa beans) was significantly higher (5.29) than the treatment T_2 (oven dried beans) of 4.89. The fat content of sun dried and oven dried cocoa beans was 48.33% and 47.93 per cent respectively. A lesser lipase activity of 0.0018(µ eq) was observed in T_1 (sun dried cocoa beans) compared to (0.0025µ eq) T_2 (oven dried beans). The free fatty acid content (1.26 %) in sun dried cocoa beans was less compared to 1.47% in T_2 (oven dried beans). The sun drying proved to be adaptive as free fatty acid content was lower than oven dried beans and were below the cut off value of 1.75per cent. The peroxide value of fermented dried cocoa beans were not detected in sun dried and oven dried beans.

Based on lower free fatty acid content the sun dried cocoa beans were selected for storage. The sun dried beans were stored in gunny bags (control), polythene covers and plastic containers under ambient condition for a period of six months. The physico-chemical qualities of cocoa beans stored in gunny bags, polythene covers and plastic containers were done initially and at an interval of one month for a duration of six months. The bean recovery per cent decreased in all three methods of storage with the duration of storage periods. The bean recovery (98%) was significantly superior in treatment T_3 (Cocoa beans stored in plastic container) at the end of sixth month of storage.

The highest moisture content among all treatments from first month after storage to the end of six month of storage was recorded in cocoa beans stored in gunny bags (T_1) ranging from 4.23 to 5.21 percent. The least moisture content was recorded in treatment T_3 (Plastic container) as from 4.22 to 4.52 per cent from first month after storage to sixth month after storage. The cocoa beans stored in polythene cover (T_3) had the moisture content of 4.79 per cent at the end of sixth month of storage.

Initially the pH of cocoa beans stored in gunny bags (T_1), polythene covers (T_2) and plastic containers (T_3) was 5.22, 5.23 and 5.22 respectively. A slight increase in pH was found in stored cocoa beans. The highest pH of 5.31was observed in treatment T_1 (Gunny bags), lowest (5.27) in treatment T_3 and 5.28 was in treatment T_2 at sixth month after storage. The initial fat content was in between 50.31 and 50.34 per cent in different treatments (T_1, T_2 and T_3). Generally a decreasing trend was recorded in all the three treatments from initial to six month after storage. The lowest fat content of 47.20 per cent was recorded in treatment T_1 (cocoa beans stored in gunny bags) and the highest fat content of 48.07 per cent was recorded in treatment T_3 (cocoa beans stored plastic container) at the end of six month after storage.

The initial lipase activity in stored cocoa beans was 0.0017 µ eq in all three treatments like cocoa beans stored in gunny bags, polythene cover and plastic container. In cocoa beans stored in gunny bags (T_1) the lipase activity varied from 0.0017 to 0.0026 µ eq from initial to six month after storage. In cocoa beans stored in polythene cover (T_2) it varied from 0.0017 to 0.0020 µ eq and in cocoa beans stored in plastic container (T_3) it varied from 0.0017 to 0.0019 µ eq. The lipase activity of cocoa beans was increased during storage. Initially the free fatty acid content was 1.12 per cent in all three treatments T_1, T_2 and T_3. An increasing trend in free fatty acid content was recorded during storage in all three treatments. The highest free fatty acid content 2.80 % was recorded in treatment T_1 (Gunny bags) and the lowest free fatty acid content of 1.68per cent was in treatment T_3 (Plastic container) at sixth month after storage. The treatment T_3 was within the cut off value of free fatty acid content 1.75 per cent at the end of six month of storage, but the treatment T_2 was within fifth month of storage and treatment T_1 was within the third month of storage.

The microbial population (bacterial and fungal count) was calculated initially and at the end of sixth month of storage of cocoa beans subjected to different storage treatments. Initially, the lowest bacterial population of l.2 x 10^4 cfu g-1 was recorded in beans stored in plastic container (T_3) followed by beans stored polythene cover i.e. T_2 (1.4 x 10^4 cfu g-1). The maximum bacterial count of 1.6 x 10^4 cfu g-1 was recorded with control (T_1) i.e. the cocoa beans stored in gunny bags. After six month after storage, the treatment T_3 (6 .0 x 10^4 cfu g-1) and T_2 (7.2 x 10^4 cfu/g) recorded the lower bacterial count. The maximum bacterial count of (10.4 x 10^{-4} cfu g-1) was observed in control (T_1) at the end of storage. The fungal population was lowest in treatment T_2 (1 x 10^{-3} cfu g-1) and T_3 (1 x 10^{-3} cfu g-1) and followed in treatment T_1 (1.2 x10^{-3} cfu g-1). The fungal population was found to increase in general at the end of storage. The highest fungal count of (13.6 x 10^{-3} cfu g-1) was recorded in cocoa beans stored in gunny bags (T_1), and the lowest fungal count of 2.2 x 10^{-3} cfu g-1 and 2.0 x 10^{-3} cfu g-1 in T_2 and T_3 respectively.

