

EXTRACTION OF COLOUR FROM CATECHU AND ITS UTILIZATION IN SELECTED DAIRY PRODUCTS



**THESIS SUBMITTED TO THE
ICAR-NATIONAL DAIRY RESEARCH INSTITUTE, BENGALURU
(DEEMED UNIVERSITY)**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF**

MASTER OF TECHNOLOGY

**IN
DAIRY TECHNOLOGY**

By

**JADHAO SWAPNIL YUVRAJSING
B.TECH. (Dairy Technology)**

**DAIRY TECHNOLOGY SECTION
ICAR-NATIONAL DAIRY RESEARCH INSTITUTE
SOUTHERN CAMPUS
ADUGODI, BENGALURU- 560030, INDIA**

2015

Regn. No. 13-M-DT-09

EXTRACTION OF COLOUR FROM CATECHU AND ITS UTILIZATION IN SELECTED DAIRY PRODUCT

By

SWAPNIL YUVRAJSING JADHAO

B.TECH. (Dairy Technology)

Thesis Submitted to the

ICAR-National Dairy Research Institute

(Deemed University)

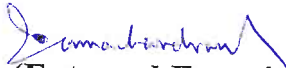
in partial fulfillment of the requirements for the degree of

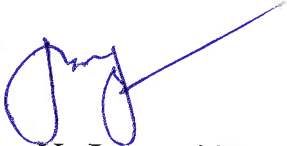
MASTER OF TECHNOLOGY

IN

DAIRY TECHNOLOGY


Approved By


(External Examiner)
(Dr. H.G. Ramachandra Rao)


(Dr. K. Jayaraj Rao)
Major Advisor & Chairman
(Guide)

Members of Advisory Committee

1. **Dr. K. Jayaraj Rao**, Principal Scientist,
(Dairy Technology Section, Bengaluru)
2. **Dr. C.N. Pagote**, Principal Scientist
(Dairy Technology Section, Bengaluru)
3. **Dr. B.V. Balasubramanyam**, Principal Scientist,
(Dairy Technology Section, Bengaluru)
4. **Dr. Menon Rekha Ravindra**, Senior Scientist,
(Dairy Engineering Section, Bengaluru)
5. **Dr. B. Surendra Nath**, Principal Scientist,
(DC&B Sec, Bengaluru & Jt. Director's nominee)











DAIRY TECHNOLOGY SECTION
ICAR-NATIONAL DAIRY RESEARCH INSTITUTE
(Deemed University)

BENGALURU- 560030 (KARNATAKA), INDIA



Dr. K. Jayaraj Rao, Ph.D
Principal Scientist
Dairy Technology Section
National Dairy Research Institute
Southern Campus
Bengaluru- 560 030

Dated: /07/2015.

CERTIFICATE

This is to certify that the thesis entitled “Extraction of colour from Catechu and its utilization in selected dairy products”, submitted by **Mr. Jadhao Swapnil Yuvrajsing** towards the partial fulfilment for the award of the degree of **MASTER OF TECHNOLOGY** in **DAIRY TECHNOLOGY** of the **NATIONAL DAIRY RESEARCH INSTITUTE (Deemed University)**, Karnal (Haryana), India, is a bonafide research work carried out by him under my supervision and guidance and no part of the thesis has been submitted for any other degree or diploma.

(Dr. K. Jayaraj Rao)
Major Advisor
(Guide)

*That I dedicate this work to
the Lord, my Guide my
loving family, and Piu*

ACKNOWLEDGEMENTS

I first thank the God for having bestowed me with all what I needed if not what I wanted and showing me the right path at every step.

It was a great pleasure to carry out my research work under the guidance of Dr. K. Jayaraj Rao, Principal Scientist, Dairy Technology Section. In his unique way, he provided me with valuable and inspiring guidance, constructive criticism and suggestions throughout the process of this research project. But for his keen interest and complete involvement, this project would have never been completed. I admire his expertise in planning the projects and immense ability of reasoning, and appreciate his perseverance in tackling research problems. He understood me thoroughly and has been very kind to me throughout the period I worked with him and I have greatly benefited from his knowledge and experience.

Every niche of this investigation is a beautiful conglomeration of ideas, and an amalgamation of rich concepts given by the members of my Advisory Committee, Dr. C. N. Pagote, Principal Scientist, Dr. Rekha Menon, Senior Scientist, Dr. B. V. Balasubramanyam, Principal Scientist, Dr. B. Surendra Nath, Principal Scientist, NDRI (SRS), Bengaluru .

I wish to convey my heartiest and humble thanks to Dr. A. K. Srivastava, Director, NDRI, Karnal, Dr. Satish Kulkarni, former Head, SRS of NDRI, Bengaluru and Dr. G.R. Patil, former Joint Director (Research) for providing all necessary facilities to carry out the present investigation.

I express my gratitude to Dr. Rekha Menon, Senior Scientist and Incharge, Education Section, and Mrs. Thivija Kumari for their help during the progress of the study; financial assistance in the form of institutional fellowship provided by NDRI, Karnal is greatly acknowledged to complete this research study.

I feel pleasure to thank Dr. B. C. Ghosh, Principal Scientist & Incharge D.T. Section for extending me all the needed support and helping hands during my research work.

I offer my cordial thanks Mr. P. Aravindakshan sir for all his kinds of assistance and flawless co-operation, encouragement and affection that he has showered on me.

I gratefully acknowledge the valuable help and all possible cooperation extended by the staff of Experimental Dairy, D.T., D.C. and D.M. Labs, SRS, NDRI. I am also thankful to my PhD seniors, Diwakar Mishra, Harin Sutariya,, Jatin Gol, Madhav Choudhary, Rahila, Nikita Sethi and Reshmi, my batch mates, Sandip Zade, Ravi, Rakesh, Roshan, Dnyaneshwar, Sudin and NDRI juniors Sachin, Spoorti and Vasant for all their help and great company.

Words do not suffice to express my devotion and gratitude to my parents who endured my absence for long and always encouraged me to go for a long innings of my academic pursuit and all teachers who came in my life, my grandfather Late. Bapusing Jadhao, My Grandmother Chandrabhagabai, my uncle Rajendra Jadhao, Vishwas Jadhao, My Aunty Rekha Jadhao, and Meena Jadhao for their blessings, enthusiastic moral support and believing me at every step of my life. My loving brothers, Amol, Atul, Shreyash, Sarthak and Sister Ankita were always there, supporting and encouraging me. It is none else but to all in my loving family that I dedicate this thesis. I thank them for having faith in me and for their support.

Date:

Jadhao Swapnil Yuvrajsing

ABSTRACT

Acacia catechu (Khair) is well known for its heartwood extract called as *katha*, commonly used in betel leaf preparation (paan) by people in Indian subcontinent. The extract gives dark red to reddish brown colour and is known to possess several medicinal properties because of its phytoconstituents. It is also being used in leather and textile industry as colouring agent. However, it has not yet been tried in food industry as colouring agent though it is a good source of natural colour and can be used to replace or supplement artificial colours. In this project, colour was extracted from catechu heartwood by heat extraction method using water as the medium. The extract was heat concentrated to get a Catechu colour concentrate (CCC). The CCC was used as colouring agent in various dairy products and its intensity was evaluated by measuring per cent reflectance using reflectance meter and colour parameters by Scanner-Adobe Photoshop method under RGBL mode. The rheological and textural parameters were measured by Ostwald viscometer and Texture Analyser. The CCC imparted brown colour to the milk and was well accepted when used at 0.8% level. The CCC coloured flavoured milk was also subjected to heat sterilization and it was found that the colour displayed good heat stability even at sterilization temperature of 121°C/15min. There was also no effect of the CCC on the viscosity of the milk during processing as well as during storage at ambient temperature. Kinematic viscosity (cS) of plain sterilized FM samples and coloured sterilized FM was 2.45 and 2.42 cS, respectively. The colour and appearance score on 9-point Hedonic scale for CCC added FM (7.8) was more than that of plain sterilised milk (7.4). It was found that caramel flavour matched well with the colour imparted by CCC. In shrikhand, the addition of CCC imparted brown colour to the product and was well accepted when used at 1.5% level. It was also observed that the CCC complemented the colour of sapota shrikhand. The sapota pulp colour matched with the catechu colour, and was well accepted when the pulp was used at 15% level. Colour was evaluated by measuring reflectance values as well as RGB values. Firmness, consistency and index of viscosity of shrikhand were also studied. The CCC was used as a colouring agent in Lassi preparation at various levels and its acceptance was studied. In case of lassi, the addition of CCC imparted optimum brown colour to the final product when used at 1.5% level. It was further observed that CCC-imparted colour matched well with cocoa flavoured lassi which was well accepted when cocoa used at 2% level. There was no influence of CCC on the pH of lassi. The CCC also imparted brown colour to set yoghurt and the optimum level of its usage was found to be 2% (v/v) of yoghurt mix. The artificial sapota flavour used @ 0.2-0.3% in yoghurt also matched with the catechu colour. The RGBL values measured indicated that their proportions changed on addition of CCC and in all the cases, the L value decreased as CCC concentration increased. There was little effect of CCC addition on the rheological and textural parameters measured in terms of viscosity of FM and firmness, consistency and index of viscosity of shrikhand, lassi and set yoghurt. It was concluded that catechu heartwood extract can be used as colouring agent in flavoured milk, lassi, shrikhand and set yoghurt. The suggested matching flavours correspondingly are caramel, cocoa, sapota and sapota. The CCC was also found to have a good stability to processing conditions as well as during storage.

