Processing of Guava fortified with Vitamin B_1

A

THESIS SUBMITTED TO THE

ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF

MASTER OF SCIENCES IN AGRICULTURE

(FRUIT SCIENCE AND HORTICULTURE TECHNOLOGY)

By

SANHATI ALIVA SETHY

Adm. No. 02FSC/14

DEPARTMENT OF FRUIT SCIENCE AND HORTCULTURE TECHNOLOGY

COLLEGE OF AGRICULTURE

ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

BHUBANESWAR- 751003, ODISHA

2016

ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
DEPARTMENT OF FRUIT SCIENCE AND HORTICULTURE TECHNOLOGY
COLLEGE OF AGRICULTURE
BHUBANESWAR-751003, ODISHA

Prof. Dillip K. Dora
Professor (Horticulture)
Dean, PGF-cum-DRI

Bhubaneswar
Date: 05/7/2016

CERTIFICATE - I

This is to certify that the thesis entitled **"Processing of Guava fortified with Vitamin B_1."** submitted in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE IN AGRICULTURE (FRUIT SCIENCE AND HORTICULTURE TECHNOLOGY)** to the Orissa University of Agriculture and Technology, Bhubaneswar is a faithful record of bonafide research work carried out by **SANHATI ALIVA SETHY** under my guidance and supervision. No part of this thesis has been submitted for the award of any other degree or diploma.

It is further certified that the assistance and help availed by her from various sources during the course of investigation has been duly acknowledged.

05/7/16

Chairman
Advisory committee

CERTIFICATE-II

This is to certify that the thesis entitled **"Processing of Guava fortified with Vitamin B_1."** submitted by **SANHATI ALIVA SETHY** to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE (FRUIT SCIENCE AND HORTICULTURE TECHNOLOGY)** has been approved by the students' advisory committee and external examiner.

Advisory committee

Chairman: **Prof. Dillip K. Dora**
Professor (Horticulture)
Dean, PGF-cum-DRI
OUAT, Bhubaneswar

12/07/16

Members :

1. **Dr. D. K. Dash**
Professor and Head
Department of Fruit Science and
Horticulture Technology
OUAT, Bhubaneswar

12-7-16

2. **Dr.(Mrs.) M. Mohapatra**
Asst. Research Engineer,
AICRP on Post Harvest Technology
Dept. of Agricultural Processing and
Food Engineering,
College of Agricultural Engineering
and Technology,
OUAT, Bhubaneswar

12/7/16

3. **Dr.(Mrs.) S. M. Samantaray**
Asst. Professor,
Department of Microbiology,
CPGS, OUAT,Bhubaneswar

12/07/16

EXTERNAL EXAMINER

Name and Designation

ACKNOWLEDGMENT

First and foremost, the author thank to Almighty God for his guidance and blessings upon her with which she was able to succeed in completing her thesis.

I would like to avail this opportunity to express my sincere gratitude to Prof. Dillip K. Dora, Dean, PGF-cum-DRI, College of Agriculture, O.U.A.T, Bhubaneswar, the chairman of the Advisory committee for his immense help and guidance in planning and execution of the experiment. His inspiration and admiration helped me a lot for writing this manuscript.

I humbly record a deep sense of gratitude to Dr. (Mrs.) S. M. Samantaray (Asst. Professor), Department of Microbiology, CPGS, O.U.A.T, Bhubaneswar, for her valuable suggestion and to make this endeavor a possibility.

My deep sense of obligation goes to Dr. (Mrs.) Minati Mohapatra (Asst. Research Engineer) Department of Agricultural Processing and Food Engineering, CAET, OUAT, Bhubaneswar for providing necessary facilities to carry out my research work.

I am extremely thankful to all the teachers and non-teaching staff members of the Department of Department of Microbiology, CPGS and Department of Agricultural Processing and Food Engineering, CAET, O.U.A.T, Bhubaneswar.

I shall never forget to put on record the help rendered of my friends during the course of the study.

All the words in the lexicon will be futile and meaningless if I fail to express the obligations from the fathom of my heart for my beloved family members in helping me to complete my degree as without their love, inspiration, moral and financial support this dream would not have been materialized.

Last but not least, I express my sincere thanks to all beloved, respected people who helped me directly or indirectly but could not find a separate mention.

Sanhati Aliva Sethy

Place: Bhubaneswar

(Sanhati Aliva Sethy)

Date: 5.7.2016

Adm.No.02FSC/14

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABBREVIATION

%	:	Percentage
MT	:	Metric Ton
kg	:	Kilogram
g	:	Gram
mg	:	Miligram
ml	:	Milliliter
t	:	Ton
i.e.	:	That is
Fig.	:	Figure
CRD	:	Completely randomized design
S.E.	:	Standard Error
CD	:	Critical Difference
CV	:	Coefficient of Variation
@	:	At the rate of
Nos.	:	Numbers
DAS		Days After Storage
Var.	:	Variety
&	:	And
cv.	:	Cultivar
CFU	:	Colony-forming Unit
Vit.	:	Vitamin

ABSTRACT

A Ready-to-serve drink (RTS) from indigenous guava fruits, widely known for having pleasant flavour and antioxidant properties was prepared supplemented with Vitamin B_1 (Thiamine). The RTS from guava was prepared taking three different concentrations of juice (10%, 20% and 30%) into account and each of which was fortified with two different concentrations of Vitamin B_1 (0.2 and 0.4 mg/200ml) referring to the recommended daily allowance (RDA) level of thiamine along with control (without vitamin). The quality parameters of all the formulations were studied. The result of the experiment showed increase in total soluble solids (TSS), acidity and ascorbic acid contents with increase in concentration of juice. The initial TSS content of the fresh sample of guava RTS was 15.8-17 ^{0}Brix which increased up to 16.2-17.3 ^{0}Brix after 21 days of storage. The titratable acidity percentage increased significantly from 0.47-0.59% over a period of 21 days storage. The initial Ascorbic acid content of the guava RTS was 3.17- 5.25 mg/100 ml in different concentrations of RTS, which significantly decreased to the level of 3.9-4.8 mg/100ml with respect to storage period from 7 days to 21 days. The observations indicated that, there was a slight decrease in the Vitamin B_1 (Thiamine) content of guava RTS due to its water soluble nature. The bacterial and fungal load increased from 0.3- 2.25 and 0.2-0.4 respectively in 10^3 dilutions in the control sample during the storage period of 21 days. Further by increasing concentration of the RTS from 10% to 30% and storage period from 1-21 days, the microbial load was also increased in a significant manner. From the sensory evaluation it could be inferred that the guava RTS containing 20% of juice fortified with either of the levels of Vitamin B_1 (Thiamine) tried was adjudged as the best.

CHAPTER - I

INTRODUCTION

INTRODUCTION

Guava (*Psidium guajava*) is of Tropical American origin and belongs to the family-Myrtaceae. Guava is valued for its delectable taste and aroma. The fruit can be considered as the 'Apple of tropics' for its high Vitamin C (i.e. 2-5 times more than orange) and mineral content. It claims to be the fourth most important fruit after mango, banana and citrus. Guava has been in cultivation in India since early 17th century and gradually became a crop of commercial significance. In India, it is found mostly in all the tropical and sub tropical regions but most abundantly grown in Uttar Pradesh followed by Maharashtra, Madhya Pradesh, Bihar and West Bengal. It is cultivated in India in 236 thousand ha with a production of 3198 thousand MT (2012-13). Besides India, it is also found in China, Thailand, Pakistan, Mexico, Indonesia, and Brazil etc. Being very hardy, it gives an assured crop even with very little care. Its cost of production is also comparatively low because its low input requirements. Therefore, it is considered to be an ideal fruit for processing, nutritional security and economic boost (Rathore, 2001).

Area and production of guava in India

Year	Area (in '000 Ha)	Production (in '000 MT)
2010-11	205	2462
2011-12	220	2510
2012-13	236	3198

Source: Horticulture Division, D/o Agriculture & Cooperation, M/o Agriculture

As a cheap nutritious fruit with a wide adoptability to diverse climatic and soil conditions guava is an ideal crop to grow in developing countries. The guava fruit contains 81.7% moisture, 2.55g protein, 0.5g fat, 14.3g CHO, 624 IU vitamin A, 228mg vitamin C, 0.06mg thiamine, 0.4mg riboflavin, 1.08mg niacin, 417mg potassium, 22mg magnesium and 2mg sodium per 100g of edible portion (USDA National Nutrient Database). It is a rich source of vitamin C and pectin (Agnihotri *et. al.*, 1962). The guava fruits are richest source of antioxidant which helps to delay the skin ageing, prevent cancer and tumour growth. It also contains fair quantity of iron, but 80% of this is found in the seed which is not utilizable (Miller and Bazore, 1945).

It has great demand as a table fruit and also raw material for the processing industries (Purseglove, 1977). Guava fruits often do not find a good place in the fresh fruit market for which guava growers do not get better return. Considering the demand of fruit juice in the present day market guava fruits could be better utilised in the processing sector for the production of large number of value added products. Fruits may be utilized to make products like jelly, jam, cheese, juice, canned segments, nectar etc. However, the most commercial use of guava is for jelly preparation due to the high pectin content. Guava juice has special characteristics in its flavour and viscosity, which is popular in many tropical countries (Yen and Lin, 1998).

Looking into the demand of the present generation, variety of soft drinks are being presently produced in the country, e.g. sweetened carbonated (aerated) soft drinks, beverages containing fruit juice/ pulp and soda water. Among these, the share of fruit juice based beverages is presently quite small as compared to synthetic carbonated drinks. Gradually there is a distinct shift towards fruit juice based beverages for obvious advantages of the higher nutritional value over the synthetic aerated waters.

Fruit juice based RTS beverages have the distinct advantage of higher nutritional value over synthetic aerated waters. Ready-to-serve (RTS) fruit drink is a type of fruit beverage which contains at least 10% fruit juice and 10% total soluble solids besides approximately 0.3% acid, intended for consumption without dilution and prepared from unfermented pure fruit juice with or without some of the pulp containing any soluble carbohydrate and water (SLS 729:1985). Since these beverages are consumed as such without dilution, hence are termed as Ready-to-serve beverage. Wide range of fruits including mango, citrus fruits, berries, litchi, guava, pineapple, grapes etc. are preferred for RTS beverages. RTS beverages are quite popular because of longer shelf-life and less loss of nutrients during processing.

Vitamin B_1, also called thiamine, is one of the eight B vitamins. Thiamine was one of the first compounds recognized as a vitamin. Thiamine is involved in many body functions, including nervous system and muscle function, the flow of electrolytes in and out of nerve and muscle cells, digestion, and carbohydrate metabolism. Thiamine is a colourless organo-sulfur compound with a chemical formula $C_{12}H_{17}N_4OS$. Thiamine is soluble in water, methanol, glycerol and partially insoluble in less polar organic solvents. It is stable at acidic pH, but is unstable in alkaline solutions. Thiamine is unstable to heat, but stable during frozen storage. Complex thiamine biosynthesis occurs in bacteria, some protozoans, plants and fungi.

Thiamine deficiency cause mental confusion, muscle weakness, impaired growth, and beriberi. Thiamine deficiency occurs in a variety of situations, including a diet high in simple carbohydrates consisting mainly of processed food (sulphites destroy thiamine), complications of alcohol misuse, total parenteral nutrition (TPN), gastrointestinal surgery, severe infection, eating disorders, cancer (especially if the patient is being treated with chemotherapy), long-term diuretic use and AIDS. The deficiency can develop within 2-3 months of a deficient intake and can cause disability and death. In general, cereal grains are the most important dietary sources of thiamine, by virtue of their wide use. Whole grains contain more thiamine than refined grains, as thiamine is found mostly in the outer layers of the grain and in the germ. Some other foods naturally rich in thiamine are oatmeal, flax and sunflower seeds, brown rice, whole grain rye, asparagus, kale, cauliflower, potatoes, oranges, liver (beef, pork, chicken) and eggs. For infants up to 12 months the Adequate Intake (AI) is 0.2-0.3 mg/day and for children ages 1–13 years the recommended daily allowance (RDA) level increases with the age from 0.5 to 0.9 mg/day (Food and Nutrition Board of U.S.).