The cocoa beans stored in plastic container were selected for secondary processing of cocoa. Secondary processing include alkalisation, roasting and chocolate making of cocoa. Based on free fatty acid content, the lowest free fatty acid content (1.68%) was observed in cocoa beans stored in plastic container at the end of storage. The stored cocoa beans in plastic container were divided into two lots. One lot was used as such and the second lot was subjected to alkalisation. For the preparation of chocolates, all the ingredients needed for chocolate making was fed in to tempering and conching machine. Twelve sets of chocolates were prepared at different time periods of 7, 9 and 11 hours at a temperature of 60^0C and 70^0C. After the preparation of different types of alkalised and non alkalised chocolates in tempering and conching machine, chocolates were subjected to analysis of physico-chemical qualities, organoleptic evaluation and for enumeration of microflora.

The textural properties of the alkalised and non alkalised chocolates were evaluated in freshly prepared chocolates. The hardness of the alkalised chocolates were varied between 72.87N and 97.70N. The maximum hardness of 97.07N was observed in treatment T_4 (chocolate prepared at 70^0C for 7 hours) and minimum (72.87N) in treatment T_3 (chocolate prepared at 60^0C for 11 hours). In non alkalised chocolates the hardness varied from 72.51 N to 99.11N. The highest value for hardness was observed in treatment $T1_0$ (chocolate prepared at 70^0C for 7 hours) and lowest in $T1_2$ (chocolate prepared at 70^0C for 11 hours).

Cohesiveness of chocolates varied in alkalised chocolates from 0.008N to 0.021N. Statistically the highest cohesiveness of 0.021N was observed in chocolates prepared at 70^0C for 11 hours (T_6) and lowest (0.008N) in treatment T1. The maximum cohesiveness of 0.022N was found in non alkalised chocolates in treatment $T1_2$ (chocolate prepared at 70^0C for 11 hours) and the minimum of 0.009N in treatment T_7 (chocolate prepared at 60^0C for 7 hours).

The adhesiveness of 0.0027N was found to be highest in treatment T_6 (chocolate prepared at 60^0C for 11 hours) and lowest in treatment T1 (chocolate prepared at 60^0C for 7 hours).In non alkalised chocolates, the adhesiveness varied from 0.0007N to 0.0021N. The maximum adhesiveness of 0.0021N was found to be in treatment T_{12} and lowest value of 0.0007N in treatment T_7 and $T_{10.}$ There was a slight increase in adhesiveness of chocolates with the duration of tempering and conching.

The gumminess of chocolates was varied with different treatments in alkalised and non alkalised chocolates. The maximum gumminess of 1.3782N was recorded in alkalised chocolates prepared at 60^0C for 11 hours (T_3) and minimum (0.9482N) in treatment T_4 (chocolate prepared at 70^0C for 7 hours). In non alkalised chocolates, statistically the highest gumminess of 1.3201 N was recorded in treatment T_9 (chocolate prepared at 60^0C for 11 hours) and lowest (1.0620N) in treatment T_7 (chocolate prepared at 60^0C for 7 hours). Gumminess was slightly decreased in alkalised and non alkalised chocolates with the duration of tempering and conching.

The moisture content generally decreased with increasing duration of tempering and conching with in a temperature range. The treatment T_1 (Chocolate prepared at 60^0C for 7 hours) had the maximum moisture content (1.33%) and T_6 (Chocolate prepared at 70^0C for 11 hours) had recorded the lowest moisture content of 1.18%. In non alkalised chocolates the highest moisture content of 1.34per cent was observed in treatment T_7 (Chocolate prepared at 60^0C for 7 hours) and the lowest moisture content in non alkalised chocolates was recorded in chocolates prepared at 70^0C for 11 hours in treatment T_{12} (1.23%).