सारांश

बबूल कत्था (खैर) अच्छी तरह से भारतीय उपमहाद्वीप में लोगों द्वारा आमतौर पर पान की तैयारी (पान) में इस्तेमाल कत्था के रूप में बुलाया अपने heartwood निकालने, के लिए जाना जाता है। निकालने भूरे रंग लाल गहरे लाल देता है और इसकी वजह से phytoconstituents के कई औषधीय गुणों के अधिकारी के लिए जाना जाता है। यह भी रंग एजेंट के रूप में चमड़ा और कपड़ा उद्योग में इस्तेमाल किया जा रहा है। यह प्राकृतिक रंग का एक अच्छा स्रोत है और जगह या कृत्रिम रंग पूरक करने के लिए इस्तेमाल किया जा सकता है, हालांकि हालांकि, यह अभी तक रंग एजेंट के रूप में खाद्य उद्योग में करने की कोशिश नहीं की गई है। इस परियोजना में, रंग माध्यम के रूप में पानी का उपयोग गर्मी निकासी विधि द्वारा कत्था heartwood से निकाला गया था। निकालने गर्मी एक कत्था रंग ध्यान (सीसीसी) प्राप्त करने के लिए ध्यान केंद्रित किया गया था। सीसीसी विभिन्न डेयरी उत्पादों में रंग एजेंट के रूप में इस्तेमाल किया गया था और इसकी तीव्रता RGLB मोड के तहत स्कैनर-एडोब फोटोशॉप विधि द्वारा reflectance meter और रंग मापदंडों का उपयोग प्रतिशत reflectance के प्रति मापने के द्वारा मूल्यांकन किया गया था। रियोलॉजिकल और गठनात्मक मापदंडों औस्टवौल्ड viscometer और बनावट विश्लेषक द्वारा मापा गया। सीसीसी दूध के लिए भूरे रंग दिया और 0.8% के स्तर पर इस्तेमाल किया जब अच्छी तरह से स्वीकार कर लिया गया। सीसीसी रंग का सुगंधित दूध भी नसबंदी गर्मी के अधीन था और यह रंग भी 121 डिग्री सेल्सियस / 15min की नसबंदी के तापमान पर अच्छा गर्मी स्थिरता प्रदर्शित कि पाया गया था। प्रसंस्करण के दौरान के रूप में अच्छी तरह से परिवेश के तापमान पर भंडारण के दौरान दूध की चिपचिपाहट पर सीसीसी का कोई प्रभाव नहीं भी आई थी। विज्ञान सम्बन्धी चिपचिपाहट सादे निष्फल एफएम नमूनों की (सीएस) और रंग का निष्फल एफएम क्रमशः, सीएस 2.45 और 2.42 था। सीसीसी के लिए 9 सूत्री Hedonic पैमाने पर रंग और रूप स्कोर सादे निष्फल दूध (7.4) की तुलना में अधिक था (7.8) एफएम गयी। यह सीसीसी द्वारा दिया रंग के साथ अच्छी तरह से मिलान कि कारमेल स्वाद पाया गया था। श्रीखंड में, सीसीसी के अलावा उत्पाद के लिए भूरे रंग दिया और 1.5% के स्तर पर इस्तेमाल किया जब अच्छी तरह से स्वीकार कर लिया गया। यह भी सीसीसी चीकू श्रीखंड का रंग पूरित कि मनाया गया। चीकू लुगदी रंग कत्था रंग के साथ मिलान किया, और लुगदी 15% के स्तर पर इस्तेमाल किया गया था जब अच्छी तरह से स्वीकार कर लिया गया। रंगीन reflectance के मूल्यों के रूप में अच्छी तरह से आरजीबी मूल्यों को मापने के द्वारा मूल्यांकन किया गया था। दृढ़ता, स्थिरता और श्रीखंड का चिपचिपापन के सूचकांक भी अध्ययन किया गया। सीसीसी विभिन्न स्तरों पर लस्सी तैयार करने में एक रंग एजेंट के रूप में इस्तेमाल किया गया था और इसकी स्वीकृति का अध्ययन किया गया था। 1.5% स्तर पर इस्तेमाल किया जब लस्सी के मामले में, सीसीसी के अलावा अंतिम उत्पाद के लिए इष्टतम भूरे रंग दिया। आगे यह भी देखा गया है कि कोको 2% के स्तर पर इस्तेमाल किया जब अच्छी तरह से स्वीकार कर लिया गया है, जो कोको के स्वाद लस्सी के साथ अच्छी तरह से मिलान सीसीसी-प्रदान रंग। लस्सी का पीएच पर सीसीसी का कोई प्रभाव नहीं था। सीसीसी भी दही सेट करने के लिए भूरे रंग दिया जाता है और इसके उपयोग का इष्टतम स्तर दही मिश्रण के 2% (वी / वी) हो पाया था। दही में 0.2-0.3% @ इस्तेमाल किया कृत्रिम चीकू स्वाद भी कत्था रंग के साथ मेल नहीं खाते। मापा RGLB मूल्यों कि वारिस अनुपात सीसीसी के अलावा पर बदल दिया और सीसीसी एकाग्रता में वृद्धि हुई है के रूप में सभी मामलों में, एल मूल्य में कमी आई संकेत दिया। एफएम की चिपचिपाहट और दृढ़ता, स्थिरता और श्रीखंड, लस्सी और सेट दही की चिपचिपाहट के सूचकांक के संदर्भ में मापा रियोलॉजिकल और गठनात्मक मापदंडों पर सीसीसी इसके अतिरिक्त की खास असर नहीं हुई थी। यह कत्था heartwood निकालने के स्वाद का दूध, लस्सी, श्रीखंड और सेट दही में रंग एजेंट के रूप में इस्तेमाल किया जा सकता है कि निष्कर्ष निकाला गया था। सुझाव मिलान जायके तदनुसार कारमेल, कोको, चीकू और चीकू हैं। सीसीसी भी प्रसंस्करण शर्तों के साथ ही भंडारण के दौरान एक अच्छा स्थिरता है पाया गया था।

CONTENTS

| CHAPTER NO. | TITLE | PAGE NO. |
|------------------------|---|---------------------|
| 1.0 | INTRODUCTION | 1-4 |
| 2.0 | REVIEW OF LITERATURE | 5-22 |
| | 2.1 RED WOOD (<i>Caesalpinia sappan</i>) | 5 |
| | 2.1.1 Red dye | 5 |
| | 2.1.2 Uses of redwood pigment | 7 |
| | 2.2 LOGWOOD (<i>Haematoxylon campechianum</i>) | 8 |
| | 2.2.1 Colour extraction | 9 |
| | 2.2.2 Uses | 10 |
| | 2.3 GLUTA APTERA WOOD | 10 |
| | 2.3.1 Colour extraction | 10 |
| | 2.3.2 Uses | 11 |
| | 2.4 ACACIA CATECHU | 12 |
| | 2.4.1 Katha | 13 |
| | 2.4.2 Beneficial attributes of catechu extract | 13 |
| | 2.4.2.1 Antibacterial | 13 |
| | 2.4.2.2 Antimycotic | 15 |
| | 2.4.2.3 Antioxidant | 16 |
| | 2.4.2.4 Other benefits | 17 |
| | 2.4.3 Uses of catechu | 17 |
| | 2.4.4 Toxicological studies | 19 |
| | 2.5 FOOD SAFETY AND STANDARS AUTHORITY | 20 |

| | | |
|------------|---|-------|
| | OF INDIA REGULATIONS | |
| 3.0 | <i>MATERIALS AND METHODS</i> | 23-38 |
| | 3.1 MATERIALS | 23 |
| | 3.1.1 Ingredients | 23 |
| | 3.1.1.1 Catechu (<i>Acacia Catechu</i>) | 23 |
| | 3.1.1.2 Milk and culture | 23 |
| | 3.1.1.3 Sugar | 23 |
| | 3.1.1.4 Caramel sugar | 24 |
| | 3.1.1.5 Sapota fruit | 24 |
| | 3.1.1.6 Cocoa powder | 24 |
| | 3.1.1.7 Chikku flavour | 24 |
| | 3.1.2 Packaging materials | 24 |
| | 3.1.3.4. BHI Broth | 24 |
| | 3.1.4 Glassware and equipment | 24 |
| | 3.1.4.1 Hot air oven | 25 |
| | 3.1.4.2 Analytical weighing balance | 25 |
| | 3.1.4.3 Induction heater | 25 |
| | 3.1.4.4 Incubators | 25 |
| | 3.1.4.5 Spectrophotometer | 25 |
| | 3.1.4.6 Colour measuring instruments | 25 |
| | 3.1.4.7 Planetary mixer | 25 |
| | 3.1.4.8 Sealing machine | 25 |
| | 3.1.4.9 pH meter | 26 |
| | 3.1.4.10 Texture analyzer | 26 |
| | 3.1.4.11 Ostwald viscometer | 26 |
| | 3.1.4.12 Autoclave | 26 |
| | 3.2. METHODOLOGY | 26 |

| | | |
|--|---|----------|
| | 3.2.1 Effect of type of extraction on catechu colour | 26 |
| | 3.2.2 Effect of various additives on catechu colour | 26 |
| | 3.2.3 Stability of extracted catechu colour under various conditions | 27 |
| | 3.2.4 Effect of heat treatment on catechu colour | 27 |
| | 3.2.5 Effect of different processing treatments on catechu colour | 27 |
| | 3.2.6 Antimicrobial property of catechu colour concentrate | 27 27 |
| | 3.2.7 Preparation catechu colour concentrate | 27 |
| | 3.2.8 Utilization of CCC as colouring agent in flavoured milk preparation | 28 |
| | 3.2.9 Utilization of CCC as colouring agent in shrikhand preparation | 29 |
| | 3.2.10 Utilization of CCC as colouring agent in plain lassi | 30 |
| | 3.2.11 Utilization of CCC as colouring agent in yoghurt preparation | 30 |
| | 3.2.12 Stability of CCC colour in products during storage | 31 |
| | 3.4 METHODS OF ANALYSIS | 32 |
| | 3.4.1 Colour measurement | 32 |
| | 3.4.1.2 Scanner-Adobe Photoshop (SAP) method | 33 |
| | 3.4.2 Sensory evaluation | 33 |
| | 3.4.3 Rheological analysis | 35 |
| | 3.4.3.1 Yoghurt and shrikhand | 35 |
| | 3.4.3.2 Flavoured milk | 35 |