AIM & OBJECTIVES OF THE PRESENT STUDY

As per the reports of National Horticulture Mission, India is the second largest producer of the fruits and vegetables in the world. Being a horticulturist our main objective is to enhance the quality of life of the farmers and the consumers with respect to the awareness and consumption of the local fruits and vegetables. Also for the benefit of the farmers value addition became a key factor in the present agriculture mission. Under the perennial fruit crop coverage of Odisha, guava comes second to mango. Being a perennial, high productive, high nutritive and rich with medicinal properties, guava became a natural choice for our study. As value addition and ready to serve became the buzzword for sustainability in the present generation, it also renewed our interest to explore the possibilities of making a ready to serve soft drink from guava fortified with Vitamin B_1 for the benefit of the farmers as well as the consumers. Such studies could be a pre-requisite for tapping the market potential of guava in Odisha context. Perusals of literatures indicate that no such kind of research has been conducted in guava. Therefore, the present investigation has been carried out with the following objectives.

1. Development of market security for indigenous guava fruit by processing it into Ready-to-serve beverage.
2. Standardization of the process of fortification by supplementation with Vitamin-B1 (Thiamine) to the processed guava RTS.
3. Assessment of sensory quality of processed RTS supplemented with Vitamin-B1 (Thiamine).

CHAPTER - II

REVIEW OF LITERATURES

REVIEW OF LITRATURES

The relative importance of the subject necessitates a critical survey of the research conducted in India and abroad pertaining to processing and preservation of indigenous guava (*Psidium guajava*) supplemented with Vitamin-B1 (Thiamine). An attempt has, therefore been made in this chapter to review a wide range of the information available to the researcher.

Due to the paucity of research findings relating to the processing and preservation of indigenous guava, various other related crops were also taken into consideration and the effects of chemical preservatives and Vitamin supplementation as reported on their preservation, palatability and characters for overall acceptance were studied and taken into account for reviewing in this chapter.

2.1 Physical characteristics

The most common colours of guava flesh were pale pink (29%), pink (20%), dark pink (18%) and cream (18%) among the 119 accessions of guava in 35 Brazilian eco-regions (Santos *et al.,* 2008). Biradar and Mukunda (2007) evaluated ten seedling progenies of cv. Taiwan guava under Bangalore conditions and revealed that maximum fruit diameter (6.52 cm) was found in control Allahabad Safeda followed by TG selection 6/10 (6.15 cm) while minimum (5.14 cm) in TG selection 5/11.

Marak and Mukunda (2007) evaluated ten seedling progenies of cv. Apple Colour under Bangalore conditions and found that maximum fruit length (5.65 cm) was recorded by cv. Allahabad Safeda, followed by A.C.Seln.10/2 (5.43 cm) and A.C.Seln.6/6 (4.26 cm). Babu *et al.* (2007) studied performance of eight years old guava selections under Meghalaya conditions and concluded that number of fruits per tree ranged between 184 (Selection-1) and 78.66 (Selection-13).

Pandey *et al.* (2007) found that among 11 newly developed guava hybrids/selections the fruit length varied between 7.27 cm (Arka Amulya) and 5.83 cm (Lalit) in an evaluation study under Lucknow condition. Under Arabhavi conditions maximum pulp weight (131.67 g) recorded in cv. Sardar followed by cvs. CIW-4 (123.50 g) and SR-1 (94.67 g), while GR-1 showed minimum pulp weight (26.28 g) (Deshpande, 2006).

Girwani *et al.* (2005) reported that the fruit weight ranged from 16 g in Chinese guava to 167.50 g in Red fleshed, whereas it was varied between 116 g (cv. Baraf Khana) and 49.50 g (cv. Lucknow-49) in an evaluation study conducted by Aulakh (2005) under Punjab conditions. The guava berry is an important tropical fruit that is mostly consumed fresh. The fruit contains several small seeds and consists of a fleshy pericarp and seed cavity with pulp (Jimenez-Escrig *et al.*, 2001; Lozoya *et al.*, 2002; Lapik *et al.*, 2005).

Ranchi conditions the fruit yield per ha ranged from 98.80q in Lucknow-49 to 14.72q in cv. Apple Colour (Reddy *et al.*, 1999). Mitra *et al.* (1983) evaluated eleven guava cultivars of four years age and reported that total weight of fruits per plant varied from 21.1 kg in cv. Lucknow-49 to 3.2 kg in cv. Seedless under West Bengal conditions. Phadnis (1970) evaluated nine guava selections under Pune conditions and found that plant height ranged between 8.1 m (Seedless) and 4.2 m (Lucknow-49). He also evaluated nine guava selections of 25 years old under Pune conditions and found that plant spread varied from 8.4 m (North-South) and 9.9 m (East-West) in Selection Seedless to 4.8 m (North-South) and 6.6 m (East-West) in Selection Lucknow-46.

Mukherjee and Dutta (1967) reported that Lucknow-49 variety took about 106 to 138 days for fruit maturity while cv. Red fleshed took 110 days. Number of days from flowering to fruit maturity varied between 121.33 (cv. GR-1) and 125.93 (cv. CIW- 5) in an evaluation study conducted by Deshpande (2006) under Arabhavi conditions.

2.2 Bio-chemical characteristics

The food value of guava fruit is ; Calories (77-86g), Moisture (2.8-5.5g), Crude fibre (0.9-1.0g), Protein (0.1-0.5g), Fat (0.43-0.7g), Carbohydrate (9.1-17mg), Calcium (17.8-30mg), Phosphorous (0.30-0.70mg), Iron (200-400 I.U), Carotene (0.046mg), Thiamine (0.03-0.04mg), Riboflavin (0.6-1.068mg), Vitamin C (350-400mg) (Kamanth *et al.*, 2008).

Paniandy *et al.*, (2000) stated the chemical composition of Guava fruit *(Psidium guajava Linn.).*Guava fruit extract contains Vitamin C, Vitamin A, iron, calcium, Manganese, phosphoric, oxalic and malic acids, saponin combined with oleanolic acid. Essential oil contains hexanal, 2,4-hexadienal, 3-hexenal, 2-hexenal, 3-hexenyl acetate and phenol, while caryophyllene, nerolidol , 3-phenylpropyl acetate, caryophyllene oxide, pentane-2-thiol, 3- penten-2-ol and 2-butenyl acetate, 3-hydroxy-2-butano3-methyl-1-

butanol, 2,3-butanediol, 3- methylbutanoic acid, (Z)-3-hexen-1-ol, 6 methyl-5-hepten-2-one, limonene, octanol, ethyl octanoate.

TSS varied between 16.90 °Brix (cv. Apple colour) and 10.40 °Brix (cv. Chittidar) in an evaluation study on 12 guava cultivars under Rahuri conditions (Gohil *et al.*, 2006). . Gohil *et al.* (2006) conducted experiment on twelve guava cultivars and concluded that reducing sugar ranged from 7.29% in cv. Seedless Basti to 4.55% in cv. Apple colour. The total sugars ranged between 15.67% (cv. Behat coconut) and 11.83% (cv. Sardar).

Aulakh (2005) evaluated 13 guava varieties and reported that higher TSS was recorded in fruits of Behat Coconut (11%) followed by Tehsildar (10.6%), Lucknow-49 (10.5%), Chittidar (10.4%) and Strawberry (10.4%). Aulakh (2004) evaluated six guava cultivars in winter season and revealed that TSS of fruit ranged between 13.5% (cv. Lucknow-49) and 10% (cv. Pear Shaped) under Punjab conditions.

Pandey *et al.* (2007) reported that the acid content varied from 0.157% (cv. Hisar Surkha) to 0.355% (cv. Sangam). Patel *et al.* (2007) revealed that the acidity ranged between 0.33% (Hybrid-11) to 0.81% (Hybrid-2) among guava varieties and hybrids studied under Meghalaya conditions. Gohil *et al.* (2006) revealed that the acidity of ripe fruit ranged from 1.08% in cv. Chittidar to 1.98% in cv. Nagpur seedless under Rahuri conditions.

Pandey *et al.* (2007) estimated the ascorbic acid content among 11 cultivars/hybrids under Lucknow conditions and reported that it ranged between 243.75 mg per 100 g in CISH-G-4 (Shweta) and 104.17 mg/100 g in cv. Pant Prabhat. Vitamin C content varied from 172 mg/100 g (cvs. Surkha) to 145mg/100 g (cv. Chittidar) under Punjab condition (Aulakh, 2005).

Guavas are considered excellent sources of antioxidant phytochemicals, which include ascorbic acid, carotenoids, antioxidant dietary fiber, and polyphenolics. After acerola cherries, guava has reported the second highest concentration of ascorbic acid (ranging from 60-1000 mg/100 g) of all fruits (Mitra, 1997). Mitra *et al.* (1983) evaluated 11 cultivars of guava at four years age and recorded that ascorbic acid content ranged from 132.5 mg/100 g (cv. Lucknow-49) to 62.5 mg/100 g (cv. Red fleshed) under West Bengal conditions.

Prakash (1976) compared two guava cultivars for quality characters of fruit and revealed that cv. Sardar recorded the highest quantity of Vitamin C per 100 g of pulp (260

mg) than cv. Dharwar (196.9 g). Pandey *et al.* (2007) estimated the content of reducing sugar among 11 guava varieties under Lucknow conditions was estimated and reported that it ranged between 6.4% in cv. Hisar Surkha and in 4.41% CISH-G-31.

Marak and Mukunda (2007) examined ten seedling progeny selections of cv. Apple colour and noticed that maximum quantity of reducing sugar (4.81%) was found in A.C.Seln.6/6 and the lesser quantity (4.08%) in control Allahabad Safeda.Biradar and Mukunda (2007) evaluated 10 seedling progeny selections of Taiwan Guava and reported that maximum percentage of non-reducing sugar ranged between 3.73 (TG selection 5/13) and 3.06 (TG selection 6/8). Patel *et al.* (2007) evaluated six guava hybrids/cultivars under Meghalaya conditions and reported that non-reducing sugars ranged from 5.37% in Hybrid-1 to 2.67% in EC-12.

The total sugar content varied from 7.48% in TG selection 5/13 to 6.72% in TG selection 7/2 among ten seedling progeny selections under Bangalore conditions (Biradar and Mukunda, 2007). Under Bangalore conditions Marak and Mukunda (2007) evaluated ten seedling progeny selections of cv. Apple Colour and reported the maximum percentage of total sugar in A.C.Seln.6/10 (8.27), followed by A.C.Seln.11/2 (8.14), and least in Allahabad Safeda.

Pandey *et al.* (1997) conducted a study on varietal evaluation of guava with nine varieties under Rewa (M.P.) conditions and concluded that maximum content of total sugars was found in Sardar variety (11.01%) whereas minimum in cv. Red-fleshed (8.71%). Under Bangalore conditions Pandey *et al.* (1997) evaluated nine cultivars and observed that sugar/acid ratio ranged from 39.44 in cv. Seedless to 24.86 in cv. Chittidar. Sadashivaiah (1989) evaluated Navalur guava selections under Dharwad conditions and recorded higher sugar/acid ratio in cv. GW-2 (24.18) followed by CIW-2 (18.37), SWY-1 (17.96), CW-1 (17.61), Sardar guava (17.52) and CIW-4 (16.92), however the lower sugar acid/ratio was observed in cv. GR-1 (10.07).

Mitra *et al.* (1983) evaluated four year old guava cultivars and reported that sugar/acid ratio varied from 28.3 in cv. Lucknow-49 to 17.2 in cv. Red fleshed under West Bengal conditions. Chandrika *et al.* (2009) compared the lycopene contents of red pulped guava cv. Horana Red and watermelon cv. Sugar baby, and revealed that red pulped guava cv. Horana Red estimated more lycopene (45.3 ± 8.0 µg per g FW) than watermelon cv. Sugar baby (37.2 ± 4.0 µg per g FW). White pulped fruits of Allahabad Safeda showed only minute quantities of lycopene pigment (0.82 mg/100 g) (Marak and Mukunda, 2005).

Budi *et al.* (2001) studied pigment contents among various Indonesian fruits and showed that among them guava was the richest in lycopene content (1150 μg/100 g) than cryptoxanthin and beta carotene (66 μg/100 g and 984 μg/100 g, respectively). The presence of lycopene pigment was attributed as the reason for pink colouration in guava pulp (Jagtiani *et al.*, 1988).