The lower calorific value of 539.80 K.cal was noticed in treatment T_1 (Chocolate prepared at 60^0C for 7 hours) and the highest calorific value of 579.04 and 579.82 was recorded in chocolates prepared at 60^0c and 70^0C for 11 hours in treatment T_3 and T_6 respectively. In non alkalised chocolates the highest energy

content of 579.62 K.cal was noticed in treatment T_{12} (chocolate prepared at 70^0C for 11 hours) followed by treatment T_9 (chocolate prepared at 60^0C for 11 hours) with 575.04 K.cal. The lowest calorific value of 539.00 K.cal was noticed in treatment T_7 followed by T_8 (543.14 K.cal).

A decrease in TSS content was seen in alkalised and non alkalised chocolates with increasing temperature and also with the duration of tempering and conching. In alkalised chocolates the highest TSS of 68^0 Bx was recorded in treatment T1 (chocolate prepared at 60^0C for 7 hours) followed by 67^0Bx in treatment T_2 (chocolate prepared at 60^0C for 9 hours) and T_3 (chocolate prepared at 60^0C for 11 hours). The same trend was also observed in non alkalised chocolates.

In alkalised chocolates the highest total sugar content of 48.02 g/100g was noticed in T_6 (chocolates prepared at 70^0C for 11 hours) and the lowest total sugar content of 46.7g/100g and 46.8g/100g was observed in T_1 (chocolates prepared at 60^0C for 7hours) and T_4 (Chocolates prepared at 70^0C for 7hours) respectively. In non alkalised chocolates the highest total sugar content of 48.02g/100g was recorded in chocolates prepared at 60^0C for 11 hours (T_9) and 70^0C for 11 hours (T_{12}). The lowest total sugar content of 46.6 g/100g and 46.7 g/100g was recorded in treatment T_7 (Chocolates prepared at 60^0C for 7hours) and T_{10} (Chocolates prepared at 70^0C for 7hours) respectively.

The highest reducing sugar content of 5.1g/100gm was recorded in treatment T_6 (70^0C for 11 hours) and the lowest reducing sugar content of 4.91g/100gm was observed in treatment T_4 (70^0C for 7 hours). In non alkalised chocolates the highest reducing sugar content of 5.3g/100g was recorded in treatment T_9 (60^0C for 11 hours). The lowest reducing content of 4.8g/ 100g was observed in chocolates prepared at 60^0C for 7 hours (T_7).

The protein content of alkalised chocolates varied between 7.30 and 10.45g/100g with the lowest protein in T_6 (70^0C for 11 hours) and highest in T_1

(60^0C for 7 hours).In non alkalised chocolates, the highest protein content of 9.45g/100g was noticed in treatment T_7 (60^0C for 7 hours) and the lowest protein content of 7.58g/100g was observed in T_{12} (70^0C for 11 hours). The total fat content of alkalised chocolates ranged from 41.1 per cent to 44.5 per cent with the lowest and highest total fat content in treatment T_1 (60^0C for 7 hours) and T_6 (70^0C for 11 hours) respectively. In non alkalised chocolates, the total fat content was found to be highest in T_{12} (46.50 per cent) and lowest in treatment T_7 (42.0 per cent).

In alkalised chocolates the highest total polyphenol of 0.21g/100g was found in treatment T_1 (Chocolates prepared at 60^0C for 7hours) and lowest 0.16g/100g of polyphenol in chocolate prepared at 70^0C for 11 hours (T_6). In non alkalised chocolates, the treatment T_7 had the maximum polyphenol content (0.23g/100g) and T_{11} and T_{12} had the lowest polyphenol content (0.20g/100g). The maximum total ash content of 1.45g/100g was recorded in treatment T_6 (70^0C for 11 hours) and the minimum total ash content of 1.41g/100g was observed in alkalised chocolates for treatment T_1 (60^0C for 7 hours). The total ash content in non alkalised chocolates was maximum (1.43g/100g) in treatments T_9 (60^0C for 11 hours), T_{11} (70^0C for 9 hours) and $T1_2$ (70^0C for 11 hours).

The treatments T_3 and T_6 had slightly high calcium content (0.04g/100gm) to other treatments (0.03g/100gm). In non alkalised chocolates the calcium content was more in treatment T_8, T_9 and T_{12} (0.04g/100gm). The maximum phosphorus content of (0.17g/100g) was recorded in treatment T_3 and T_6 followed by T_1, T_2 and T_5 (0.16g/100g). In non alkalised chocolates the phosphorus content was maximum in treatment $T1_2$ (0.17g/100g) followed by treatments T_9 and T_{11} (0.16g/100g).