| | | |
|------------|--|-------|
| | 3.4.3.3 Lassi | 37 |
| | 3.4.4 pH | 37 |
| | 3.4.5 Antimicrobial activity of catechu extract | 37 |
| | 3.4.6 Statistical analysis | 37 |
| 4.0 | <i>RESULTS AND DISCUSSION</i> | 39-76 |
| | 4.1 STUDIES ON EXTRACTION OF CATECHU HEARTWOOD COLOUR AND ITS PROPERTIES | 39 |
| | 4.1.1 Effect of type of extraction on catechu colour | 39 |
| | 4.1.2 Effect of various additives on catechu colour | 39 |
| | 4.1.2.1 Effect of betel leaf extract and calcium hydroxide on catechu colour | 39 |
| | 4.1.2.2 Effect of calcium chloride on catechu colour | 40 |
| | 4.1.2.3 Effect of acidification on catechu colour | 40 |
| | 4.1.2.4 Effect of alkalisation on catechu colour | 40 |
| | 4.1.2.5 Effect of turmeric on catechu colour | 40 |
| | 4.1.2.6 Effect of turmeric and calcium hydroxide on catechu colour | 41 |
| | 4.1.3 Stability of extracted catechu colour under various conditions | 42 |
| | 4.1.3.1 At 37°C storage | 42 |
| | 4.1.3.2 Exposure to atmospheric air | 42 |
| | 4.1.3.3 Light | 42 |
| | 4.1.4 Effect of heat treatment on catechu colour | 43 |
| | 4.1.5 Effect of different processing treatments on catechu colour | 43 |

| | | |
|--|--|----|
| | 4.1.6 Antimicrobial property of catechu colour concentrate | 45 |
| | 4.2 METHOD OF PREPARATION OF CATCHU COLOUR CONCENTRATE | 47 |
| | 4.3 UTILIZATION OF CATECHU COLOUR CONCENTRATE (CCC) IN SELECTED DAIRY PRODUCTS | 47 |
| | 4.3.1 Optimization of CCC in selected dairy products | 47 |
| | 4.3.1.1 Effect of CCC on sensory quality of flavoured milk | 47 |
| | 4.3.1.2 Effect of CCC level on instrumental colour parameters of flavoured milk | 49 |
| | 4.3.1.3 Effect of CCC and heat treatment on instrumental colour parameters of flavoured milk | 50 |
| | 4.3.1.4 Effect of CCC on viscosity of flavoured milk | 52 |
| | 4.3.1.5 Effect of storage on colour stability of CCC added sterilised flavoured milk | 52 |
| | 4.3.1.6 Effect of CCC on sensory scores of shrikhand | 53 |
| | 4.3.1.7 Effect of CCC addition on instrumental colour parameters of shrikhand | 55 |
| | 4.3.1.8 Effect of CCC on textural properties of shrikhand | 55 |

| | | |
|--|---|----|
| | 4.3.1.9 Effect of CCC on sensory scores of sapota pulp added shrikhand | 56 |
| | 4.3.1.10 Effect of CCC on instrumental textural properties of sapota pulp added shrikhand | 57 |
| | 4.3.1.11 Effect of CCC and sapota on instrumental colour parameters of shrikhand | 57 |
| | 4.3.1.12 Effect of CCC on sensory scores of lassi | 59 |
| | 4.3.1.13 Effect of CCC on instrumental colour parameters of lassi | 59 |
| | 4.3.1.14 Effect of CCC on pH of lassi | 59 |
| | 4.3.1.15 Effect of CCC on viscosity measure of lassi | 60 |
| | 4.3.1.16 Effect of CCC on sensory scores of cocoa lassi | 61 |
| | 4.3.1.17 Effect of CCC on instrumental colour parameters of cocoa lassi | 61 |
| | 4.3.1.18 Effect of CCC on sensory quality of cocoa lassi | 62 |
| | 4.3.1.19 Effect of CCC on sensory scores of yoghurt | 64 |
| | 4.3.1.20 Effect of CCC on instrumental textural properties of yoghurt | 65 |
| | 4.3.2 Stability of CCC colour in products during storage | 66 |
| | 4.3.2.1 Effect of storage on instrumental colour parameters of CCC coloured cocoa lassi | 66 |
| | 4.3.2.2 Effect of storage on sensory scores of CCC coloured cocoa lassi | 67 |

| | | |
|--|--|-------|
| | 4.3.2.3 Effect of storage on instrumental colour parameters of CCC coloured sapota shrikhand | 68 |
| | 4.3.2.4 Effect of storage on sensory scores of CCC coloured sapota shrikhand | 70 |
| | 4.3.2.5 Effect of storage on instrumental colour parameters of CCC coloured yoghurt | 70 |
| | 4.3.2.6 Effect of storage on sensory scores of CCC coloured yoghurt | 71 |
| | 4.3.2.7 Effect of storage on instrumental colour parameters of sterilised flavoured milk | 73 |
| | 4.3.2.8 Effect of storage on sensory scores of CCC coloured flavoured milk | 74 |
| | <i>SUMMARY AND CONCLUSION</i> | 77-80 |
| | <i>BIBLIOGRAPHY</i> | |
| | <i>APPENDIX</i> | |

LIST OF TABLES

| TABLE NO. | TITLE | PAGE NO. |
|----------------------|---|---------------------|
| 2.1 | Table-2.1: FSSAI permitted colour | 20 |
| 4.1 | Effect of storage at 37°C on colour of catechu extract | 42 |
| 4.2 | Effect of exposure to air on the colour of catechu extract (a) Exposed to atmospheric air (b) Air tight closure | 42 43 |
| 4.3 | Effect of exposure to light on the colour of catechu extract (a) Presence of light (b) Absence of light | 44 45 |
| 4.4 | Instrumental colour parameters of catechu extract as influenced by various heat treatments | 45 |
| 4.5 | Table-5: Effect of various processing treatments on catechu colour | 46 |
| 4.6 | Table-6: Antimicrobial activity of aqueous catechu extract *on <i>E. coli</i> | 46 |
| 4.7 | Table- 7: Effect of CCC level on sensory acceptance score of flavoured milk | 49 |
| 4.8 | Effect of CCC level on instrumental colour parameters of flavoured milk | 50 |
| 4.9 | Effect of CCC level on sensory scores of plain and sterilized flavoured milks | 51 |
| 4.10 | Effect of CCC and heat treatment on instrumental colour parameters of flavoured milk | 51 |
| 4.11 | Effect of CCC on viscosity of flavoured milk | 52 |

| | | |
|------|--|----|
| 4.12 | Effect of storage on kinematic viscosity of plain and sterilized flavoured milk | 54 |
| 4.13 | Effect of CCC on sensory scores of shrikhand | 54 |
| 4.14 | Effect of CCC addition on instrumental colour parameters of shrikhand | 55 |
| 4.15 | Effect of CCC on textural properties of shrikhand | 56 |
| 4.16 | Effect of CCC on sensory scores of sapota pulp added shrikhand | 57 |
| 4.17 | Effect of CCC on instrumental textural properties of sapota pulp added shrikhand | 58 |
| 4.18 | Effect of CCC and sapota on instrumental colour parameters of shrikhand | 58 |
| 4.19 | Effect of CCC on sensory scores of lassi | 60 |
| 4.20 | Effect of CCC on instrumental colour parameters of lassi | 60 |
| 4.21 | Effect of CCC level on pH of lassi | 61 |
| 4.22 | Effect of CCC level on viscosity measure of lassi | 61 |
| 4.23 | Effect of CCC on sensory scores of cocoa lassi | 62 |
| 4.24 | Effect of CCC on instrumental colour parameters of cocoa lassi | 63 |
| 4.25 | Effect of CCC on sensory quality of cocoa lassi | 63 |
| 4.26 | Effect of cocoa on instrumental colour parameters of cocoa lassi | 64 |
| 4.27 | Effect of CCC on pH of yoghurt | 64 |
| 4.28 | Effect of CCC on sensory scores of yoghurt | 65 |
| 4.29 | Effect of CCC on instrumental textural properties of yoghurt | 66 |
| 4.30 | Effect of storage on instrumental colour parameters of CCC | 67 |

| | | |
|------|---|----|
| | coloured cocoa lassi | |
| 4.31 | Effect of storage on instrumental colour parameters of CCC coloured sapota shrikhand | 69 |
| 4.32 | Effect of storage on instrumental colour parameters of CCC coloured yoghurt | 72 |
| 4.33 | Effect of storage on instrumental colour parameters of sterilized flavoured milk | 74 |

LIST OF FIGURES

| FIGURE NO. | TITLE | PAGE NO. |
|-----------------------|---|---------------------|
| 1.1 | Representation of plant parts used in preparation of natural dyes | 2 |
| 2.1 | Redwood extract, cross section of redwood and haematoxylin extracted from redwood | 6 |
| 2.2 | The structure of natural dyestuff components: brazilin (A) and brazilin (B) | 6 |
| 2.3 | Fig.2.3.Sappan wood extraction | 7 |
| 2.4 | Logwood powder and cross section of logwood | 8 |
| 2.5 | The complex phenolic structure of the dyes haematoxylin and brazilin | 9 |
| 2.6 | <i>Gluta aptera</i> wood | 11 |
| 2.7 | Molecular structure of the flavones | 11 |
| 2.8 | <i>Acacia catechu</i> | 13 |
| 2.9 | Cross section of catechu plant stem | 13 |
| 2.10 | Catechu heartwood extract | 14 |
| 2.11 | Catechu powder | 14 |
| 3.1 | <i>Acacia catechu</i> heartwood pieces | 23 |
| 3.2 | Flow chart for the preparation of various types of flavoured milks | 28 |
| 3.3 | Flow chart for the preparation of shrikhand | 29 |
| 3.4 | Flow chart for the preparation of lassi | 30 |

| | | |
|-----|---|----|
| 3.5 | Flow chart for the preparation of yoghurt | 31 |
| 3.6 | Reflectance meter | 32 |
| 3.7 | Measurement of colour parameters by SAP method (a) JPEG format of scanned image in Adobe-Photoshop window (b) Measurement of colour parameters under RGB mode | 34 |
| 3.8 | Typical force – time curve of yoghurt and shrikhand | 36 |
| 3.9 | Inhibition zones of CCC obtained by agar diffusion technique | 38 |
| 4.1 | Effect of various additives on catechu colour | 41 |
| 4.2 | Colour of catechu extract as affected by various heat treatments | 44 |
| 4.3 | Instrumental colour parameters of catechu extract as influenced by various heat treatments | 44 |
| 4.4 | Flow chart for method of preparation of catechu colour concentrate (CCC) | 48 |
| 4.5 | Effect of storage on reflectance of plain and sterilised flavoured milks | 53 |
| 4.6 | Effect of storage on sensory scores of CCC coloured cocoa lassi | 68 |
| 4.7 | Effect of storage on sensory scores of CCC coloured sapota shrikhand | 71 |
| 4.8 | Effect of storage on sensory scores of CCC coloured yoghurt | 73 |
| 4.9 | Effect of storage on sensory scores of CCC coloured flavoured milk | 75 |