2.3 Medicinal properties

Psidium guajava L. (Myrtaceae) has been used traditionally against gastrointestinal disturbances and respiratory ailments. The chemical composition of the essential oil of both leaves and fruits were elucidated by gas–liquid chromatography/ mass spectrometry (GLC/MS). The dominant compounds were β caryophyllene (17.6%) and limonene (11.0%) for the fruit oil and β-caryophyllene (16.9%) and selin-7(11)-en-4α-ol (8.3%) for the leaf oil. The radical scavenging activities of both essential oils were assessed by the diphenyl picrylhydrazyl (DPPH•) and deoxyribose degradation assays. The anti-inflammatory activity was explained via virtual docking of the major identified compounds to the main sites in the 5-LOX crystal structure (Sherweit *et al.*, 2013). Studies with humans have found that the consumption of guava for a period of 12 weeks reduced blood pressure by 8%, total cholesterol levels by 9%, triacylglycerides by almost 8%, and induced an 8% increase in the levels of HDL-c (Otoboni *et al.*, 2012).

2.4 Processed products from guava

Chopda *et al.* (2001) optimized the enzyme treatment of guava puree for yield and clarity of guava juice. Clarified guava juice was clearer (89.6%) when prepared using ultra filtration (MW cut-off 40-60kDa) rather than plate and frame filtration (82.8%); however the latter was higher in both soluble solids and ascorbic acid. Clarified guava juice powders were made using freeze-drying, spray drying and tunnel drying. The freeze-dried product had superior quality; however the spray-dried product was stable and may be more economical. Sensory panellists ranked the cloudy juice prepared from aseptic guava puree highest, and there were no significant differences between the juices from pasteurized, clear nectar, freeze-dried puree powder or juice powder.

Gow-Chin Yen (1998) prepared guava puree from fresh fully ripe guava (*Psidium guajava* L. cv. Chung shan). The puree was packed in a 500 mL plastic bottle and stored at -40 °C until use. Guava puree cloud was found to be composed of 43-45% protein, 25-26%

carbohydrate, 5-8% pectin, 3-5% crude fiber, 6.5-9.3% ash, and 0.18% crude fat. The analyses of protein pattern and amino acid composition showed that the protein in cloud was of a low molecular weight, with a high isoelectric point and a compact structure; thus, it could combine with pectin and become suspended in the guava puree.

Thongsombat *et al.* (2007) prepared guava juice fortified with soluble dietary fibre as pectin extracted from guava cake (peel, pulp, and seeds). The waste guava cake from juice processing plant was used for pectin extraction using sodium hexametaphosphate method followed by pectin precipitation using acidified ethanol method. A yield of 30.50±0.34% crude pectin was achieved. Crude pectin also contained 4.71±0.18% moisture, 0.34±0.21% protein, 0.68±0.00% ash, 20.70±0.16 g (%dwb) soluble dietary fibers. By the consideration from the greatest perceived scores of all sensory evaluation attributes including colour, turbidity, odour, flavour and overall acceptability, the °Brix-acid ratio of 40.0 was selected for guava juice processing. The clarified guava juice was then fortified with pectin powder extracted from previous experiments using various pectin.

Kuchi *et al.* (2014) standardize the recipe of guava jelly bar. Firm ripe guava fruits of Lucknow-49 harvested from college farm were used for the study. Jelly bar of 2 cm x 7 cm pieces were made and packed in LDPE and laminated aluminium foil pouches. Storage study was conducted in ambient and refrigerated conditions for two months. Among different treatment combinations, the recipe with 50 % sugar, 0.3% citric acid and 0.5 % pectin added to pulp extract recorded highest organoleptic score.

The study was conducted for processing of jelly from guava juices at different stages of extraction. Sensory attributes and storage studies of the jellies were also evaluated. The fresh and fully mature guavas were used for this experiment. Chemical characteristics such as moisture, ash, acidity, Vitamin C, sugar and total soluble solids (TSS) of edible fruits, juice and jellies were determined. Characteristics of chemical variation were observed among the juice and jelly samples. The jelly from composite of first and second extractions juice was found better than other jellies as per chemical composition and sensory evaluation. Storage study was conducted on the jellies for nine months at room temperature (23-30°C) and relative humidity 80 to 85% (Hossen *et al.*, 2009).

Bal *et al.* (2014) studied the preparation of nectar using guava cv. Lalit with respect to pulp percentage and TSS (°Brix) as per the treatments and the processed nectar was

analyzed in CRD (Completely Randomized Design). Physico-chemical parameters viz., TSS, acidity, ascorbic acid, non-reducing sugars, total sugars and viscosity as well as organoleptic attributes viz., colour, flavour, taste and overall acceptability of nectar were evaluated at an interval of 2 months up to 8 months of storage. The variety Lalit is commercially used in processing industry due its attractive pulp colour and could make significant contribution to food industry.

Kocher *et al.* (2011) prepared a wine from guava. Guava juice requires 'chaptalization' so as to adjust its Brix and prepare a perfect wine out of it. The chaptalized juice ("must") is treated with pectinase or a combination of enzymes and fermented with traditional yeasts at a temperature range of 22 to 30°C and inoculum size of 6 to 11% (v/v). The addition of N and P improves ethanol production and quality parameters of guava wine. Racking and ageing of guava wine also improves the sensory and organoleptic characteristics of guava wine.

Bhat and Singh (2014) prepared whey-guava beverage and studied its shelf life. The ratio of whey and guava pulp that was used for the preparation of beverage is 67.5:20 (%). Treatments which include different temperature and time combination were 60°C, 65°C and 70°C for 15, 25 and 35 minutes. However the beverage which was pasteurized at 65°C for 25 minutes was scored much than the others treatments. Samples were evaluated initially and after that at an interval of 15, 30, 45, 60, 75 and 90 days for sensory analysis which included taste, colour, flavour, appearance and overall acceptability.

Selvi et al. (2013) was conducted a study to evaluate the formulation of therapeutic drink guava-lime-ginger RTS beverage (GLG RTS) to boon health. The fixed ratio of fruit juices in guava-lime-ginger RTS beverage was 10:3:2. The prepared RTS was bottled in glass bottles and stored at room (R1) and refrigerated (R2) temperature. The freshly prepared guava-lime-ginger RTS beverages had TSS of 15obrix and slight reduction was noticed during storage. It was found that maximum profit could be obtained per litre of RTS production Rs. 6.26 per kg.

CIPHET (2008) has standardized the technology for preparation of guava RTS beverages with and without carbonation. Plain guava beverages were prepared by mixing fruit pulp with water. The recipe containing 10-15 % fruit pulp, 10-13 % sugar and 0.28-0.30 % acidity were found to be most ideal for such beverages.

R. Sasikumar (2015) prepared a therapeutic ready-to-serve (RTS) made from blend of aloe vera, and aonla fruit juice. The blended juice of different ratio of aloe vera and aonla fruit juice 60:40(A), 65:35(B), 70:30(C) 75:25(D) and 80:20(E) with 15% of sugar, 0.3% of acidity and 100 ppm of Potassium metabisulfite and blends were homogenized and pasteurized. The developed therapeutic RTS could be recommended for the large scale production at industrial level.

Nilugin *et al.* (2010) was conducted a study to develop a ready-to-serve (RTS) beverage using palmyrah fruit pulp at different concentrations of 8, 10, 12, 14 and 16% with sugar, citric acid, distilled water and potassium metabisulphite, considering the recommendations of Sri Lanka standards for RTS fruit beverages. From the results of quality assessments, the formulated beverage with 12% of pulp concentration was found to be superior in quality and could be stored at 30±2°C for a minimum period of six months without any significant changes in quality.

2.5 Physico-chemical changes during storage of products

Bhat and Singh (2014) analyzed the chemical and microbiological activity of whey-guava RTS at regular intervals. Storage study showed an increasing trend in TSS, acidity, reducing sugar and a decreasing trend in the pH, lactose and ascorbic acid. Total viable count was analyzed using standard methods.

The acid content of RTS beverage increased. The freshly prepared guava-lime-ginger RTS beverages had TSS of 15 °Brix and slight reduction was noticed during storage. A gradual increase in reducing sugar content of the RTS was observed. A gradual reduction in the ascorbic acid content was observed in all the samples during storage. A slight increase in the microbial load was noted in the formulated value added fruit products during storage (Selvi *et al.*, 2013).

Kadam *et al.*, (2012) reported that ascorbic acid content decreased with the decrease in TSS during product preparation like RTS, Nectar and guava bar. Microbial examination revealed that the product is safe to consume.

Santos *et al.* (2010) recorded that the production of nectar from fresh guava reduced Vitamin C, lycopene and titratable acidity, by contrast soluble solid and pH increased significant. It was observed that TSS, pH and acidity of jelly did not show any remarkable changes. Colour and flavour was acceptable up to 210 days but after 210 days the colour and

flavour of jellies were changed due to fungal growth and incipient spoilage (Hossen *et al.*, 2009).

Correa *et al.* (2009) recorded that there was no significant effect of thermal treatment on the volatile compound concentrations, except for a significant decrease ($p = 0.0001$) in hexanal and (*Z*)-hex-3-enyl acetate ($p = 0.0029$). As for the storage time, there was a much greater decrease in the esters contents, such as (*Z*)-hex-3-enyl and phenyl-3-propyl acetates. Cinnamyl acetate had the greatest decrease over storage time. Refrigeration was better than room temperature for guava nectar volatile compounds stability over storage time, mainly for esters compounds, which are important for the product aroma and flavour.

Jawaheer *et al.*, (2003) reported that the postharvest storage of the guava fruits resulted in a loss of ascorbic acid over six days. During the juice making process, the highest percentage of loss of ascorbic acid was due to peeling (6%) followed by exhausting (4.5%). Storage of jam at room temperature resulted in a significant decrease in ascorbic acid content over the storage period. Storage of juice at 4°C did not significantly decrease the ascorbic acid content over time.

Gow-Chin Yen and Hsin-Tang Lin (1999) observed that heat processing (95°C, 5 min) caused decrease in the majority of flavour components in the juice when compared with freshly extracted juice. High-pressure treatment at 600 MPa for 15 min can effectively sterilize microbes but partially inactivate enzymes of guava juice.

2.6 Vitamin Supplementation

Leskauskaite *et al.*, (2016) conducted a research on Fortification of Dairy Products with Vitamin D3 and reported that Oilinwater emulsions stabilized by whey proteins alone and by whey proteins plus carboxymethyl cellulose were used. No change in Vitamin D3 added to the yoghurt and sour cream in the form of both emulsions was observed after storage at 7 days in light and 14 days in dark at 4°C. The results of bioavailability tests, using rats, for Vitamin D3 from the fortified emulsions and yoghurt indicated that it is feasible to use stabilized emulsions as delivery systems of Vitamin D3 in fortified products.

Fortification of dairy products with Vitamins A and C leads to improvement in their nutritive quality and consequently, increases their acceptability (Gahruie *et al.*, 2015). Lebiedzinska *et al.* (2004) provides information about the concentrations of Vitamins B (thiamine, riboflavin, pyridoxine and niacin) in cereal and soy-products, grain and seeds. The concentrations of Vitamins were determined by microbiological analytical methods. The results demonstrated that there are great differences in Vitamin B composition within

varieties of the analysed products. Whole grain products and seeds, are better sources of the Vitamin B group than technologically processed products, and therefore more nutritionally efficacious.

Leonard *et al.*, (2004) conduct a research on Vitamin E bioavailability from fortified breakfast cereal is greater than that from encapsulated supplements and reported that the low bioavailability of Vitamin E from the 400-IU capsule and the variability observed when the capsule was consumed with cereal suggest that encapsulated Vitamin E is poorly absorbed when consumed with a low-fat meal and that bioavailability can be enhanced by food fortification with Vitamin E. Tangpricha *et al.* (2003) Vitamin D fortification at 1000 IU/240 ml orange juice for 12 wk safely increased 25(OH) D3 concentrations in adults.

CHAPTER - III

MATERIALS AND METHODS

MATERIALS AND METHODS

In this chapter a detailed account of the materials used and the methods employed which ultimately determines the proficiency and exactness and finally the success of the experiment has been explained.