The maximum iron content of 18.45 mg/100gm was observed in the treatment T_3 followed by T_2 (17.93mg/100gm). The lowest iron content of 15.09 mg/100gm was found to be in chocolate prepared at 70^0C for 7 hours. In non alkalised chocolates, the iron content was highest in treatment T_9 (16.89mg/100gm).

In alkalised chocolates the lipase activity was decreased with increasing the temperature and duration of tempering and conching of chocolates. The lipase activity of alkalised chocolates ranged from 0.0010 to 0.0015 µ eq and in non alkalised chocolates varied from 0.0010 µ eq to 0.0017 µ eq. The treatment T_1 (chocolate prepared at 60^0C for 7 hours) had the maximum free fatty acid content (2.80 %) and the treatment T_6 (chocolate prepared at 60^0C for 11 hours) had recorded the lowest free fatty acid content of 1.67per cent, which is a desirable property for good chocolate. In non alkalised chocolates the highest free fatty acid content of 2.61per cent was observed in treatment T_7 (chocolate prepared at 60^0C for 7 hours) and the lowest free fatty acid content in non alkalised chocolates also recorded in chocolates prepared at 70^0C for 11 hours in treatment T_{12} (1.68%).

Based on organoleptic evaluation, the comparison of temperature during tempering and conching of chocolates was done based on overall acceptability and total score of sensory evaluation parameters like appearance colour, taste, texture, flavour and overall acceptability. The maximum score for overall acceptability (8.87) was obtained for the treatment T_6 (70^0C for 11hours) in alkalised chocolates and 8.89 was for chocolate prepared at 70^0 for 11hours ($T1_2$) in non alkalised chocolates. The highest total score was also recorded in treatment T_6 (53.13) in alkalised chocolates and total score of 52.63 in treatment T_{12} in non alkalised chocolates. So the temperature of 70^0C was suitable for tempering and conching of chocolate preparation. The bacterial, fungal and yeast population was not detected in all treatments ie, chocolates prepared with alkalised and non-alkalised cocoa beans.

Chocolates prepared from alkalised beans and subjected to tempering and conching operations at 70^0C for 11 hours were used for the preparation of blended chocolates. Chocolates were blended with dehydrated grapes, dehydrated dates, osmodehydrated jackfruit, osmodehydrated pineapple, almond, cashew nut, powdered dehydrated mint leaves and white pepper powder. Standardisation of

mint powder and white pepper added in the chocolates were standardised based on organoleptic evaluation.

After preparing blended chocolates, the organoleptic evaluation was done in alkalised and non alkalised chocolates. From the, organoleptic evaluation of different treatments, based on appearance, colour, flavor, texture, taste, overall acceptability and total score the best rated treatments from alkalised chocolates were T_3 (95 % alkalised chocolates +5% osmodehydrated jack fruit), T_6 (95 % alkalised chocolates +5% almond) and T_8 (95 % alkalised chocolates +5% white pepper) and from non alkalised chocolates the treatments T_{11} (95 % non alkalised chocolates +5% osmodehydrated jack fruit), T_{14} (chocolate blended with 95 % non alkalised chocolates and 5% almond) and T_{16} (95 % non alkalised chocolates and 5% white pepper powder) were selected for further storage studies.

The selected blended chocolates from alkalised and non alkalised chocolates were packed in aluminium foils and stored in refrigerator for six months. The chocolates were analysed for its physical and chemical qualities initially and during six month of storage. The textural properties of blended chocolates were evaluated initially and at the end of six month of storage and it was compared with commercial chocolates. The hardness of all blended chocolates and controls (alkalised and non aljkalised chocolates) increased at the sixth month of storage compared to initial hardness. Initially the lowest hardness of 74.41N was recorded in control (T_0 - NAC), chocolates prepared with 100% non alkalised beans followed by treatment T_0 (76.71N) with 100% alkalised beans. Initial cohesiveness of blended chocolates varied from 0.022N to 0.028N and at the end of storage it varied from 0.025N to 0.031N. The initial adhesiveness of blended chocolates varied between 0.0017N and 0.0028N and at the end of storage adhesiveness varied from 0.0014N to 0.0026N. The initial gumminess of blended chocolates varied from 1.8813N to 1.2401N and at the end of storage the maximum gumminess of 1.9841 N was recorded in T_4 (95 % NAC+5% ODJ) and the lowest gumminess of 1.3798 N was recorded in T_3 (95 % AC+5% WPP).