LIST OF ABBREVIATIONS AND SYMBOLS

| | | |
|-------|---|-------------------------|
| % | : | Per cent |
| µl | : | microliter |
| °C | : | Degree Celsius |
| ANOVA | : | Analysis of Variance |
| B | : | Blue |
| B&T | : | Body and Texture |
| C&A | : | Colour and Appearance |
| Conc. | : | Concentrated |
| cS | : | centi Stokes |
| df | : | Degrees of freedom |
| DH | : | Degree of Hydrolysis |
| Fl | : | Flavour |
| G | : | Green |
| g | : | Gram |
| hr | : | hours |
| kg | : | Kilogram |
| L | : | Luminosity |
| mg | : | Milligram |
| min | : | Minutes |
| ml | : | Milliliters |
| mm | : | Millimeter |
| N | : | Newton |
| O&A | : | Overall Acceptability |
| R | : | Red |
| RT | : | Room Temperature |
| SAP | : | Scanner Adobe Photoshop |
| S-M | : | Sample moisture |
| SNF | : | Solids not fat |
| SPC | : | Standard plate count |
| W/V | : | weight/volume |

Chapter- 1

Introduction

1.0 INTRODUCTION

Colour is the first sensory quality by which foods are judged; food quality and flavours are closely associated with colour. Colour powerfully influences the consumer's ability to identify the flavours and quality. The colours of foods are result of natural pigments or of added colours. Colours are used to preserve product identity, ensure colour uniformity of food products that naturally vary in colour, intensify the colours of certain manufactured foods, protect flavours and light sensitive vitamins during storage by a sunscreen effect, serve as a visual indication of quality, and give colour to certain foods such as sugar confectionery, soft drinks, sauces, ice lollies and soft drinks, which would otherwise be virtually colorless.

Natural colours:

Although the use of natural colours in food is an ancient practice, it is currently gaining increasing importance because consumers are becoming aware of the hazards of using synthetic colours. Natural colourants are those that are extracted from vegetables, fruits, minerals and spices used to colour foods e.g. carotenoids from annatto, paprika, saffron, anthocyanin's, caramel, chlorophyll and turmeric. Carotenoids are used the most followed by the red pigment and brown coloured caramels.

Various plant parts viz., leaves, fruits, seeds, bark, stem etc., (Fig.1) are traditionally used for extracting dyes by utilizing traditional extraction techniques. The contribution of different parts of the plant in percentages used for the preparation of natural colours is represented in Fig. 1.1.

Natural colours are also extracted from microorganisms which are sometimes called bio colours. Plant pigments, by virtue of their natural occurrence in edible plants, are generally considered to be harmless. Nature produces a variety of brilliant coloured pigments viz., water soluble anthocyanins, betanins and fat soluble pigments carotenoids and chlorophylls used for colouring foods (Madhava Naidu and Sowbhagya, 2012).

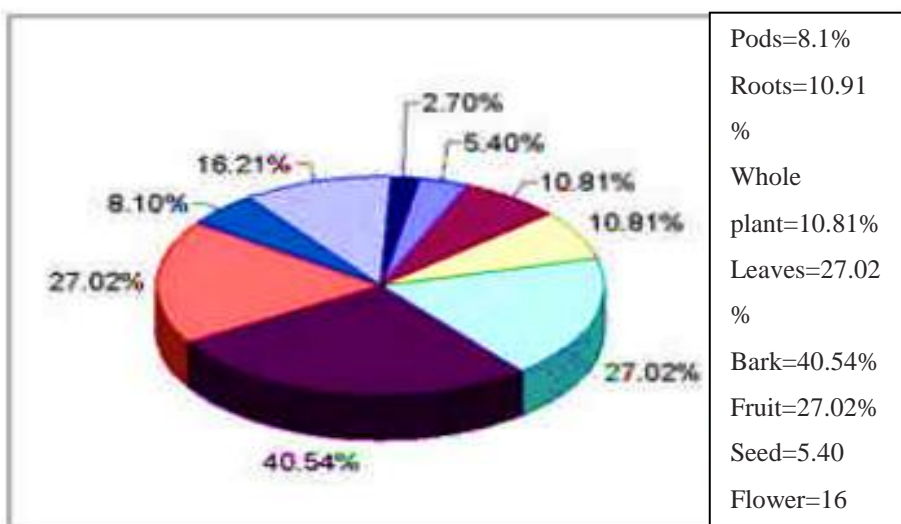


Fig.1.1 : Representation of plant parts used in preparation of natural dyes
(Source: Mahanta and Tiwari, 2005)

Nature identical synthetic colours:

These are synthesized in the laboratories and a very limited range is available. Nature-identical colours are man-made pigments which are identical to the ones found in nature viz., carotene, canthaxanthin and riboflavin.

Artificial / synthetic colours:

Synthetic colours are synthesized and not found in nature, whereas natural colors are obtained from plant and also animal sources. These are two types FD and C dyes and FD and C lakes. Dyes are water-soluble compounds that produce colour in solution. Lakes are made by combining dyes with alumina to form insoluble colourants. Coal tar is available in wide range of colours. Indigo carmine is an example of synthetic colour.

Inorganic colours: FSSAI prohibits use of inorganic colour except titanium dioxide, which is permitted in chewing gum (max limit 1.0 %).

The side effects of artificial food colouring

According to the consumer advocacy group Centre for Science in the Public Interest, artificial colours may cause damage to DNA, bladder tumours and other forms of cancer, but no human trials have found links between cancer and the dyes in humans. Artificial food colors are linked to several behavioral and health problems, brain tumors and attention deficit hyperactivity disorder or ADHD. Chemicals used as

ingredients in making these artificial food colors--such as tar derivatives, petrochemicals and hydrocarbons--are said to be the cause of the problems. Artificial food colors have no nutritional or health benefits. They are simply used to make the food look appetizing. Symptoms of food allergies include cramping stomach pain, diarrhea, shortness of breath, chest pain and swelling of the airways.

Natural dyes

Plants produce plethora of colours with amazing shades, many of which are unmatched by even the well-developed colour industry developed by man. In fact the entire spectrum of colours human has evolved systematically is based on the observations of naturally existing colours in plant kingdom. It is not yet clear why plants produce such wonderful shades of colour, reasons may be many like attracting insects aiding pollination, protection from enemies etc., yet evolutionary reasons may be many. No doubt, humans have immensely benefitted by the colour or pigment production by the plants. Since their development is part of evolution chain, many of them are compatible with other living beings. The discovery of the first synthetic colorant by William Perkin in 1856 ushered in a new era of dyes. Till then all the colour used was of natural origin. Synthetic dyes caught the attention of people so much that it led to belief that work on natural dyes was a waste of time and energy. However, today the importance of natural colours again is being realised because of their health beneficial aspects.

Natural dyes are considered as sustainable and eco-friendly dyes. They can produce different shades of colours, but have lower colour fastness than synthetic dyes. The products of natural dyes are very much in demand due to their ability to produce different shades and eco-friendly nature.

Wood based colours

Woody plants can synthesize and accumulate in their cells a great variety of phytochemicals including alkaloids, flavonoids, tannins, cyanogenic glycosides, phenolic compounds, saponins and lignin's. Colour producing compounds in woody parts are of three classes: Porphyrin, Carotenoids and Flavonoids. These are embedded within the vascular tissues or within the cell walls of the stem tissues. When the wood is cut open, these pigments are visible or ooze out. There is

wonderful range of colours produced in wood portion of plants. The extraction of most of the wood portion colours has not been studied, so there remains a large scope for this study. Textile industry and tanning industry are the major beneficiaries of the wood portion colours; however food industry has yet to try out such colours. Of course their effect on human health, allergies and any other undesirable effects need to be studied thoroughly before recommending for human consumption.

Catechu

Catechu is a plant belonging to Mimosaceae family whose heartwood finds several applications in industries. It yields a red to dark brown dye in water which is used in textile and leather industries. The heartwood of the plant reportedly has several phytochemicals including catechins and tannins, which are good antioxidants. Catechu extract has not yet been tried in any food products, but the dried catechu heartwood extract is popularly used in paan not only for developing red colour in mouth but also for good digestibility attribute of paan. A few natural colours of seed, vegetable and fruit origin are being used in dairy products, for example annatto seeds, turmeric, mango, berries etc., but catechu has not been tried so far in dairy products.

In this project, catechu heartwood has been tried in some selected dairy products. The objectives of the study were:

- 1) To optimize conditions for extraction of colour from catechu
- 2) To utilize the extracted catechu colour in selected dairy products and
- 3) To study the stability of extracted colour in selected dairy products.

Chapter- 2

Review of Literature

2.0 REVIEW OF LITERATURE

Natural colours are derived from various parts of plants. The present project was taken up to extract dye from heartwood of Catechu plant, which is a woody plant, and utilise it as colouring agent in some selected dairy products. Woody portion of other plants also yield colours useful in several industries. Some of these are briefly reviewed.