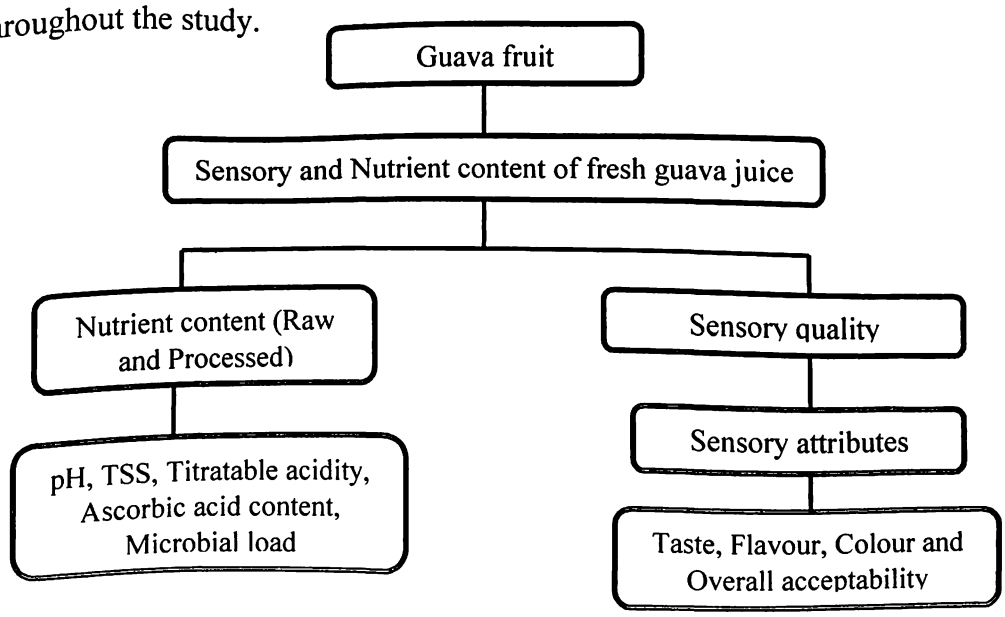
3.1 Experimental site

Studies on processing of RTS from indigenous guava fruit supplemented with vitamin-B1 (Thiamine) and preservation were carried out in the research laboratory at Department of fruit science and horticulture technology, college of agriculture with active support of Department of Agricultural Processing and Food Engineering (CAET) and Department of Microbiology (CPGS), Orissa University of Agriculture and Technology, Bhubaneswar, Odisha during 2015-16.

The experiment was carried out in the laboratories of the experimental site in the year 2015-16 without changing the site. This experiment was carried out for about 21 days. The RTS from guava prepared and supplementation of Vitamin-B1 was done before filling the bottles. The materials used and methods followed to determine proficiency, exactness and ultimately the success of the experiment are as follows.

3.2 Conceptual framework

The broad conceptual framework of this research is given in figure-. The conceptual framework indicates the steps followed during the process of data collection to answer the research questions of this study. The design broadly outlines the step and methods that followed throughout the study.



3.3 Preparation

3.3.1 Collection of Guava fruits

The properly matured and undamaged guava fruits (2kg) were brought from the local market during May 2016. The fruits were brought to the processing laboratory of Department of agricultural processing and food engineering. Then those fruits were washed and cleaned properly.

3.3.2 Preparation of RTS

The unwanted parts (30-40g) were separated from guava fruits through a knife and then the whole fruits were cut into small pieces. Those pieces were then put into a grinder jar, filled with water i.e. half to the weight of pulp and blended. The mixture was collected in a sieve for extraction of juice (1800ml). Then the juice was mixed with strained syrup solution i.e. the mixture of sugar, water and citric acid. The content was heated just to dissolve. Guava RTS was prepared with different juice concentration (10%, 20% and 30%) with constant amount of sugar (350g) and citric acid (2.5g) in each concentration.

3.3.3 Supplementation of Vitamin-B1 (Thiamine)

Thiamine tablets were collected which was named as '*HealthAid® Vitamin B1 100mg*'. Each tablet contains (average) 100mg Vitamin B1 (thiamine). One tablet was crushed into powder form properly then two different amounts were taken considering the recommend daily allowance (RDA) level viz. 0.2mg/200ml (2 serve/day) and 0.4mg/200ml (1 serve/day) and added to the RTS prepared with different concentration of juice. The control was taken without vitamin content.

3.3.4 Preservation of Guava RTS fortified with Vitamin-B1

The High-density polyethylene plastic bottles with the capacity of 200ml were brought from market for preservation of RTS. These bottles can with stand in somewhat higher temperature. The HDPE bottles were sterilized in hot water for 10min, and then the final product was filled into bottles and capped properly. After 30min these bottles were stored under low temperature (4^0C) in refrigerator to study the storage life and quality standards of the product.

Plate 1. Different concentrations of fortified guava RTS prepared in the laboratory for the study.

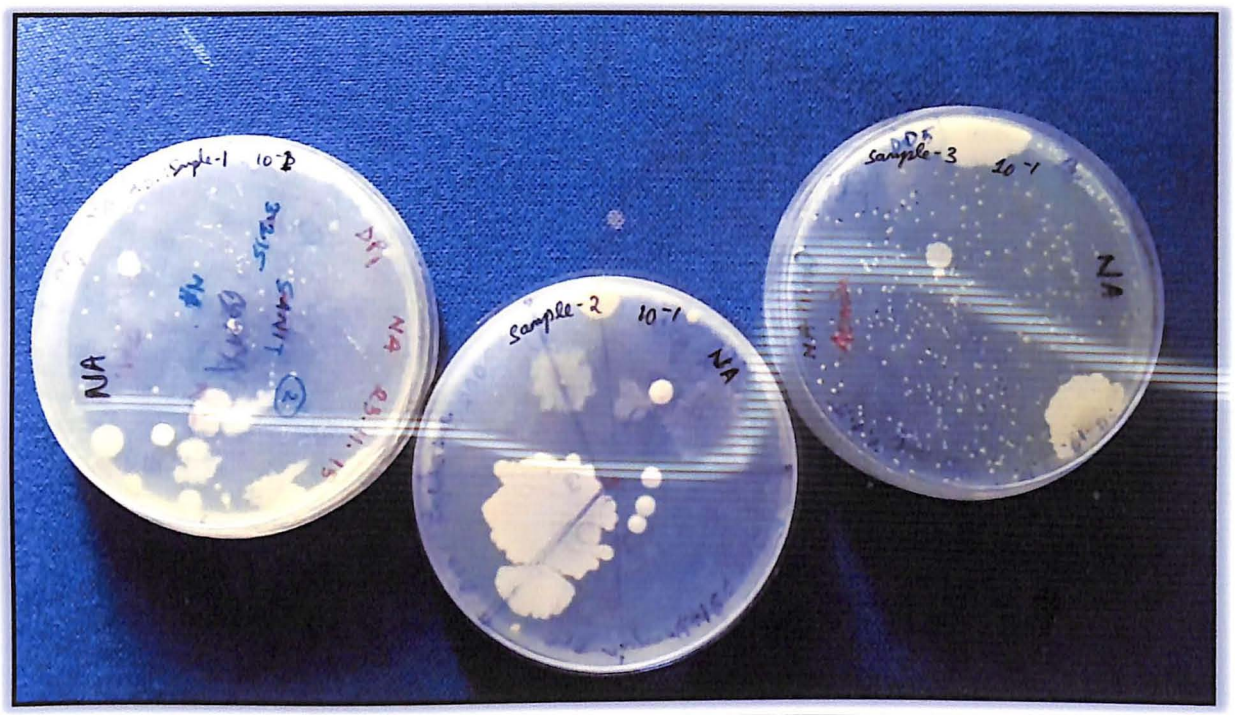


Plate 2. Observations on bacterial load of fortified guava RTS under Nutrient Agar medium.

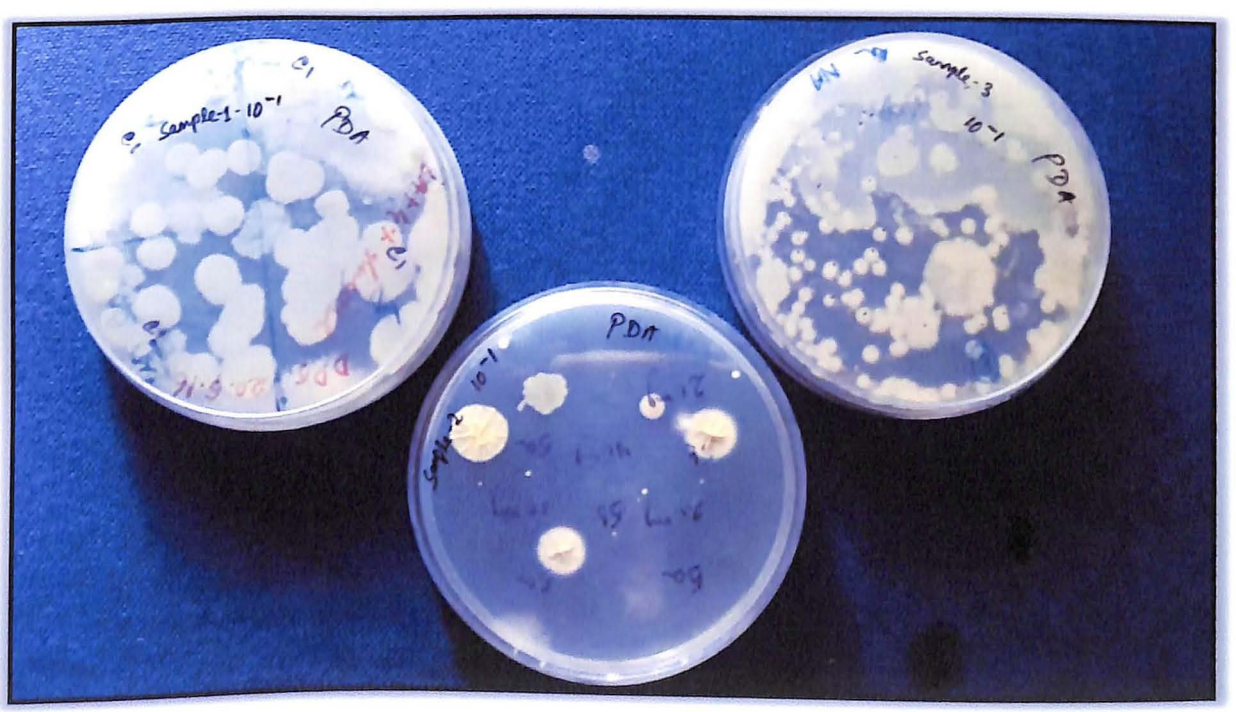


Plate 3. Observations on fungal load of fortified guava RTS under Potato Dextrose Agar medium.

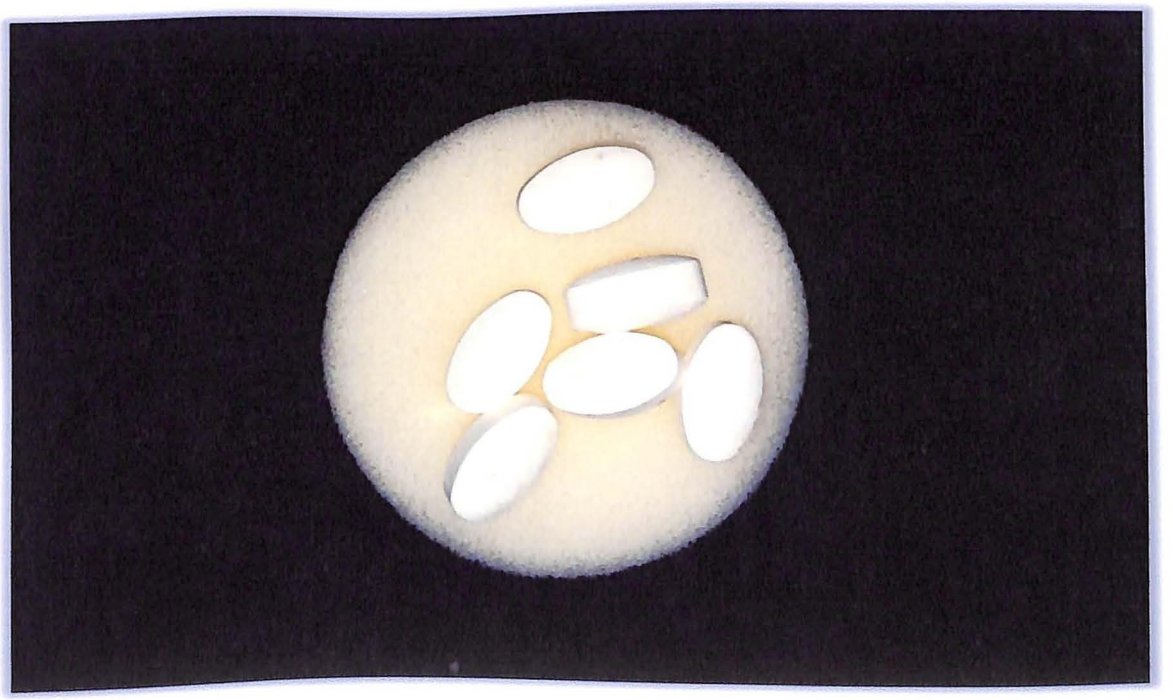


Plate 4. The Health Aid Vitamin B_1 tablet used for fortification of guava RTS for conducting the present experiment.