The moisture content was increased during storage in blended chocolates. Initially the moisture content in blended chocolates ranged between1.16 to 1.24 per cent and at the end of storage it varied from 1.43 to 1.52 per cent. The energy content of blended chocolates was observed in all treatments, initially the highest energy content of 582.98K.cal and at the end of sixth month of storage the maximum energy content of 580.15K.cal was found in treatment T_5 (95 % NAC+5% A). An increase in TSS content of blended chocolates was recorded in all treatments during storage. Initially the total sugar content of blended chocolates varied from 40.23 g/100gm to 41.59 g/100gm. From initial to sixth month of storage the maximum total sugar was in T_1 (95 % AC+5% ODJ). Initially the reducing sugar content of blended chocolates varied from 5.23g/100g to 6.35 g/100g. The highest reducing sugar content among all treatment from initial to the end of six months of storage was recorded in T_1 which is reduced from 6.35g/100g to 5.60g/100g. Initially the maximum protein content (7.74 g/100g) was noticed in treatment T_2 (95 % AC+5% A) and T_5 (95 % NAC+5% A) and lowest (7.31g/100g) in T_0 (100% AC). A steady decrease in fat content during storage was observed among all treatments. The treatment T_5 (95 % NAC+5% A) had high fat (48.35 %) content and the lowest (46.40%) fat content was recorded in T_4 (95 % NAC+5% ODJ).

The initial polyphenol content of different blended chocolates varied from 0.18g/100g to 0.23g/100g. Among the treatments the highest polyphenol content ranging from 0.23g/100g to 0.19g/100g was seen in chocolates prepared with hundred per cent non alkalised chocolates (T_0 -100% NAC) throughout the storage periods. Initially, the total ash content of blended chocolates are varied from 1.47g/100g to 1.81g/100g. At sixth month of storage the maximum ash content (1.67g/100g) was in T_3 (95 % AC+5% WPP) and T_6 (95 % NAC+5% WPP). Initially, the calcium content of blended chocolates varied from 0.53g/100g to 0.80g/100g. After storage, a reduction in calcium content was observed in all treatments and at the end of sixth month calcium content varied from 0.39g/100g to 0.63g/100g. A reduction in phosphorus content of blended chocolates was

recorded in all treatments during storage. At the end of sixth month of storage the treatments T_5 and T_6 was with maximum phosphorus content (0.16g/100g). Initially the iron content in blended chocolates ranged from 17.78mg/100g to 19.96mg/100g and the maximum iron content (18.23mg/100g) at sixth month of storage was recorded in T_5 (95 % NAC+5% A).

Initially and during first month of storage the lipase activity was recorded as 0.0010µ eq in all treatments. At the end of sixth month of storage the lowest lipase activity (0.0013 µ eq) was in T_2 (95 % AC+5% A) and T_5 (95 % NAC+5% A) and highest ((0.0015 µ eq) in T_3 (95 % AC+5% WPP). Initially among the treatments the lowest (1.52%) free fatty acid content was observed in T_1 (95 % AC+5% ODJ), T_2 (95 % AC+5% A) and T_5 (95 % NAC+5% B) and highest (1.52%) in T_6 (95 % AC+5% WPP). An increase in trends in free fatty acid content of blended chocolates was recorded during storage and it was within 1.75per cent up to fourth month of storage.

The organoleptic qualities of the selected chocolates during storage were analysed and the mean score and mean rank score obtained for different quality attributes of appearance, colour, flavour, texture, taste and overall acceptability of alkalised and non alkalised blended chocolates during storage were also observed. The initial score for appearance of blended alkalised and non alkalised chocolates varied from 8.89 to 9.00. A gradual decrease was observed in the mean score for appearance in all the treatments during storage. The highest score of 9.00 for colour was attained for the treatments T_1 (95 % AC+5% ODJ), T_2 (95 % AC+5% A). Initially, the score for flavour of blended chocolates varied from 8.87 to 9.00. The highest mean score and rank score for flavour during all the six months of storage period was recorded for the alkalised chocolate blended with almond ie, T_2 (95 % AC+5% A). The texture of blended chocolates showed a reduction with the advancement of storage periods. Before storage and fifteenth day of storage, the highest mean score of 9.00 for texture was for the treatments T_0 (controls – alkalised and non alkalised), T_3 (95 % AC+5% WPP) and T_6 (95 % NAC+5% WPP). The