2.1 RED WOOD (*Caesalpinia sappan*)

The common names are: (English) Indian brazilwood, Indian redwood or Sappan wood; (Hindi) Sappan; (Malayalam) Pathimukam. Redwood tree is a medium sized, shrubby tree. In healthy soil it can grow up to 10 m tall. The main trunk wood can become up to 14 cm in diameter. It is a thorny plant with stipulated leaves about 25 cm long and 10 cm broad.

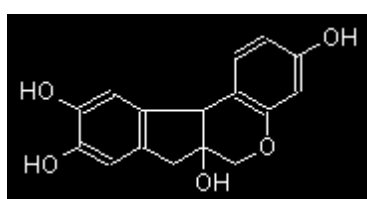
Redwood tree is mostly found in the regions of central and southern India, especially in Kerala. Agro-Climatic conditions for growing Redwood trees are hilly regions, rocky and stony besides warm climate. Watery and much wet soil condition is not suitable for plantation of this. Suitable annual temperature is 24 to 28 degree Celsius. Redwood tree is cultivated in China, India, Malaysia, Myanmar and Sri Lanka. So Redwood Tree is cultivated and used much in Kerala because of its medicinal value. It takes 7 to 10 years for the harvest of the redwood. The sapwood is thin and the heartwood is hard and heavy. The heartwood yields valuable crystalline dye for colouring cotton, silk and wool fabrics. It is also used for making health drinks. The tree is uprooted and all the heartwood is collected. The tree can also be cut about two feet above the ground level. There is every chance of shoots or sprouts growing from it.

2.1.1 Red dye

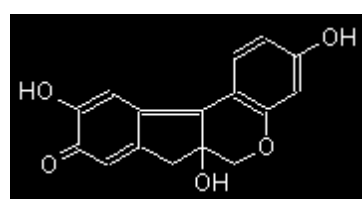
The important part of this plant is the heartwood (Fig. 2.1) that contains water-soluble dyes (Fig.2.1) such as brazilian, protosappanins, sappanchalcone and haematoxylin (Fig.2.1).



Fig.2.1: Redwood extract, cross section of redwood and haematoxylin extracted from redwood



(A)



(B)

Fig.2.2. The structure of natural dyestuff components: brazilin (A) and brazilin (B)
(Kim *et al.*, 1997).

Brazilian (Fig.2.2 A & B) on oxidation yields a red dye called brazilin (deep red) and brazilin (intense red) *Extraction of Red wood dye:* Without extraction, the dried heartwood of *C. sappan* as such has been used as a red dye stuff for a long time. However, the wood is normally extracted in water because many of these compounds are water soluble. In one of the methods of extraction, 20 g of sappan wood was washed with distilled water for 30 min and dried. Ten grams of dried sappan wood was poured in a flask, and the first extraction was obtained by heating with 500 ml of distilled water for 60 min at 80°C. The second extraction was obtained by heating the remaining sappan wood after mixing the first extraction and an additional 300 ml of distilled water. The third extraction was obtained by heating with an additional 100 ml of water for 60 min. A mixture of the first, second and third exactions was used as the dye for emulsification (Fig.2.3) (Yong *et al.*, 1999). In another method, the aqueous solution was prepared by *C. sappan* of heartwood (38g) with water (1000ml) repeatedly for 48h. They found that this method gives good intense of colour (Srinivasan *et al.*, 2012).

The wood can also be extracted in ethanol which has different extraction property than water. The dried heartwood was subjected to an extraction with 50% ethanol (1:3 v/v) at 80°C for 30 min. Brazilein with a molecular weight of 284 was the major dyestuff component of the ethanol extract of brazilwood. They found that it can be utilized for the production of brazilein standard for the laboratory use to analyse brazilein in foods and beverages (Lioe *et al.*, 2012). In another study, the wood was cut into small pieces and dried at room temperature (37°C). The air dried sample was ground into fine powder and then directly extracted by Soxhlet method by using Ethanol/petroleum ether (500ml) and then the extract was concentrated by using reduced pressure to yield a light brown coloured mass. It was found that ethanol or methanol dye extract of brazilwood after crystallization had a reddish brown colour (Kim *et al.*, 1997).

2.1.2 Uses of redwood pigment

About 10 g of redwood is put into one litre of boiling water. The water becomes reddish. If the water is not fully reddish, we can add more redwood. We can also add

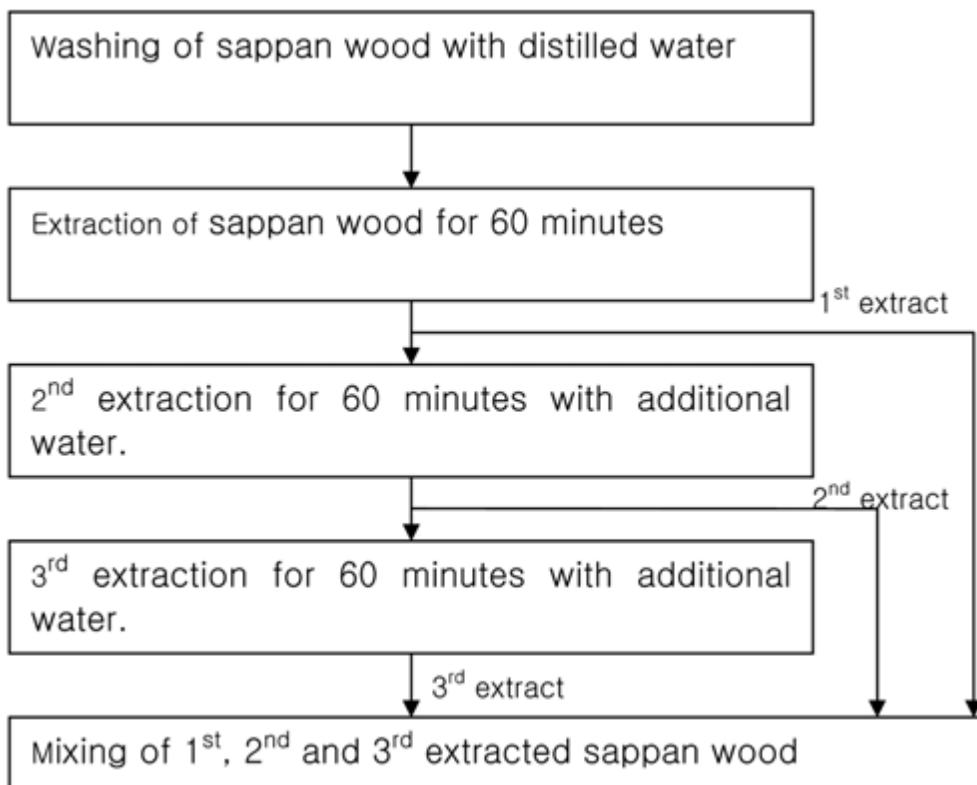


Fig.2.3.Sappan wood extraction

jeera, cardamom and such spices for additional flavours. We can drink this instead of ordinary plain water.

The most important part of the tree is the heartwood, which is used for making woodcrafts and for extracting dyes, used as a colouring agent for mats, woollen and cotton fabrics. The bark and pods of the tree contain a red dye, which is used for colouring cakes and alcoholic drinks. In addition, the wood contains several medicinal properties and is used for treating dysentery, diarrhoea and skin diseases in alternative systems of medicine. Lioe *et al.* (2012) used brazilwood colour traditionally for food and beverage. Water boiled with Redwood is liberally served during meals in most of the rice hotels in South India and more especially in Kerala. Sreenivas KN *et al.* (2015) used pathimugam (*Caesalpinia sappan Linn.*) as natural colouring agent in Sandesh

2.2 LOGWOOD (Campeachy wood) (*Haematoxylon campechianum*)

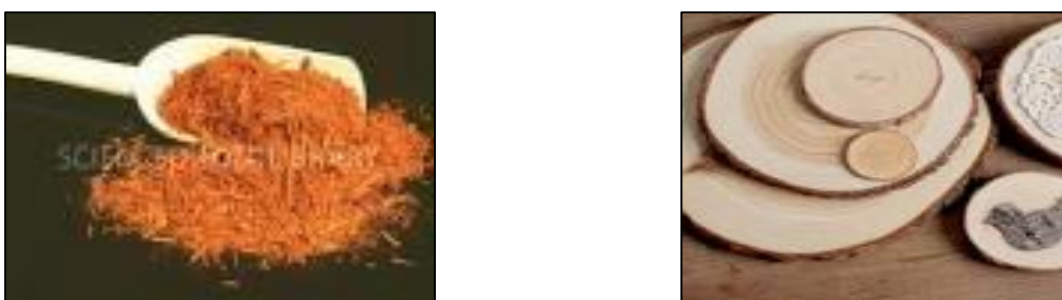


Fig.2.4: Logwood powder and cross section of logwood

The common names are: (English) Campeachy wood; The tree's scientific name means "bloodwood" (*haima* being Greek for blood and *xylon* for wood). Logwood is a large sinuous tree that grows naturally in tropical regions of South and Central America to the height of 30-45 feet. The logwood powder and cross section of logwood are shown in Fig. 2.4.

The dyestuff originates in the yellow heartwood which contains the substance *haematoxylin* (C₁₀H₁₄O₆). When exposed to air, *haematoxylin* is oxidized, and the purple compound *haematine* (or *haematein*) is formed. The plant bears relation to soluble, dye-producing redwoods of the same family, including Peachwood, Sappanwood, and Pernambuco. The latter are used in combination to produce brazil wood dye, a red-purple dye that contains *brazilein* as the principle colouring matter.

Logwood may contain amounts of *brazilein* (Carlo, *et al.*, 2002), as the redwoods listed above contain some of its primary colouring matter (Cannon and Cannon 1994). The two compounds have been proven to be quite similar in constitution (Mayer and Cook 1943).