3.3.5 Sensory evaluation

Fifty panellists (male=20, female=30) belonging to the age group of 24-40 years evaluated the sensory quality of the processed and preserved Guava RTS fortified with vitamin-B1 (Thiamine). The panel consisted of some chosen faculty members and students of Orissa University of Agriculture and Technology, Bhubaneswar, Odisha.

The panellists who usually consume ready-to-serve drinks and other processed products (more than 4-5 servings a month) and willing to participate in the study were selected for the evaluation. Two sensory evolution sessions comprising 25 consumers for each session were conducted during May, 2016. Prior to evaluation, an introductory session was held to familiarize panellists with the product. After introductory session, the panellists were served with three samples of prepared guava RTS, which had been labelled with random number. Panellists were instructed to expectorate and rinse with water prior to evaluation. Time delay between judgments of two consecutive samples was fixed at 30 seconds to avoid respondent's fatigue. Samples were served to different order to each panellist (MacFie*et al.* 1989). Each consumer evaluated the samples for their sensory attributes (Meilgaurd*et al 2007*). The sensory attributes were quantified using a 5-point hedonic scale (1=dislike extremely, 2.5=neither like nor dislike, 5=like extremely). Consumers also indicated their acceptability on a nominal scale.

3.4 Biochemical Analysis

3.4.1 Total soluble solids (^{0}Brix)

The freshly extracted guava juice and processed RTS with different juice concentration (10%, 20%, and 30%) were used for measuring total soluble solids. Total soluble solids were determined by using a Hand Refractrometer (Erma Japan) 0 to 32 per cent range. The measurements were taken at 7 days interval and expressed in ^{0}Brix.

3.4.2 Titratable acidity (%)

The acidity was determined by diluting the 10ml of sample with 50ml of distilled water then titrated against standard NaOH (0.1 N) using a phenolphthalein indicator. The appearance of light pink colour was recorded as the end point. The values were expressed in terms of citric acid percent of the fruit (AOAC, 1984). Acidity, as citric acid, was calculated using the following formula.

$$\% \text{ Acidity} = \frac{\text{Equivalent weight of acid (64)X Titer X Normality of NaOH (0.158)}}{\text{10X Volume of sample taken (10ml)}}$$

3.4.3 pH

The pH of the processed RTS was measured using digital pH meter (Model: Analog, research, USA). Standard buffer solution of pH 4.01 and 9.08 were used as reference to calibrate the instrument.

3.4.4 Ascorbic Acid (Vitamin C)

Ascorbic acid content was determined by using 2,6-Dichlorophenol-Indophenol Visual titration method of Ranganna (2001). Five ml of sample was taken and volume was made up to 50ml with 3% Metaphosphoric acid (HPO3). An aliquot of 10ml of the HPO3 extract of the sample was taken and titrated with the standard dye to a pink end point. The titration was repeated for 2-3 times for accuracy. It is expressed in mg of Ascorbic acid/100ml.

$$\text{Ascorbic acid} = \frac{\text{Dye factor X Titer X Volume made up X 100}}{\text{Aliquot of extract taken (10ml) X Volume of sample taken (5ml)}}$$

3.5 Microbial Analysis

The presence of microorganisms in any processed RTS drink is obvious until and unless they are treated with some sorts of sterilisation or pasteurisation mechanisms. The shelf life and quality of any processed food mostly depends on its chemical properties, microbial load and types of microbes present in it. Thus looking into these aspects, the microbiological analysis of the RTS of the Guava for detection of the presence of microbes (Bacteria & Fungi) were conducted by following the tenfold serial dilution and spread plate technique.

One ml. of processed RTS was diluted into 9ml of sterile NSS (0.85 % of NaCl) solution. The sample was further diluted up to 10^5 dilutions. 100 µL of the diluted sample was spreaded over sterile Plate Count agar and Rose Bengal agar plate with the help of a L-shaped spreader for the growth of the bacteria and fungi respectively. The processed plates were incubated at 30^0 C±2 for 24 to 48 hours. The colony forming units per ml of the RTS were calculated as per the formula given below.

$$\text{CFU/ml of RTS} = \frac{\text{Mean plate count}}{\text{Quantity taken x dilution factor}}$$

3.6 Estimation of vitamin B_1 (Thiamine)

For estimation of Vitamin B1 content in the guava RTS, the sample was submitted to the animal nutrition department of OVC, OUAT. The results of the processed and treated RTS samples were depicted in Table no. 9 & 10.

3.7 Statistical analysis of the data

The data recorded on various pysico-chemical and microbial characteristics of fortified guava RTS were subjected to Fisher's method of analysis of variance and interpretation of data were taken up as per Sukhatme and Amble (1995). The level of significance used in 'F' test was p = 0.05. Least significant difference or C.D. values were calculated whenever the f' test was significant.

CHAPTER - IV
EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS

The results of the experiment entitled **"Processing of guava RTS fortified with Vitamin B_1"** conducted during 2015-16 have been presented in this chapter. The results are categorized under the following heads.

- Physico-chemical analysis of fortified guava RTS under varying storage period.
- Microbial load of fortified guava RTS under varying storage period.
- Sensory evaluation of fortified Guava RTS under varying storage period.

4.1 Physico-chemical analysis of fortified guava RTS under varying storage period

4.1.1 Total soluble solids(°Brix)

The data relating to TSS of fortified guava RTS observed during 1st day, 7 days, 14 days and 21 days after storage are presented in Table 1. The results revealed that the TSS content of fortified guava RTS recorded on 1st day of storage was significantly influenced by juice percentage and vitamin content. The maximum TSS content (17.00 °Brix) was noticed in T_4 and T_7 which was statistically at par with T_6 (16.80 °Brix). The minimum TSS was recorded in T_1 (15.77 °Brix).

Table 1.TSS content (^{0}Brix) of fortified guava RTS as influenced by juice concentrations during storage

Treatments	Total soluble solids (^{0}Brix)			
	1st day of storage	7 DAS	14 DAS	21 DAS
T_1 (Control)	15.77	15.90	16.02	16.20
T_2 (10% Juice + 0.2mg Vit-B_1)	15.80	15.90	16.12	16.25
T_3 (20% Juice + 0.2mg Vit-B_1)	16.67	17.00	17.12	17.10
T_4 (30% Juice + 0.2mg Vit-B_1)	17.00	17.02	17.10	17.35
T_5 (10% Juice + 0.4mg Vit-B_1)	15.80	15.85	16.07	16.27
T_6 (20% Juice + 0.4mg Vit-B_1)	16.80	16.87	17.05	17.10
T_7 (30% Juice + 0.4mg Vit-B_1)	17.00	17.00	17.07	17.47
SE(m)±	0.04	0.04	0.04	0.04
CD (5%)	**0.12**	**0.13**	**0.12**	**0.12**
CV (%)	0.004	0.005	0.004	0.004

The TSS content of fortified guava RTS recorded at 7 days after storage was significantly influenced by juice concentration. The maximum TSS content was recorded in

T_4 (17.02 °Brix) which was statistically at par with that of T_3 (17.00 °Brix), T_6 (16.87 °Brix) and T_7 (17.00 °Brix). The minimum TSS content was noticed in the treatment T_1 and T_2 (15.90 °Brix).

The data of the concerned character recorded on 14 days after storage was highly influenced by juice concentration and storage period. TSS content recorded in T_3 (17.12 °Brix) was statistically at par with treatments T_4 (17.10 °Brix), T_7 (17.07 °Brix) and T_6 (17.05 °Brix). The minimum TSS content was observed in T_2 (16.02° Brix).

The TSS content of fortified guava RTS recorded after 21 days of storage was significantly influenced by juice concentration. The maximum TSS was recorded in T_7 (17.47 °Brix) which was statistically at par with T_4 (17.35 °Brix). The minimum TSS content was recorded in T_1 (16.20 °Brix).

4.1.2 Titratable Acidity (%):

The data patterning to titratable acidity of fortified guava RTS recorded during 1st day, 7 days, 14 days and 21 days after storage are presented in Table 2. The results indicated that the titratable acidity of guava RTS have been significantly influenced by the juice % fortified with Vitamin B_1. The maximum titratable acidity was recorded in both T_4 and T_7 (0.46%) which was found to be statistically superior to rest of the treatments. The minimum titratable acidity of guava RTS was observed in T_1 (0.33%).

Table 2. Titratable acidity (%) of fortified guava RTS as influenced by juice concentrations during storage

Treatments	Titratable Acidity (%)			
	1st Day of storage	7 DAS	14 DAS	21 DAS
T_1 (Control)	0.33	0.34	0.41	0.50
T_2 (10% Juice + 0.2mg Vit-B_1)	0.34	0.35	0.41	0.50
T_3 (20% Juice + 0.2mg Vit-B_1)	0.39	0.40	0.50	0.54
T_4 (30% Juice + 0.2mg Vit-B_1)	0.46	0.46	0.50	0.59
T_5 (10% Juice + 0.4mg Vit-B_1)	0.34	0.35	0.41	0.47
T_6 (20% Juice + 0.4mg Vit-B_1)	0.39	0.39	0.49	0.56
T_7 (30% Juice + 0.4mg Vit-B_1)	0.46	0.47	0.54	0.59
SE(m)±	0.005	0.005	0.005	0.006
CD (5%)	**0.01**	**0.01**	**0.02**	**0.02**
CV (%)	0.026	0.025	0.022	0.023

The titratable acidity of fortified guava RTS recorded on 7 days after storage was significantly influenced by juice concentration. The maximum acidity recorded in T_7 (0.47

%) which was statistically at par with T_4 (0.46%). The minimum titratable acidity was found in the treatment T_1 (0.34%).

The titratable acidity of fortified guava RTS on 14 days after storage indicated that the concerned character was highly influenced by juice concentration and storage period. The maximum titratable acidity in T_7 (0.57%) was found to be statistically at par with rest of the treatments and minimum titratable acidity value (0.41%) recorded in T_1, T_2 and T_3.

The data of 21 days after storage indicated that the titratable acidity of guava RTS have been significantly influenced by the juice % and storage period. The highest titratable acidity value was recorded in both T_4 and T_7 (0.59%). Whereas T_5 (0.47%) has the minimum titratable acidity percentage.

4.1.3 Changes in Ascorbic Acid (mg/100ml)

The data relating to ascorbic acid content of fortified guava RTS noticed during 1st day, 7 days, 14 days and 21 days after storage are presented in Table 3. The results clearly revealed that the ascorbic acid content of fortified guava RTS significantly influenced by the juice percentage. The maximum ascorbic acid content was found in both T_4 and T_7 (5.25 mg/100ml) which were statistically at par with rest of the treatments. The minimum ascorbic acid content of guava RTS recorded in T_1 (3.16 mg/100 ml).

Table 3. Ascorbic acid content (mg/100ml) of fortified guava RTS as influenced by juice concentrations during storage

Treatments	Ascorbic acid content (mg/100ml)			
	1st Day of storage	7 DAS	14 DAS	21 DAS
T_1 (Control)	3.16	3.15	3.14	3.07
T_2 (10% Juice + 0.2mg Vit-B_1)	3.17	3.16	3.14	3.09
T_3 (20% Juice + 0.2mg Vit-B_1)	4.24	4.22	4.18	4.14
T_4 (30% Juice + 0.2mg Vit-B_1)	5.25	5.10	4.95	4.75
T_5 (10% Juice + 0.4mg Vit-B_1)	3.17	3.16	3.15	3.10
T_6 (20% Juice + 0.4mg Vit-B_1)	4.24	4.21	4.18	4.14
T_7 (30% Juice + 0.4mg Vit-B_1)	5.25	5.15	5.00	4.80
SE(m)±	0.01	0.02	0.02	0.02
CD (5%)	**0.05**	**0.07**	**0.06**	**0.06**
CV (%)	0.007	0.012	0.009	0.010

The ascorbic acid content at 7 days after storage was significantly influenced by juice concentration and storage period. The maximum ascorbic acid content observed in T_7 (5.15 mg/100ml) which was statistically at par with T_4 (5.10 mg/100 ml). The minimum ascorbic acid content was recorded in T_1 (3.15 mg/100 ml).