taste of the chocolates varied with the storage periods of the bended alkalised and non alkalised chocolates. T_1 (95 % AC+5% ODJ), T_2 (95 % AC+5% A), T_3 (95 % AC+5% WPP), T_4 (95 % NAC+5% ODJ), T_5 (95 % NAC+5% A), and T_6 (95 % NAC+5% WPP), had the maximum score (9.00) before storage and fifteen days after storage and also showed maximum score in each storage periods. The chocolates (alkalised and non alkalised) bended with almond (T_2 -95 % AC+5% A and T_5 - 95 % NAC+5% A) had the highest score for taste before storage (9.00) and at the end of the storage (8.82).

The initial score for overall acceptability of blended chocolates varied from 8.76 to 9.00, with the highest score for T_1 (95 % AC+5% ODJ), T_2 (95 % AC+5% A), T_3 (95 % AC+5% WPP), T_4 (95 % NAC+5% ODJ), T_5 (95 % NAC+5% A), and T_6 (95 % NAC+5% WPP. A gradual decrease was observed in the mean score for overall acceptability in all treatments. The highest mean score and rank score during all the six months of storage period was recorded for the chocolates blended with almond (T_2 -95 % AC+5% A and T_5 -95 % NAC+5% A). Based on organoleptic score, the treatment T_2 (95 % AC+5% A) attained the highest rank I which was followed by the ranks II and III for the treatments T_5 (95 % NAC+5% B) and T_1 (95 % AC+5% ODJ) respectively.

The blended chocolates were evaluated for the presence of bacteria, yeast and mould initially and monthly intervals for a period of six months. The bacterial count was not detected from initial to forth month of storage in all treatments during storage. At the end of sixth month of storage the highest bacterial count of 2.0×10^4 cfu g-1 was found in treatments T_1, T_2,T_3,T_4,T_5 and T_6 and lowest (1.66×10^4 cfu g-1) in controls (T_0 -100% AC and T_0 -100% NAC). Regarding storage periods. The fungal count was not detected from initial to fifth month of storage in all treatments during storage. During sixth month of storage, the fungal count was detected and the lowest count (1.33×10^3 cfu g-1) was in treatment T_0 (100% AC), T_2 (95 % AC+5% A),T_3 (95 % AC+5% WPP), T_0 (100% NAC) and T_4 (95 % NAC+5% ODJ) and highest (1.66×10^3 cfu g-1) in T1(95 % AC+5% ODJ), T_5 (95 % NAC+5% A)

and T_6 (95 % AC+5% WPP). The yeast count of blended chocolates during storage was not detected initially and up to fifth month of storage. During sixth month of storage, the presence of yeast was detected and the lowest (2.0 x 10^3 cfu/g) yeast count in blended chocolates was in treatment T_1 (95 % AC+5% ODJ), T_0 (100% NAC), T_4 (95 % NAC+5% ODJ) and T_5 (95 % NAC+5% A) and highest (2.66 x 10^3 cfu g-1) in T_6 (95 % AC+5% WPP).

The cost of production was varied from Rs.46.54 to Rs.50.62 / 40 g of chocolate bar. The lowest cost was found to be in the chocolates(Controls- T_0 (AC)/ T_0 (NAC) prepared from alkalised and non alkalised cocoa nibs and the highest cost (Rs.50.62) was for the chocolates blended with 5% almond (T_2 -95 % AC+5% A and T_5 -95 % NAC+5% A). The cost of the chocolates prepared in the present study was lesser as compared to the commercial chocolates.

The process optimisation of cocoa from bean to chocolates based on free fatty acid content viz. fermentation methods, drying of cocoa beans, conching and tempering, blending and storage of chocolates were standardised. Blending of chocolates with osmodehydrated jack fruit, almond and white pepper powder increased the nutritional and organoleptic acceptability of the products.