The actual dye from logwood is hematoxylin, a complex phenolic compound similar to the flavonoid pigments of flowers. The chemical structure of hematoxylin is practically identical with the dye brazilin from brazilwood, except that hematoxylin has one additional atom of oxygen. Hematoxylin is extracted by boiling chips or raspings of logwood in water. By exposure to the air the orange-red crystals of hematoxylin are gradually oxidized to metallic green crystals of another popular dye called hematein. The presence of a considerable amount of tannin in the purplish-red dye bath allows the logwood extract to react with iron salts to give a permanent black color (Fig. 2.5). The complex phenolic structure of the dyes hematoxylin and brazilin are similar to bioflavonoids, such as the pigments of flowers. They are classified as phenolic compounds because of the hydroxyl (OH) groups attached to the benzene rings. Hematoxylin is practically identical with brazilin, except that hematoxylin has one additional atom of oxygen.

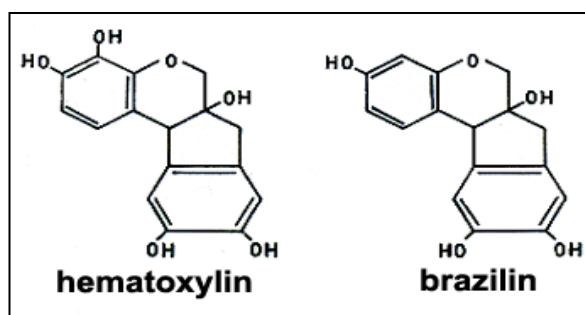


Fig. 2.5: The complex phenolic structure of the dyes haematoxylin and brazilin

2.2.1 Colour extraction

According to (Ainsworth and Hepworth 1916) there were three methods employed in the production of commercial logwood extracts. (1) The finely divided chips are frequently digested with hot water under a pressure of 1 to 2 atmospheres, this process yielding a large extract, which, however, contains a considerable proportion of resins, fats, and other impurities; (2) the French method of boiling chips with water at the ordinary pressure, which yields a smaller though purer extract; and (3) a

diffusion process, in which an apparatus similar to that used in the sugar industry is employed. The yield by this process is smaller than in (1) or (2), but the shades of colour are finer. (Mitchell and Hepworth 1916).

2.2.2 Uses

Logwood was used for a long time as a natural source of dye, and still remains an important source of haematoxylin, which is used in histology for staining. The bark and leaves are also used in various medical applications. In its time, logwood was considered a versatile dye, and was widely used on textiles but also for paper. The dye's colour depends on the mordant used as well as the pH. It is reddish in acidic environments but bluish in alkaline ones. Logwood dyes have been used extensively for cotton and woollen goods, leather, furs, silk and inks. Both haematoxylin and haematin are still commonly used for bacteriological and histological stains. Logwood and brazilwood dyes are also popular stains for fine wood finishing.

2.3 *GLUTA APTERA* WOOD (Fig. 2.6)

Gluta aptera wood (Rengas), obtained from Wood Engineering Laboratory at Forest Research Institute of Malaysia (FRIM), was tried and used as natural dye source due to the colour of the wood which is blood-red (Fig. 2.7).

2.3.1 Colour extraction

The boiling and solvent extraction methods were used to obtain the dyes. Two types of extraction methods were used in the study. In the boiling method, a liquor ratio of 1:20 (weight of materials in gram: amount of water in mL) was used to boil crushed wood in distilled water for 60 min. The mixture was then cooled down before being strained and the extract was used to dye fabrics. In the solvent extraction method, a liquor ratio of 1:20 (weight of materials in gram: amount of methanol in ml) was used to soak crushed wood in methanol and 0.1% HCl was added for acidified methanol. The mixtures were soaked 48 h at room temperature and placed inside dark room. The mixtures were then filtered to obtain the extracts. The extract was evaporated using rotary evaporator to produce crude (dyes in form of paste). The crudes from both methanol and acidified methanol were used to dye the fabrics. In order to convert into powder, the crude was dried inside desiccators for 24 h. The dried crude

was mixed with beta-cyclodextrin with a ratio of 1:1 and ground manually using mortar and pestle to form powder.



Fig. 2.6: *Gluta aptera* wood

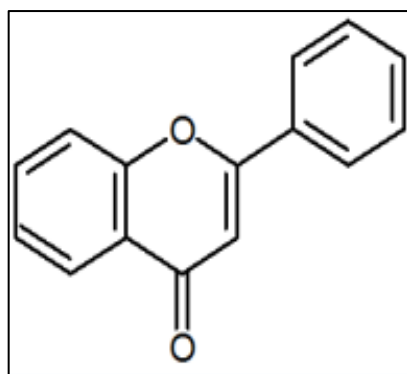


Fig. 2.7: Molecular structure of the flavones (2-phenyl-1,4-benzopyrone)

2.3.2 Uses

Natural dyes are seen as eco-friendly dyestuff because they are derived from natural sources (Ali *et al.*, 2009; Samanta *et al.*, 2010; Montazer and Parvinzadeh 2007). The dyes also have better biodegradability in comparison with synthetic dyes (Kulkarni *et al.*, 2011). Natural dyes can offer not only a rich and variation of source of dyestuff but also have a far superior aesthetic quality, which is more pleasing to the eye due to unique natural colour (Vankar, 2008). Natural dyes can be obtained from vegetations (extracted from root, leaf bark, trunk, fruit and flower of plants), animals (derived from cochineal and shellfish), and minerals (derived from soils, clay sources) (Manhita *et al.*, 2011; Bechtold and Mussak, 2009; Senthilkannan, 2010). The most common colours of natural dyed products on fabric are yellow and brown.

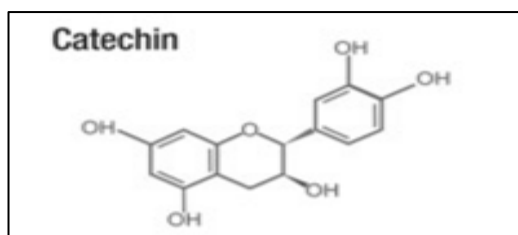
Natural dyes are normally extracted using boiling method. However, colours from plants can be extracted using chemical which is an organic solvent. The colour compound in plants may vary in polarities depending on their chemical structure. Therefore, they need different solvents for extraction (Shrivastava and Dedhia, 2006). According to Wounter and Verhecken (1989), the most common method for natural dyes extraction are involved the use of hydrochloric acid (HCl) and methanol (MeOH). The application of waste materials as sources of natural dyes may cut down cost of natural dyeing as well as can preserve the environment. *Gluta aptera* (Rengas) is one of the famous hard-medium woods which can be found in Sumatra, Peninsular

Malaysia and Borneo. It's a medium to large tree in size which the wood is blood-red in colour, streaky and moderately durable if exposed to the weather (Wan Yunus *et al.*, 2011). In general, the woods are used to make structural components, roofing, staircase, flooring, walling as well as door and window frames.

2.4 ACACIA CATECHU

Acacia catechu (Fig. 2.8) belongs to Mimosaceae family and is commonly known as Khair or Cutch tree. The cross section of stem of the tree is shown in Fig. 2.9. It is also referred to as Khadira, Raktasaara , Dantdhaavan, Kantaki, Baalpatra, Gayatri (Sushruta) and somavalka (charaka). The white variety is known as Shweta Saara, Kaarmuka, kubja kantaka in Ayurveda, Kattaha, khair and kaat (Arabic, persian) in Unani. The *Acacia catechu* is a shrub or tree, growing in the mountainous districts of Bengal, coromandel, and other parts of Hindustan. The bark is dark brown; the prickles are stipular and acute. It occurs throughout India in dry types of mixed forests. Common from Punjab to Assam to an altitude of 1200m. Common in peninsular region. It is a moderate sized tree 20 m in height, spiny with thorny branches and rough bark. The tree grows extensively in Uttar Pradesh, Himachal Pradesh, Madhya Pradesh, Gujarat, Bihar, Maharashtra and to a lesser extent in the southern states.

The heartwood of the tree is a potential source of *katha* and cutch . The average yield of *katha* is 3.0-4.5 % of dry weight of heartwood and the average yield of 'cutch' is about 6-8 %. *Katha* predominately consists of a mixture of catechin and its isomers in 55 % (in good quality *katha*) whereas 'cutch' contains 25-35 % of catechu tannic acid, 2-10 % ocetechin and small proportions of catechu red, quercetin and gum (Patel, 2011). Deshpande and Patil (1981) reported the flavonoids like kaempferol, dihydrokaempferol, taxifolin, isorhamnetin, (+)-afzelchin, and a dimeric procyanidin AC along with quercetin and (-)-epicatechin in heartwood of *Acacia catechu* Willd. The colour compound of catechu is identified as catechin, whose chemical structure is given below:



2.4.1 Katha

Katha preparation involves cutting the inner wood into pieces and placing them in containers. They are then boiled along with some water. The hot water is strained into separate containers and evaporated to a thick consistency which is then poured in quadrangular moulds for setting. Fig. 2.10 shows catechu heartwood extract.

Processing involves three stages: production of a crude extract "black catechu"; isolation of "katha" and solidification of the residue, "cutch". Fig. 2.11 shows katha powder.