The ascorbic acid content after 14 days of storage indicated that the character was highly influenced by juice concentration and storage period. The maximum ascorbic acid content observed in T_7 (5.00 mg/100ml) which was superior to T_4 (4.95 mg/100ml). Minimum ascorbic acid content observed in both T_1 and T_2 (3.14 mg/100ml).

The result of the concerned character recorded on 21 days after storage was highly influenced by juice concentration and storage period. The treatment T_7 (4.80 mg/100ml) was statistically at par with T_4 (4.75 mg/100ml). The minimum ascorbic acid content was recorded under T_1 (3.07 mg/100 ml).

4.1.4 pH

The results of pH of fortified guava RTS recorded during 1st day, 7 days, 14 days and 21 days after storage are presented in Table 4. The results divugled that the pH of fortified guava RTS have been significantly influenced by the juice concentrations. The maximum pH was recorded in both T_1 and T_5 (3.91) which were statistically at par with T_2 (3.90). The minimum pH of guava RTS was observed in T_7 (3.84).

Table 4. pH of fortified guava RTS as influenced by juice concentrations during storage

Treatments	pH values			
	1st Day of storage	7 DAS	14 DAS	21 DAS
T_1 (Control)	3.91	3.90	3.86	3.73
T_2 (10% Juice + 0.2mg Vit-B_1)	3.90	3.89	3.85	3.72
T_3 (20% Juice + 0.2mg Vit-B_1)	3.85	3.83	3.78	3.69
T_4 (30% Juice + 0.2mg Vit-B_1)	3.85	3.81	3.77	3.67
T_5 (10% Juice + 0.4mg Vit-B_1)	3.91	3.89	3.85	3.73
T_6 (20% Juice + 0.4mg Vit-B_1)	3.85	3.85	3.80	3.71
T_7 (30% Juice + 0.4mg Vit-B_1)	3.84	3.82	3.79	3.70
SE(m)±	0.004	0.005	0.005	0.005
CD (5%)	0.01	0.02	0.02	0.01
CV (%)	0.002	0.003	0.003	0.002

The pH at 7 days after storage was significantly influenced by juice concentration and the maximum pH value recorded in T_1 (3.90) which was statistically at par with both T_2 and T_5 (3.89). The minimum pH value was observed in T_4 (3.81).

The pH value of fortified guava RTS after 14 days storage reported that the concerned character was highly influenced by juice concentration and storage period. The maximum pH value recorded in both T_2 and T_5 (3.85) which were statistically at par with T_1 (3.86). The minimum pH value was recorded in T_4 (3.77).

The pH at 21 days after storage was significantly influenced by juice concentration and storage period. The highest value for the concerned character was observed in both T_1 and T_5 (3.73) which were significantly at par with T_2 (3.72). The minimum pH value was recorded in T_4 (3.67).

4.1.5 Vitamin B_1 (Thiamine) content (mg/200ml)

The data patterning to Vitamin B_1 content of guava RTS recorded during 1st day, 7 days, 14 days and 21 days after storage are presented in Table 5. The results clearly revealed that the Vitamin B_1 content of guava RTS have been significantly influenced by juice concentrations. The maximum Vitamin B_1 content recorded in T_5, T_6 and T_7 (0.40 mg/200 ml) on the 1st day of storage were significantly at par with other treatments. The minimum value was recorded in both T_2 and T_3 (0.19 mg/200 ml).

The Vitamin B_1 content of guava RTS recorded at 7 days after storage was significantly influenced by juice concentration and storage period. The maximum vitamin content observed in T_5, T_6 and T_7 (0.39 mg/200ml) were statistically at par with other treatments. The minimum vitamin content was found in T_2, T_3 and T_4 (0.19 mg/200ml).

The Vitamin B_1 content of guava RTS recorded on 14 days after storage was significantly influenced by juice concentration and storage period. The highest value for Vitamin B_1 content was observed in T_5, T_6, and T_7 (0.38 mg/200ml) which were statistically at par and the minimum vitamin content was recorded in both T_2 and T_3 (0.18 mg/200 ml).

Table 5. Vitamin B_1 content (mg/200ml) of fortified guava RTS as influenced by juice concentrations during storage

Treatments	Vitamin B_1 content (mg/200ml)			
	1st Day of storage	7 DAS	14 DAS	21 DAS
T_1 (Control)	0	0	0	0
T_2 (10% Juice + 0.2mg Vit-B_1)	0.19	0.19	0.18	0.17
T_3 (20% Juice + 0.2mg Vit-B_1)	0.19	0.19	0.18	0.17
T_4 (30% Juice + 0.2mg Vit-B_1)	0.20	0.19	0.19	0.18
T_5 (10% Juice + 0.4mg Vit-B_1)	0.40	0.39	0.38	0.36
T_6 (20% Juice + 0.4mg Vit-B_1)	0.40	0.39	0.38	0.38
T_7 (30% Juice + 0.4mg Vit-B_1)	0.40	0.39	0.38	0.38
SE(m)±	0.002	0.002	0.002	0.002
CD (5%)	**0.005**	**0.008**	**0.008**	**0.006**
CV (%)	0.013	0.020	0.020	0.015

The data for the concerned character was indicated that the Vitamin B_1 content was significantly influenced by juice concentration and storage period of RTS after 21 days of storage. The maximum value for Vitamin B_1 content was recorded in both treatment T6 and T7 (0.38 mg/200ml) which were statistically at par with rest of the treatments. The minimum value for this character was recorded in both T_2 and T_3 (0.17 mg/200ml).

4.2 Changes in microbial load of fortified guava RTS under varying storage period

4.2.1 Bacterial load in guava RTS (CFU/ml)

The data relating to bacterial load of fortified guava RTS observed during 1st day, 7 days, 14 days and 21 days after storage are presented in Table 6. The results revealed that the bacterial load of fortified guava RTS recorded on 1st day of storage was significantly influence by juice percentage. The maximum bacterial load (0.77 CFU/ml) was noticed in T_7 which was statistically at par with T_6 (0.72 CFU/ml). The minimum bacterial load was recorded in T_1 (0.20 CFU/ml).

The bacterial load of fortified guava RTS recorded at 7 days after storage was significantly influenced by juice concentration and storage period. The maximum load of bacteria was observed in T_7 (1.57 CFU/ml) which was statistically at par with T_4 (1.67 CFU/ml). The minimum bacterial load was noticed in T_1 (0.92 CFU /ml).

Table 6. Bacterial load (CFU/ml) of fortified guava RTS as influenced by juice concentrations during storage

Treatments	Bacterial load (CFU/ml)			
	1st Day of storage	7 DAS	14 DAS	21 DAS
T_1 (Control)	0.20	0.92	2.10	2.25
T_2 (10% Juice + 0.2mg Vit-B_1)	0.30	1.10	2.02	3.05
T_3 (20% Juice + 0.2mg Vit-B_1)	0.42	0.95	1.30	1.42
T_4 (30% Juice + 0.2mg Vit-B_1)	0.55	1.67	2.15	3.17
T_5 (10% Juice + 0.4mg Vit-B_1)	0.45	1.07	1.92	2.95
T_6 (20% Juice + 0.4mg Vit-B_1)	0.72	1.12	1.32	2.60
T_7 (30% Juice + 0.4mg Vit-B_1)	0.77	1.57	2.35	3.20
SE(m)±	0.06	0.05	0.04	0.08
CD (5%)	**0.20**	**0.15**	**0.12**	**0.25**
CV (%)	0.245	0.082	0.004	0.061

The data for the concerned character recorded on 14 days after storage was highly influenced by juice concentration and storage period. The bacterial load recorded in T_7 (2.35 CFU/ml) was statistically at par with the rest of the treatments. The minimum bacterial load was observed in T_3 (1.30 CFU/ml).

The result after 21 days storage indicated that the fortified guava RTS was significantly influenced by juice concentration and storage period bacterial load. The highest value recorded in T_4 (3.17 CFU/ml) was statistically at par with the treatment T_7 (3.20 CFU/ml), T_2 (3.05 CFU/ml) and T_5 (2.954 CFU/ml). The minimum load of bacteria was recorded in T_3 (1.42 CFU/ml).

4.2.2 Fungal load in guava RTS (CFU/ml).

The data pertaining to fungal load of fortified guava RTS recorded during 1st day, 7 days, 14 days and 21 days after storage presented in Table 7. The results indicated that the fungal load of guava RTS have been significantly influenced by the juice percentage in the 1st day of storage. The maximum fungal load was recorded in T_7 (0.90 CFU/ml) which was found to be statistically at par with T_6 (0.85 CFU/ml). The minimum fungal load was observed in T_1 (0.17 CFU/ml).

Table 7. Fungal load (CFU/ml) of fortified guava RTS as influenced by juice concentrations during storage

Treatments	Fungal load (CFU/ml)			
	1st Day of storage	7 DAS	14 DAS	21 DAS
T_1 (Control)	0.17	0.30	0.40	0.42
T_2 (10% Juice + 0.2mg Vit-B_1)	0.27	0.95	1.02	1.20
T_3 (20% Juice + 0.2mg Vit-B_1)	0.37	1.32	1.70	1.85
T_4 (30% Juice + 0.2mg Vit-B_1)	0.40	1.35	1.40	2.35
T_5 (10% Juice + 0.4mg Vit-B_1)	0.65	1.00	1.10	1.15
T_6 (20% Juice + 0.4mg Vit-B_1)	0.85	1.25	1.35	1.60
T_7 (30% Juice + 0.4mg Vit-B_1)	0.90	1.37	1.45	1.72
SE(m)±	0.05	0.10	0.05	0.07
CD (5%)	**0.15**	**0.27**	**0.18**	**0.21**
CV (%)	0.198	0.167	0.097	0.096

The fungal load of fortified guava RTS recorded at 7 days after storage was significantly influenced by juice concentration and storage period. The maximum fungal load was observed in T_7 (1.37 CFU/ml) which was statistically at par with T_3 (1.32 CFU/ml), T_4 (1.35 CFU/ml) and T_6 (1.25 CFU/ml). The minimum load was observed in T_1 (0.30 CFU/ml).

The data for the concerned character recorded on 14 days after storage was highly influenced by juice concentration and storage period. The fungal load recorded in T_1 (1.70 CFU/ml) was statistically at par with the rest of the treatments. The minimum fungal load was observed in T_1 (0.40 CFU/ml).

The result after 21 days storage indicated that the fortified guava RTS was significantly influenced by juice concentration and storage period. The fungal load in T_4 (2.35 CFU/ml) was statistically at par with the rest of the treatments and the minimum fungal load recorded in T_1 (0.42 CFU/ml).

4.3 Sensory evaluation of fortified guava RTS under varying storage period.

The sensory scores as evaluated by the panellists for different juice concentration of RTS taken as per 5 point hedonic scale at 1st day, 7 days, 14 days and 21 days after storage are graphically presented in Fig. 1, 2 and 3. The results revealed that the highest score for taste were recorded in T3 and T6 at 1st day, 7 days, 14 days and 21 days after storage. The score for flavour of fortified guava RTS were recorded highest in T3, T4, T6 and T7 at 1st day, 7 days, 14 days and 21 days after storage. The overall acceptability score given by the

panellists recorded the highest in T3, T4, T6 and T7 at 1st day, 7 days, 14 days and 21 days after storage. However, as per the evaluation most of the panellist liked T3 and T6 for better taste, flavour and overall acceptability.