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APPENDIX – I

Score card for organoleptic evaluation of alkalised chocolates made at 60^0C and 70^0 C

Name of the judge:

Date:

Signature

Parameter	Treatments					
	T_1	T_2	T_3	T_4	T_5	T_6
Appearance						
Colour						
Flavour						
Texture						
Taste						
Overall acceptability						

Nine point hedonic scale

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

APPENDIX – II

Score card for organoleptic evaluation of non alkalised chocolates made at 60^0C and 70^0 C

Name of the judge:

Date:

Signature

Parameter	Treatments					
	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
Appearance						
Colour						
Flavour						
Texture						
Taste						
Overall acceptability						

Nine point hedonic scale

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

APPENDIX – III

Score card for organoleptic evaluation for the standardisation of dehydrated mint powder in the chocolate

Name of the judge:

Date:

Signature

Parameter	Treatments				
	T_1	T_2	T_3	T_4	T_5
Appearance					
Colour					
Flavour					
Texture					
Taste					
Overall acceptability					

Nine point hedonic scale

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

APPENDIX- IV

Score card for organoleptic evaluation for the standardisation of white pepper powder in the chocolate

Name of the judge:

Date:

Signature

Parameter	**Treatments**				
	T_1	**T_2**	**T_3**	**T_4**	**T_5**
Appearance					
Colour					
Flavour					
Texture					
Taste					
Overall acceptability					

Nine point hedonic scale

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

APPENDIX –V

Score card for organoleptic evaluation for the standardisation alkalised chocolates with dried fruits, nuts, dehydrated mint leaves and white pepper

Name of the judge:

Date:

Signature

Parameter	**Treatments**									
	T_0	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**T_7**	**T_8**	**T_9**
Appearance										
Colour										
Flavour										
Texture										
Taste										
Overall acceptability										

Nine point hedonic scale

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

APPENDIX-VI

Score card for organoleptic evaluation for the standardisation non alkalised chocolates with dried fruits, nuts, dehydrated mint leaves and white pepper

Name of the judge:

Date:

Signature

Parameter	Treatments									
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
Appearance										
Colour										
Flavour										
Texture										
Taste										
Overall acceptability										

Nine point hedonic scale

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

APPENDIX-VII

Sensory evaluation of alkalised and non alkalised blended chocolates with jack fruit, almond and white pepper

Name of the judge:
Date:
Signature

Parameter	**Treatments**							
	Alkalised chocolates				**Non alkalised chocolates**			
	T_0	**T_1**	**T_2**	**T_3**	**T_0**	**T_1**	**T_2**	**T_3**
Appearance								
Colour								
Flavour								
Texture								
Taste								
Overall acceptability								

Nine point hedonic scale

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

PROCESS OPTIMISATION AND QUALITY EVALUATION OF COCOA BASED CHOCOLATES

By

SHAHANAS E.

(2016 - 24 - 002)

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirements for the degree of

Doctor of philosophy in Community Science

(FOOD SCIENCE AND NUTRITION)

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF COMMUNITY SCIENCE

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VELLANIKKARA, THRISSUR – 680656

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2019

ABSTRACT

Cocoa (*Theobroma cacao*) is a supporting crop to farmers due to its remunerative income, which forms the basis for one of the world's most popular food products chocolate. The quality of final product depends upon the fermented dried beans. High free fatty acid content is a serious quality defect and reduces the economic value of the cocoa beans. Recently, the cocoa trade has assumed a more scientific position and a lot of emphasis are placed on the content of free fatty acid. A reduction in the free fatty acids level will definitely have a positive impact. As such there is a need for the farmers to carry more intensive research and to develop and technology for the standardisation of free fatty acid content in the processing stages of cocoa in both primary and secondary processing.

The present study was undertaken to develop protocol for primary processing of cocoa beans based on free fatty acid content (<1.75 %), to standardise the time and temperature of chocolate making using machine and to evaluate the quality attributes and shelf life of the products.

Cocoa fermentation was carried out with three different types of fermentation methods like basket, heap and sack method for the periods of 5, 6 and 7 days of fermentation with nine different treatments. The physico-chemical qualities of fermented cocoa beans were evaluated. The highest (84.99%) fermentation index or cut test score was recorded in heap method at seventh day of fermentation (T_6). The highest fermentation bean recovery of 84.33% was noticed in treatment T_4 in heap method at fifth day of fermentation.

The moisture content vary from 50.40 %, 53.67% and 54.40% to 39.80%, 37.83% and 39.43% from initial to seventh day of fermentation in sack, heap and basket method respectively.