Fig 2.8: *Acacia catechu*



Fig. 2.9: Cross section of catechu plant stem

2.4.2 Beneficial attributes of catechu extract

2.4.2.1 Antibacterial

Katha i.e. catechu extract is attributed with several beneficial properties. Catechin has significant antioxidant and antimicrobial effects. As analysed by disc diffusion technique, the ethanolic extracts also exhibited significant inhibitory activity against bacteria like *Staphylococcus aureus*, *Bacillus subtilis*, *E.coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* (Lakshmi *et al.*, 2011b). Similar study was conducted to evaluate the potency of *Acacia catechu* heartwood extract against dental caries causing microbes and organism associated with endodontic infections like *Streptococcus mutans*, *Streptococcus salivarius*, *Lactobacillus acidophilus* and



Fig. 2.10: Catechu heartwood extract



Fig. 2.11: Catechu powder

Enterococcus faecalis using disc diffusion method (Geetha *et al.*, 2011) *Streptococcus mutans* and *Lactobacillus acidophilus* are potent initiator for dental caries that results in destruction of mineralised tissues in the teeth . The study showed that *Acacia catechu* heartwood extract is highly active on oral pathogens and can be applied in dental practice in the field of periodontal diseases like dental caries, gingivitis, mouth sores and in endodontal treatment as *Enterococcus faecalis* is found to be the root cause of failure in root canal treatment (Isabelle *et al.*, 2003). The antimicrobial property was also reported by Khan *et al.* (2011) and Tawde *et al.* (2012). Due to its antimicrobial effect, the extract has been considered as valuable ingredient for dental care preparation. Taxifolin in catechu is a potent antibacterial agent (Lakshmi *et al.*, 2011a).

The Antimicrobial screening and phytochemical analysis were performed to prove the antibacterial property and presence of active phytochemicals in extracts of *Acacia catechu*. A phytochemical analysis was done using a HPTLC instrument. Antimicrobial testing demonstrated excellent results with the petroleum ether extract against *Pseudomonas aeruginosa* (10 µg/ml), followed by the aqueous extract against *Bacillus subtilis* (20 µg/ml) and the chloroform extract against *Staphylococcus aureus* (30 µg/ml). Two major phytochemical constituent's, epicatechin and quercetin, were identified by HPTLC as active ingredients in the extract (Patel *et al.*, 2009).

Gulzar *et al.* (2010) also has conducted a similar study on Preliminary phytochemical and antimicrobial activity of the crude extract obtained from the leaves of *Acacia catechu* (AC.).The presence of carbohydrates, steroids, alkaloids, glycosides, tannins, sapiens, flavones and phenolic compounds was indicated by the tests conducted. Antimicrobial activity of petroleum ether, ethanolic and ethanol: water (1:1) extracts

of leaves of *Acacia catechu* was evaluated against some pathogenic fungi and gram positive and negative bacteria. Ethanolic extract was found to possess the broadest and potent antimicrobial activity (Gulzar *et al.*, 2010).

2.4.2.2 Antimycotic

Antimicrobial activity of ethanol and acetone extracts against fungi like *Aspergillus niger*, *Fusarium oxysporum*, *Alternaria alternata*, *Rhizopus stolonifer* and *Macrophoma phaseolina* was reported by Nagaraj *et al.* (2008).

A study was conducted to evaluate the anti mycotic activity of heartwood of *Acacia catechu* on selected fungal species like *Candida albicans*, *Aspergillus niger*, *Aspergillus fumigates*, *Mucor spp* and *Penicilium marneffeii*. Disc diffusion technique was followed for screening anti-fungal activity. The results obtained from the study shows that ethanolic extract has got a very good anti mycotic activity against the selected fungal species. (Geetha *et al.*, 2011)

An attempt was made to assess the antimycotic activity of *Acacia catechu* extracts using three different solvents such as ethanol, acetone and hexane. Agar well diffusion technique was followed for screening against chosen fungi like *Aspergillus niger*, *Fusarium oxysporum*, *Alternaria alternata*, *Rhizopus stolonifer* and *Macrophoma phaseolina*. The maximum inhibition was recorded in ethanol, acetone and hexane roots extracts. *A. niger* growth was controlled by acetone extract of bark, whereas *F. oxysporum*, *A. alternata*, *R. stolonifer* and *M. phaseolina* by acetone extracts of *A. catechu* extract (Anita Roy *et al.*, 2011).

The antifungal activity of the aqueous and methanol extract of twenty Indian medicinal plants against fourteen human pathogenic fungi. Antifungal assay was done using agar disc diffusion method. The result showed that the aqueous extract of *Andrographis paniculata* was only found active while the methanol extract of seven plants i.e. *Acacia catechu*, *Hemidesmus indicus*, *Andrographis paniculata*, *Pongammia pinnata*, *Carica papaya*, *Cannabis sativa* and the *Oroxylum indicum* exhibited significant antifungal activity against one or more test organism. The methanol extract of *Acacia catechu* was established most promising, and found active against *Candida*, *Dermatophytes* and *Aspergillus species* (Nagaraja *et al.*, 2008).

2.4.2.3 Antioxidant

Ethyl acetate fraction of catechu extract was shown to possess high antioxidant capacities i.e. free radical scavenging activity (Sanjay *et al.*, 2011). Monga *et al.* (2011) reported that this antioxidant property was responsible for the anti-tumour property of the catechu extract. According to Valte (2012), the same property was also responsible for hepatoprotective activity.

Gayathri Devi *et al.* (2011) conducted a pharmacognostical study on *Acacia catechu* and the antioxidant principles were analysed by Dot-blot assay and quantitative analysis by DPPH (1, 1, - diphenyl-2-picryl hydroxyl) radical scavenging assay with ascorbic acid as standard. The results of dot-blot assay showed yellow coloured spots when sprayed with DPPH solution. When the plates were sprayed with DPPH solution in methanol (0.4mM), the regions where substances with antioxidant capacity occurred stained yellow in the purple back ground. The TLC of the alcoholic plant extract conducted using CEF (chloroform-ethyl acetate -formic acid, 5:4:1) as mobile phase and DPPH as spray reagent, gave six major spots with yellow colour indicating that there are at least six antioxidant constituents in the extract. The more intense the yellow colour, the greater the antioxidant activity. Radical scavenging activity was estimated by DPPH assay and the results were compared with that of ascorbic acid. Ascorbic acid at 66.12 μ M could scavenge half of DPPH (IC₅₀) when reacted for 30 minutes. IC₅₀ value of the plant extract was found to be 61.72 μ M which is comparable with the values obtained for ascorbic acid (Gayathri Devi *et al.*, 2011).

Similar study was carried out to determine the antioxidant and iron chelating property of 70% methanolic extract of 'katha'. The extract was found to be an antioxidant with a TEAC value of 0.72 ± 0.02 . The extract has shown its scavenging activity for different radical and $17.8 \pm 0.63 \mu\text{g/ml}$, $39.55 \pm 1.36 \mu\text{g/ml}$, $55.31 \pm 7.12 \mu\text{g/ml}$, $746.85 \pm 30.46 \mu\text{g/ml}$, $44.2 \pm 4.48 \text{ mg/ml}$, $57.5 \pm 4.23 \mu\text{g/ml}$, $155.48 \pm 3.78 \mu\text{g/ml}$ were determined as IC₅₀ value for DPPH, superoxide, nitric oxide, peroxy nitrite, hydrogen peroxide, singlet oxygen and hypochlorous acid radicals, respectively. The plant was found to inhibit lipid peroxidation with an IC₅₀ of $12.35 \pm 0.43 \mu\text{g/ml}$. The extract also has the ability to chelate iron with an IC₅₀ of $810.8 \pm 20.74 \mu\text{g/ml}$ as well

as DNA protective property with *P+50 of 60.44 ± 5.73 $\mu\text{g/ml}$. It was also observed that the plant extract (100 mg) has 97.13 ± 0.006 mg/ml gallic acid equivalent phenolic and 383.66 ± 0.014 mg/ml quercetin equivalent flavonoid content. The results provide evidence that 70% methanol extract of 'katha' acts as an antioxidant, iron chelator and DNA protector which is partly due to the phenolic and flavonoid compounds present in it (Hazra *et al.*, 2010). Four aqueous extracts from different parts of medicinal plants used in Ayurveda viz., *Momardica charantia* Linn (AP1), *Glycyrrhiza glabra* (AP2), *Acacia catechu* (AP3), and *Terminalia chebula* (AP4) were examined by Naik *et al.* (2003) for their potential as antioxidants. The antioxidant activity of these extracts was tested by studying the inhibition of radiation induced lipid peroxidation in rat liver microsomes at different doses in the range of 100-600 Gy as estimated by thiobarbituric acid reactive substances (TBARS) (Patil *et al.*, 2003). The extracts were found to restore antioxidant enzyme superoxide dismutase (SOD) from the radiation induced damage. The antioxidant capacities were also evaluated in terms of ascorbate equivalents by different methods such as cyclic voltametry, decay of ABTS (-) radical by pulse radiolysis and decrease in the absorbance of DPPH radicals (Naik *et al.*, 2003). (Patil *et al.*, 2003) conducted a free radical scavenging study on aqueous extracts of *Acacia catechu* and *Rotula aquatica*. His study revealed that the polyphenols present in the polar extracts possess a stronger antioxidant activity which may be useful in treatment of cancer and in cancer patients undergoing radiation therapy (Braca *et al.*, 2000).

2.4.2.4 Other benefits

Other benefits of catechu include antidiabetic Vaishali *et al.*, 2003; Phuong *et al.*, 2004; Nagarajan *et al.*, 2005; Nalamolu *et al.*, 2006; Jarald *et al.*, 2009;). Immuno modulatory (Ismail and Asad, 2009), Anti-pyretic activity (Ray *et al.*, 2006) hepato protective (Rage *et al.*, 1984; Jayasekhar *et al.*, 1997; Ray *et al.*, 2006; Pingale, 2010; (Reitman and Frankel, year 1957), Anti-diarrhoeal (WHO, 1985; Ray *et al.*, 2006) and antiulcer Karwani (2011) .

2.4.3 Uses of catechu

Katha is used from ancient times in betel leaf chewing together with lime, and now-a-days consumed in larger extent for the preparation of *pan* masala (Patel, 2011). There

is a very large internal demand for it for masticatory use in *pan* preparations and in medicine. It is an indispensable ingredient of *pan* preparations. In combination with lime, it gives the characteristic red colouration resulting from the chewing of *pan* (Jha, 2002).

Other uses of the tree

It is used for many industrial purposes such as dyeing cotton and silk (Gaur, 2008) and preserving of fishing nets, sailing ropes and mail bags; in water softening and in the manufacture of stencils and printers ink (Agroforestry Database 4.0) (Orwa *et al.*, 2009).