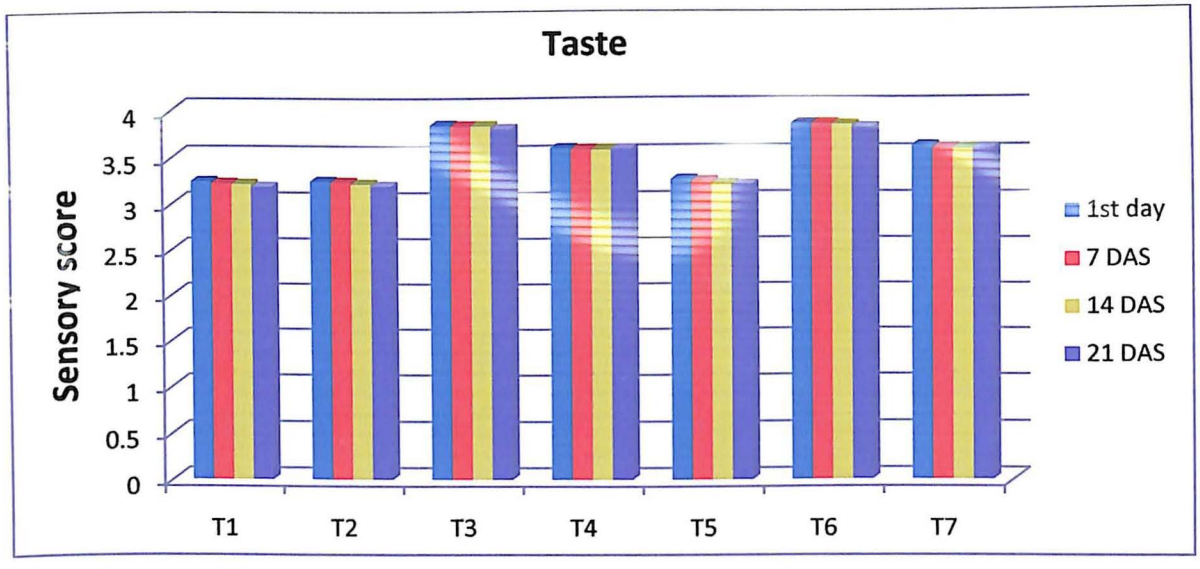


Fig. 1. Sensory evaluation for taste of fortified guava RTS as influenced by varying concentration of juice and storage period.

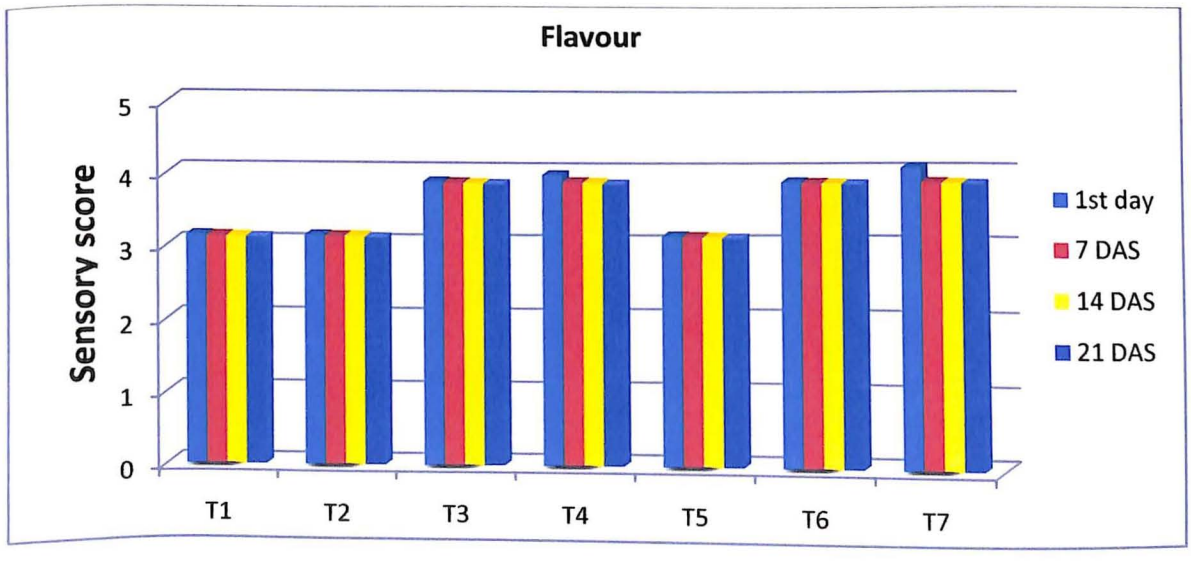


Fig. 2. Sensory evaluation for flavour of fortified guava RTS as influenced by varying concentration of juice and storage period.

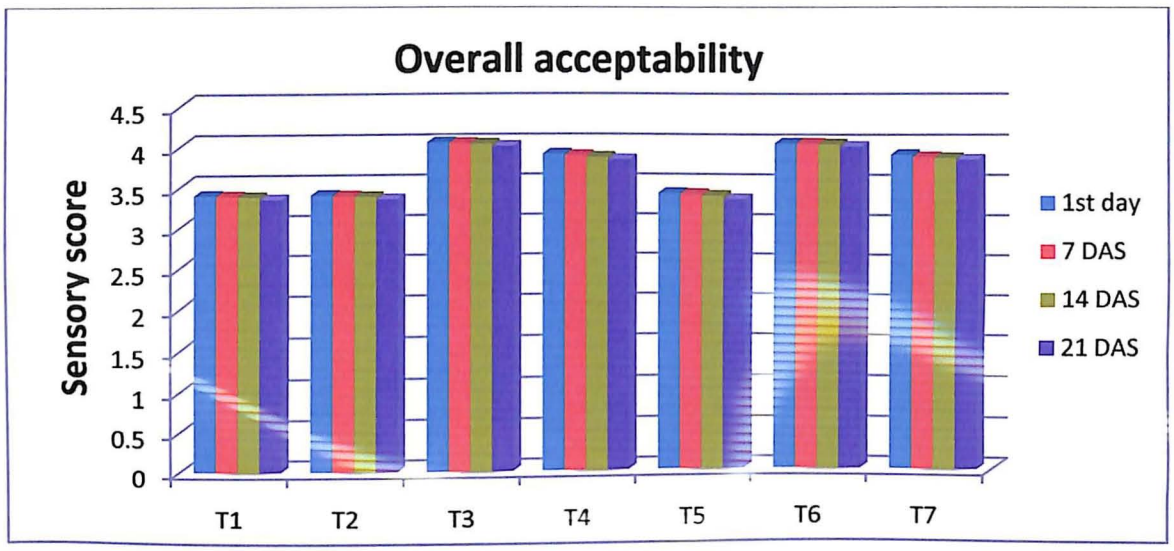


Fig.3. Sensory evaluation for overall acceptability of fortified guava RTS as influenced by varying concentration of juice and storage period.

T_1 (Control)	T_5 (10% Juice + 0.4mg Vit-B_1)
T_2 (10% Juice + 0.2mg Vit-B_1)	T_6 (20% Juice + 0.4mg Vit-B_1)
T_3 (20% Juice + 0.2mg Vit-B_1)	T_7 (30% Juice + 0.4mg Vit-B_1)
T_4 (30% Juice + 0.2mg Vit-B_1)	

CHAPTER - V

DISCUSSION

DISCUSSION

In the present chapter the results of the study have been discussed in the light of scientific facts and findings of earlier research workers from this country as well as abroad. The results are discussed under the following heads.

- Physico-chemical analysis of fortified guava RTS under varying storage period.
- Microbial load of fortified guava RTS under varying storage period.
- Sensory evaluation of fortified Guava RTS under varying storage period.

5.1 Physico-chemical analysis of fortified guava RTS under varying storage period

5.1.1 Total soluble solids (°Brix)

The maximum TSS content of guava RTS was observed with T_4 (17.02 ^{0}Brix), T_3 (17.12 ^{0}Brix) and T_7 (17.47 ^{0}Brix) at 7 DAS, 14 DAS and 21 DAS respectively. The results of the study inferred that with increased concentration of juice in the fortified guava RTS the TSS also went on increasing with the passage of storage period.

The conversion of polysaccharides into sugar might be the reason of an increase in TSS. The findings of present studies are in conformity with Bhat and Singh (2014) who observed an increasing trend of TSS in whey-guava RTS over the storage period.

In mango-ginger RTS, mango pulp used in beverage development was containing 21.80% TSS whereas ginger juice was containing 1.80% TSS and it increased with storage period (Deen *et al.*, 2014).

5.1.2 Titratable Acidity (%)

The maximum titratable acidity content of guava RTS was observed with T_7 (0.47%), T_3 (0.54%) and T_7 (0.59%) at 7 DAS, 14 DAS and 21 DAS respectively. The results of the study informed that with increased concentration of juice in the fortified guava RTS the titratable acidity also went on increasing with the passage of storage period.

It was reported that pectin substances increased the acidity of fruit products (Conn and Stumf, 1976). Also increase in percentage of acidity might be due to the slight growth of micro-organism in the beverage during storage period.

The similar trend was observed in the acid content of the guava-lime-ginger RTS. The acid content of RTS beverage increased from 0.252 to 0.305% during storage (Selvi *et*

al., 2013). Sakhale *et al.* (2012) prepared whey based RTS beverage from mango cv. Kesar and the study indicated that the acidity of RTS showed increasing trend (0.36 - 0.38%).

5.1.3 Ascorbic Acid (mg/100ml)

The maximum ascorbic acid content of guava RTS was observed with T_7 (5.15mg/ml), T_3 (5.00mg/100ml) and T_7 (4.80mg/100ml) at 7 DAS, 14 DAS and 21 DAS respectively. The results of the study inferred that with increased concentration of juice in the fortified RTS the ascorbic acid content also increased, but with the passage of storage period the ascorbic acid content was decreased.

The decreasing trend of ascorbic acid content might be due to oxidation of ascorbic acid to dehydro-ascorbic acid followed by further degradation to 2, 3-diketogulonic acid and finally to furfural compounds (Kuchi *et al.*, 2014). These losses of ascorbic acid were attributed to the effect of processing, storage time and exposure to light (Pandey, 2004). Deen *et al.* (2014) also found the similar decreasing trend of ascorbic acid (Vitamin C) content in mango-ginger RTS. The decreasing trend in the ascorbic acid content of the guava-lime-ginger RTS was observed during storage (Selvi *et al.*, 2013).

5.1.4 pH

The maximum pH value of guava RTS was observed with T_1 (3.90, 3.86, 3.73 at 7 DAS, 14 DAS and 21 DAS respectively) and T_5 (3.73 in 21 DAS). The results of the study revealed that the increased concentration of juice in the fortified guava RTS with respect to its storage period the pH value decreased.

The pH value of juice decreases with increase in acidity. Variations in pH during storage might be due to change in chemical properties which are affected by storage conditions. The findings of present studies are in confirmity with the slight decreasing trend of pH in whey guava beverage during the storage period (Singh *et al.*, 2014). Nilugin *et al.*, (2010) revealed the decreasing trend of pH in RTS prepared from palmyrah fruit pulp (3.17-3.12).

5.1.5 Vitamin B_1 (Thiamine) content

The two different concentrations of vitamin i.e. 0.2mg/200ml and 0.4mg/200ml were added to each concentration of RTS. The maximum Vitamin B_1 content of guava RTS was observed in both T_5 and T_6 (3.90, 3.88, 3.88 mg/200ml) at 7 DAS, 14 DAS and 21 DAS respectively. The results of the study indicated the vitamin B_1 content was decreased with that of increase in storage period.

Thiamine is soluble in water and unstable to heat, but up to some extend it is stable at acidic pH. It might be the cause of slight decrease in thiamine content in guava RTS over the storage period. The similar work was also done in fortification of dairy products with Vitamin A and C which leads to the improvement in their nutritive quality and consequently increases their acceptability (Gahruie *et al.*, 2015).

5.2 Microbial load of fortified guava RTS under varying storage period

The maximum bacterial load in fortified guava RTS was observed with T_7 (1.57 CFU/ml), T_7 (2.35 CFU/ml) and T_4 (3.17 CFU/ml) and maximum fungal load was recorded with T_7 (1.37 CFU/ml), T_3 (1.70 CFU/ml) and T_3 (1.85 CFU/ml) at 7 DAS, 14 DAS and 21 DAS respectively). The results of the study divulged that the increase in concentration of juice in guava RTS and its storage period the microbial load was increased.

Ready to serve food items have always gone through a stringent quality control measures with regards to its physical, chemical and microbiological parameters. During our experimental formulation of the RTS of guava it observed that there was a presence of the microorganisms in a lower order. The presence of the microbes cannot be eliminated because the RTS was not processed for any sterilization or pasteurization techniques. Due to the acidic nature of the RTS and addition of citric acid as preservative, this might have helped in lowering the microbial population. Citric acid is a chelator; i.e. a lipophobic, dissociated acid inhibiting growth of micro-organisms by chelating divalent metal ions from the medium (Brul and Coote, 1999; Stratford, 1999). Though the RTS was stored in a 4^0 C, the prolonged storage period up to 21 days is showing a gradual increase in the microbial population over time. Though the storage temperature of 4^0 C helps to control the growth of majority of mesophilic bacterial species by decreasing its metabolic activity, it is not sufficient to control the growth of the psychrophilic bacterial population.