The pH of fermented beans varied from 6.11 to 4.26 in all methods. Among the fermentation methods the maximum fat content was in heap method from initial 42.66% to 32.89%. The lipase activity, decreased with fermentation periods in all three fermentation methods. In heap method it varied from 0.0022 to 0.0005. The heap method with seven days of fermentation were selected to sun drying and oven drying based on the lowest free fatty acid content (0.80%).

The bean recovery of fermented dried beans was high (41.00 %) in sun dried cocoa beans as compared to oven dried beans (40.12 %). The free fatty acid content (1.26 %) in sun dried cocoa beans was less compared to 1.47% in T_2 (oven dried beans). The moisture content (3.76%) and lipase activity (0.0018µ eq) was less but, the fat content and pH was high in sun dried beans. The sun drying proved to be adaptive as free fatty acid content was lower than oven dried beans and were below the cut off value of 1.75%.

The sun dried beans were stored in gunny bags (control), polythene covers and plastic containers under ambient condition for a period of six months and the physico-chemical qualities of stored cocoa beans were done initially and at an interval of one month for a duration of six months. The highest bean recovery (98%) and least moisture content (4.22 to 4.52) was in cocoa beans stored in plastic container at the end of sixth month of storage. The lowest free fatty acid content of 1.68% was in cocoa beans stored in plastic container at sixth month after storage.

The stored cocoa beans in plastic container were divided into two lots, one lot was used as such and the second lot was subjected to alkalisation. The chocolates was prepared with alkalised and non alkalised cocoa beans in tempering and conching machine for different time periods of 7, 9 and 11 hours at a temperature of 60^0C and 70^0. After the preparation of different types of alkalised and non alkalised chocolates in tempering and conching machine, chocolates were subjected to analysis of physico-chemical qualities, organoleptic evaluation and for enumeration of microflora. The treatment T_6 (Chocolate prepared at 70^0C for 11 hours) and T_{12} (70^0C for 11hours) had the lowest free fatty acid content of 1.67% and 1.68% and maximum score for overall acceptability (8.89 and 8,87).

Chocolates prepared at 70^0C for 11 hours from alkalised and non alkalised beans were blended with dehydrated grapes, dehydrated dates, osmodehydrated jackfruit, osmodehydrated pineapple, badam, cashew nut, powdered dehydrated mint leaves and white pepper powder. From the, organoleptic evaluation of different treatments, the best rated treatments from alkalised chocolates were T_3 (95 % alkalised chocolates +5% osmodehydrated jack fruit), T_6 (95 % alkalised chocolates +5% badam) and T_8 (95 % alkalised chocolates +5% white pepper) and from non alkalised chocolates the treatments T_{11} (95 % non alkalised chocolates +5% osmodehydrated jack fruit), T_{14} (chocolate blended with 95 % non alkalised chocolates and 5% badam) and T_{16} (95 % non alkalised chocolates and 5% white pepper powder) were selected,

packed in aluminium foil and stored in refrigerator for six months. Initially the lowest hardness of 74.41N and 76.71N was recorded in controls (T_0 - AC) and (T_0 – NAC. The maximum energy content of 580.15Kcal was found in treatment T_5 (95 % NAC+5% B). Initially the maximum protein content (7.74 g/100g) was noticed in treatment T_2 (95 % AC+5% B) and T_5 (95 % NAC+5% B). The highest polyphenol content ranging from 0.23g/100g to 0.19g/100g was seen in T_0 (100% NAC) throughout the storage periods. A reduction in mineral contents like calcium, phosphorus and iron content was observed in all treatments and at the end of sixth month calcium content varied from 0.39g/100g to 0.63g/100g. An increase in lipase activity and free fatty acid content of blended chocolates was recorded during storage and FFA was within 1.75% up to fourth month of storage.

Based on organoleptic score, the treatment T_2 (95 % AC+5% B) attained the highest rank. The blended chocolates were evaluated for the presence of bacteria, yeast and mould initially and monthly intervals for a period of six months, At the end of sixth month of storage the highest bacterial count of 2.0 x 104 cfu/g was found and during sixth month of storage. The cost for blended chocolates was varied from Rs.46.54 to Rs.50.62 / 40 g of chocolate bar. The cost of the chocolates prepared in the present study was lesser as compared to the commercial chocolates.

The present study found that good quality, nutritious and healthy blended chocolates using cocoa could be prepared without adding any preservatives. The blended chocolates contain treasure of nutrients and bioactive compounds polyphenol which is essential for the growth and development of children and also give several health benefits.