Thus in this context further study is essential to process the RTS for pasteurization or sterilization process to remove any microbial contamination which will help in enhancing the storage period of the RTS with a greater market value. A slight increase in the microbial load was noted in the formulated value added fruit products during storage. Initially the bacteria, fungal and yeast count was below detectable level (BDL) and at the

end of storage period microbial load slightly increased in guava-lime-ginger RTS (Selvi *et al.*, 2013).

As the storage period proceed the total plate count increases and after the completion of one month (30 days) in Whey based RTS Beverage from Mango cv. Kesar (Sakhale *et al.*, 2012). Similar results of total plate count were reported for whey-based mango beverage (Ismail *et al.*, 2011). The supplementation of Vitamin B_1 has no significant effect in any quality attributes of prepared guava RTS.

5.3 Sensory Evaluation

The RTS prepared with 20% and 30% guava juice have higher score regarding the taste, flavour and overall acceptability than the RTS prepared with 10% guava juice might be due to lower juice concentration. RTS with 30% guava juice was not preferred by panellists might be due to its more sweet taste. So, RTS containing 20% juice was adjudged as the best for RTS preparation with either concentration of Vitamin B_1. The scores for all the sensory parameters were decreased with passage of storage period. It might be due the changes of physico-chemical properties and increase in microbial load during the storage period.

The taste, flavour and overall acceptability value of orange based blended RTS beverages decreased with the increase in storage period up to 90^{th} days (Gupta et al., 2015). The storage study of whey based mango RTS revealed that all the characteristics i.e. appearance, colour, flavor, taste and overall acceptability of sensory evaluation was in decreasing trend. This might be due to changes occurred during storage of beverage (Sakhale *et al.*, 2012).

From the results of the present study it could be inferred that guava RTS prepared using 20% of juice with either of the concentration of Vitamin B_1 (0.2 and 0.4 mg/200ml) tried could be acceptable to consumers as rated by the panelists.

CHAPTER - VI

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The research work entitled **"Processing of guava RTS fortified with Vitamin B_1"** was carried out in the laboratory at Department of Fruit Science and Horticulture Technology, College of Agriculture with active support of Department of Agricultural Processing and Food Engineering (CAET) and Department of Microbiology (CPGS), Orissa University of Agriculture and Technology, Bhubaneswar, Odisha during 2015-16. The main objective of the research was to process the guava RTS fortified with Vitamin B_1 in order to develop the market security for indigenous guava fruit with addition of nutritive value to it. The studies were undertaken regarding the physico-chemical properties, microbial load and sensory evaluation of the fortified guava RTS. Basing the results obtained, the salient findings are summarized as follows.

1. The total soluble solids (TSS) of fortified guava RTS was significantly influenced by juice concentration and storage period. The total soluble solids content recorded at 7 DAS, 14 DAS and 21 DAS was found highest in T_4 (17.02 ^{0}Brix), T_3 (17.12 ^{0}Brix) and T_7 (17.47 ^{0}Brix) as compared to T_1 (15.90, 16.02 and 16.20 ^{0}Brix), respectively.

2. The titratable acidity percent of fortified guava RTS was significantly influenced by juice concentration and storage period. The titratable acidity recorded at 7 DAS, 14 DAS and 21 DAS was found to be highest in T_7 (0.47%), T_3 (0.54%) and T_7 (0.59%) as compared to T_1 (0.34, 0.41 and 0.50 %) respectively.

3. The results of ascorbic acid content of fortified guava RTS were significantly influenced by juice concentration and storage period. The ascorbic acid content observed at 7 DAS, 14 DAS and 21 DAS was found to be highest in T_7 (5.15mg/ml), T_3 (5.00mg/100ml) and T_7 (4.80mg/100ml) as compared to T_1 (3.15, 3.14 and 3.07 mg/100ml) respectively.

4. The pH value of fortified guava RTS was highly influenced by juice concentration and storage period. The pH value recorded at 7 DAS, 14 DAS and 21 DAS was found to be highest with. T_1 (3.90, 3.86, 3.73) respectively.

5. The maximum Vitamin B_1 content of guava RTS observed at 7 DAS, 14 DAS and 21 DAS was recorded to be highest in both T_5 and T_6 (3.90, 3.88, 3.88 mg/200ml) respectively as compared to control i.e. without Vitamin B_1 content.

6. The results of microbial load of fortified guava RTS were significantly influenced by juice concentration and storage period.The maximum bacterial load recorded at 7 DAS, 14 DAS and 21 DAS was found to be highest in T_7 (1.57 CFU/ml), T_7 (2.35 CFU/ml) and T_4 (3.17 CFU/ml) as compared to T_1 (0.92, 2.10 and 2.25 CFU/ml) respectively.

7. The highest fungal load in fortified guava RTS recorded at 7 DAS, 14 DAS and 21 DAS was found to be highest in T_7 (1.37 CFU/ml), T_3 (1.70 CFU/ml) and T_3 (1.85 CFU/ml) as compared to T_1 (0.30, 0.40 and 0.42 CFU/ml) respectively.

8. The highest score for taste, flavour and overall acceptability recorded at 7 DAS, 14 DAS and 21 DAS were found to be highest with T_3 (20% juice + 0.2mg Vitamin B_1) and T_5 (20% juice + 0.4mg Vitamin B_1) as compared to other juice concentrations of guava RTS with either of the concentration of Vitamin B_1.

From the results of the present study it could be inferred that guava RTS prepared using 20% of juice with either of the concentration of Vitamin B_1 (0.2 and 0.4 mg/200ml) tried could be acceptable to consumers as rated by the panelists. Further taking into account the demand for the processed juice more and more research work could be taken up to fortify the fruit juices with different vitamins, minerals and if possible probiotics. This kind of venture would not only become more health friendly but also add value to the produce and value to the human life style.

REFERENCES

REFERENCES

Abano EE and Dadzie RG. 2014. Simultaneous detection of water-soluble vitamins using the High Performance Liquid Chromatography (HPLC) - a review, *Croation Journal of Food Science and Technology*, **6 (2):**116-123.

Ahmady SH, Ashour ML and Wink M. 2013. Chemical composition and anti-inflammatory activity of the essential oils of *Psidium guajava* fruits and leaves, *The Journal of Essential Oil Research,* **01**:14.

Bal LM, Ahmad T, Senapati AK and Pandit PS. 2014. Evaluation of Quality Attributes During Storage of Guava Nectar Cv. Lalit from Different Pulp and TSS Ratio. *Journal of Food Processing and Technology*, **5**: 329.

Barbalho SM, Farinazzi-Machado FMV, Goulart RA and Brunnati ACS. 2012. *Psidium guajava* (Guava): A plant of multipurpose medicinal applications, *Medicinal & Aromatic Plants*, **1**:104

Calvo MS and Whiting SJ. 2003. Prevalence of vitamin D insufficiency in Canada and the United States: importance to health status and efficacy of current food fortification and dietary supplement use. *Nutrition Reviews*, **61**:107–13.

Deen B and Kumar A. 2014. Development and storage of mango ginger RTS beverage, *International Journal of Food, Agriculture and Veterinary Sciences,* **4:** 15-20.

Divya and Archana K. 2009. Effect of different temperatures, timings and storage periods on physico-chemical and nutritional characteristics of whey guava beverage, *World Journal of Dairy Food Science,* **4:** 118-122.

Gopalan C. 2013. The changing nutrition scenario, *Indian Journal of Medical Research,* **138**:392-397.

Gupta R, Malav M, Kushwaha NK and Pandey A. 2015. Studies on organoleptic qualities of orange based blended ready-to-serve (RTS) beverages, *The Bioscan*, **10(3):** 1041-1043.

Hossen S, Kabir MS, Uddin MB, Rahman AKML and Mamun MRA, Effect of different extractions of juice on quality and acceptability of guava jelly, *Journal of innovation and development strategy.* **3(4):** 27-35.

Jain PK and Asati VK. 2004. Evaluation of guava cultivars for pulp preparation, *Journal of Food Science and Technology,* **41**: 684-86.

Kadam DM, Kaushik P and Kumar K. 2012. Evaluation of guava products quality, *International Journal of Food Science and Nutrition Engineering,* **2(1):** 7-11.

Kocher GS and Pooja. 2011. Status of wine production from guava (*Psidium guajava* L.): A traditional fruit of India, *African Journal of Food Science,* **5:** 851-860.

Kuchi VS, Gupta R, and Tamang S. 2014. Standardization of recipe for preparation of guava jelly bar, *Journal of Crop and Weed,* **10(2):**77-81.

Lebiedzinska A and Szefer P. 2006. Vitamins B in grain and cereal–grain food, soy-products and seeds, *Food Chemistry,* **95:** 116–122.

Lebiedzinska A, Marszall ML, Kuta J and Szefer P. 2007. RP-HPLC method with coulometric electrochemical and ultraviolet detection for the quantification of Vitamins B1, B6 and B12 in animal and plant foods, *Journal of Chromatography A,* **1173 (1-2):**71-80.

Malav M, Gupta R and Nagar T. 2014. Studies on bio-chemical composition of orange based blended ready-to-serve (RTS) beverages, *Bioscience Biotechnology Research Communications,* **7(1):** 78-83.

Nielsen MK and Arneborg N. 2007. The effect of citric acid and pH on growth and metabolism of anaerobic *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii* cultures, *Food Microbiology,* **24**:101–105.

Nilugin SE and Mahendran T. 2010. Preparation of ready-to-serve (RTS) beverage from palmyrah (*Borassus flabellifer* L.) Fruit pulp, *The Journal of Agricultural Sciences,* **5:**80-88.

Pandey AK. 2004. Study about the storage stability of guava beverage, *Prog Hort,* **36**: 142-145.

Ritu G and Gupta A. 2014. Fortification of Foods with Vitamin D in India *Nutrients,* **6**:3601-3623.

Sakhale BK, Pawar VN and Ranveer RC. 2012. Studies on development of soymilk based mango RTS beverage, *Electronic Journal of Environmental, Agricultural and Food Chemistry,* **11(5):** 523-528.

Santos CAF, Correa LC and da Costa SR. 2011. Genetic divergence among Psidium accessions based on biochemical and agronomic variables. *Crop Breeding and Applied. Biotechnology*, **11**:149–156.

Sasikumar R. 2015. Preparation of therapeutic RTS beverage from *aloe vera* gel and aonla fruit juice and evaluation of storage stability, Asian Journal Dairy & Food Research, **34(2):** 151-155.

Selvi J, Banumathi P, Kanchana S and Ilamaran M. 2013. Formulation of therapeutic drink to boon human health (guava-lime-ginger RTS beverage), *Food Science,* **4:** 141-146.

Shirur DS, Adhikari R, Sanjay ST and Sajjekhan S. 2013. A review on the medicinal plant *Psidium guajava* L. (Myrtaceae), *Journal of Drug Delivery & Therapeutics,* **3(2):**162-168.

Singh RB, Rastogi SS, Singh NK, Ghosh S and Gupta S. 1993. Can guava fruit intake decrease blood pressure and blood lipids. *Journal of Human Hypertension,* **7**: 33- 38.

Singh RB, Rastogi SS, Singh NK, Ghosh S and Niaz MA.1992. Effects of guava intake on serum total and high-density lipoprotein cholesterol levels and on systemic blood pressure. *American Journal of Cardiology,* **70**: 1287-1291

Tangpricha V, Koutkia P, Rieke SM, Chen TC, Perez AA and Holick, MF. 2003. Fortification of orange juice with vitamin D: A novel approach for enhancing vitamin D nutritional health, *American Journal of Clinical Nutritio*, **77**:1478–1483.

Thongsombat W, Sirichote A and Chanthachum S. 2007. The production of guava juice fortified with dietary fiber, *Songklanakarin Journal of Science and Technology*, **29:** 187-196.

Yen G and Song T. 1998. Characteristics of Clouding Substances in Guava Puree, *Journal of Agricultural Food Chemisrty,* **46**: 3435-3439.