Fodder: Branches of the tree are quite often cut for goat fodder and are fed to cattle

Fuel: The tree is often planted for use as firewood and its wood is highly valued for furniture and tools.

Timber: Timber is used for agricultural implements and wheels. Spent chips left over after extraction of Katha and cutch can be used for the manufacture of hardboards.

Tannin or dyestuff: Cutch, which is marketed as a solid extract, is isolated from the heartwood. The dark catechu or Pegu cutch is used to tan heavy hides into sole leather, often in a mixture of tan stuffs. Catechu extract is also used for dyeing silk, cotton, canvas, paper and leather to a dark-brownish colour (Lemmens and Wulijarni-Spetjiptoed, 1991)

Gum or resin: The bark exudes a light gum of very good quality and is one of the best substitutes for gum arabic.

"Black catechu" has been traditionally employed in the producer countries for crude dyeing, leather tanning and in indigenous medicines.

The further processed "cutch" is employed as a cheap brown dye and preservative for canvas, fishing nets and similar items and also as a tanning agent for leather, particularly in India. In more recent times, cutch has found use as a viscosity modifier in oil well drilling.

Trade in cutch appears restricted to the traditional usage areas of the Indian sub-continent and certain countries in Southeast Asia. The major producer of cutch and katha is India where production is based in the northern provinces on wild and cultivated trees and involves both cottage-scale and industrial-scale processing (with some factories having a throughput capacity of 1,000 tonnes of raw material per

month). Up-to-date information is not available for India on output levels or on the breakdown in domestic usage of catch between dyestuff, tanning and medicinal applications. However, factories have recently reported difficulties in operating at full capacity owing to raw material supply problems; the tree is valued as a fuelwood and for other purposes. During the mid-1970s, some 63,000 tonnes of wood were consumed annually by the katha/catch industry and this is estimated crudely as providing around 5,000-6,000 tonnes of catch. India recorded exports of 1,000-1,300 tonnes of catch annually over 1988-1993 with Pakistan as the major destination. Minor producers of unrefined "black catechu" include Myanmar, Thailand, Bangladesh and Pakistan. Reliable data are available only for Thailand; current production is cottage-industry type, based on a limited natural resource with sales mainly to the domestic traditional medicine market but involving small exports to Pakistan, Nepal and Bangladesh.

The absence of reliable data makes it impossible to predict a market trend for catch as a dyestuff or tanning agent. However, a continued substantial demand may be expected in the traditional regional market and this could possibly increase along with population growth, demand for manufactured goods and industrialization. The major perceived constraint lies with the availability of the wood raw material which is already under pressure from alternative uses, particularly as a fuelwood, in many countries.

2.4.4 Toxicological studies

Catechin from *Acacia catechu* was tested for safety. Acute and subchronic toxicity testing demonstrated no abnormalities in any toxicological endpoints examined including animal body weights, gross organ pathology and tissue histology, and blood chemistries or serology. The extract, when dosed in 344 rats, a model for gastric toxicity, showed no evidence of ulceration. No mutagenicity or drug interactions were seen. When the extract was compared with placebo after administration to a healthy human population, no changes in blood chemistry or serology were observed. Based on these findings, the catechin appears to possess a safety profile. No toxicity of catechin was reported in trials with rats (Yimam *et al.*, 2010). However, its consumption is not advocated for more than 2-3 weeks for those suffering from

kidney inflammation. Cutch is subjected to legal restrictions in some countries (Khare, 2004).

2.5 FOOD SAFETY AND STANDARDS AUTHORITY OF INDIA REGULATIONS

Unauthorized addition of colouring matter is prohibited. The addition of colouring matter to any article of food except as specifically permitted by these regulations is prohibited. Natural colouring matters which may be used except as otherwise provided in these regulations and Appendices, and the following natural colouring principles whether isolated from natural colours or produced synthetically that may be used in or upon any article of food are: a) Carotene & Carotenoids including: I) beta-carotene II) Beta-apo 8'-carotenal III) Methyl ester of Beta-apo 8' carotenoic acid IV) Ethylester of Beta-apo 8' carotenoic acid and V) Canthaxanthin; b) Chlorophyll, c) Riboflavin (Lactoflavin), d) Caramel, e) Annatto, f) Saffron and g) Curcumin or turmeric

Explanation: In the preparation of the solution of annatto colour in oil, any edible vegetable oil to these regulations may be used either singly or in combination and the name of the oil or oils used shall be mentioned on the label of Food Safety and Standards Packaging and Labelling Regulations, 2011.

Table-2.1: FSSAI permitted colours

| S.No. | Colour | Common name | Colour Index (1956) | Chemical |
|-------|--------|--|-------------------------|--------------------------------|
| 1. | Red | Ponceau 4R Carmosine Erythrosine | 16255 14720 45430 | Azo Azo Xanthene |
| 2. | Yellow | Tartrazine Sunset Yellow FCF | 19140 15985 | Pyrazolone Azo |
| 3. | Blue | Indigo caramine Brilliant Blue PCF | 73015 42090 | Indigoid Triary Imethane |
| 4. | Green | Fast Green FCF | 42053 | TriaryImethane |

Addition of inorganic colouring matters and pigments prohibited.-Inorganic colouring matters and pigments shall not be added to any article of food unless otherwise provided in these regulations and appendices. Synthetic food colours which may be

used:-No synthetic food colours or a mixture thereof except the following shall be used in food (Table.2.1).

Use of lake colours as colorants in foods: Aluminium Lake of sunset Yellow FCF may be used in powdered dry beverages mixes (powdered soft drink concentrate) up to a maximum limit of 0.04 per cent by weight. The maximum limit of colour content in final beverage for consumption shall not exceed 8.3 ppm and that of aluminium content shall not exceed 4.4 ppm of the final beverage for consumption: Provided the powdered dry beverage mix (powdered soft drink concentrate) label shall give clear instruction for reconstitution of product for making final beverage.

Use of permitted synthetic food colours in or upon any food other than those enumerated below is prohibited:- i) Ice cream, milk, lollies, frozen desserts, flavoured milk, yoghurt, ice cream mix powder ii) Biscuits including biscuit wafer, pastries, cakes, confectionery, thread candies, sweets, savouries (*dalmoth, mongia, phululab, sago, papad, dal biji*, only) iii) Peas, strawberries and cherries in hermetically sealed containers, preserved or processed papaya, canned tomato juice, fruit syrup, fruit squash, fruit crushes, fruit cordial, jellies, jam, marmalade, candied crystallised or glazed fruits iv) Non-alcoholic carbonated and non-carbonated ready to serve synthetic beverages including synthetic syrups, sherbets, fruit bar, fruit beverages, fruit drinks, synthetic soft-drink concentrates v) Custard powder vi) Jelly crystal and ice-candy and vii) Flavour emulsion and flavour paste for use in carbonated or non-carbonated beverages.

Maximum limit of permitted synthetic food colours: - The maximum limit of permitted synthetic food colours of mixture thereof which may be added to any food article. These regulations shall not exceed 100 parts per million of the final food or beverage for consumption, except in case of food articles mentioned in clause(c), where the maximum limit of permitted synthetic food or beverage for consumption.

Colours to be pure – The colours specified in these regulations, when used in the preparation of any article of food shall be pure and free from any harmful impurities.

Chapter- 3

Materials and Methods

3.0 MATERIALS AND METHODS

This chapter deals with various materials used and methodologies employed during the present investigation relating to the technological, analytical, microbiological, sensory aspects of Catechu coloured products.

3.1 MATERIALS

3.1.1 Ingredients

3.1.1.1 Catechu (*Acacia Catechu*)

Catechu (*Acacia Catechu*) heartwood was used in this project. Catechu heartwood procured from Buldhana dist. of Maharashtra. Wood of reddish brown colour was selected and cut from the plant and brought to the laboratory. It was further cut into ~ 4cm pieces (Fig. 3.1) and used for colour extraction.



Fig. 3.1: *Acacia catechu* heartwood pieces

3.1.1.2 Milk and culture

Fresh cow milk was procured from Institute's Cattle Yard. Yoghurt culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus*) and dahi cultures (*Lactococcus lactis spp. lactis*) required for the study were obtained from the Dairy Chemistry & Bacteriology Section of the Institute.

3.1.1.3 Sugar

Crystalline sugar obtained from the market was used as the sweetening material in the preparation of flavoured milk, shrikhand, yoghurt and lassi.

3.1.1.4 Caramel sugar

Crystalline sugar obtained from the market was caramelised and used as caramel flavour in flavoured milk.

3.1.1.5 Sapota fruit

Good quality sapota fruit was obtained from local market and its pulp was prepared using mixer and used as flavouring ingredient in shrikhand.

3.1.1.6 Cocoa powder

Cocoa powder was obtained from local market and used as flavouring agent in lassi

3.1.1.7 Chikku flavour

Artificial Chikku Flavour (LUX ESSENCE, Chennai) was obtained from local market and used as flavouring agent in yoghurt.

3.1.2 Packaging materials

Polystyrene (PS) cups of 100ml capacity with polystyrene lids were procured from the local market for packaging the samples under study. For storage studies, the cups and lids were washed, dried and sterilised under an UV lamp for 60 min prior to use in the experiments. LDPE milk pouches were procured from the local market for packaging of lassi and pasteurised flavoured milk.

3.1.3.4. BHI Broth

BHI broth (Hi-Media, Mumbai) was used for growing *E.Coli* culture overnight @ 37°C. Thirty seven grams of BHI broth was dissolved in 1000ml of distilled water . The mixture was then dispensed into bottles or tubes and sterilised by autoclaving at 15 lbs 121°C for 15 min.

3.1.4 Glassware and equipment

Glassware of Borosil and Schott Duran brands were used in the determination heat stability of catechu extract as well as scanning, chemical and microbiological analysis of pasteurized flavoured milk, lassi, yoghurt and shrikhand samples. The glassware were washed by using soap solution in laboratory after use and dried in hot air oven before they were re-used.

The equipment's used during the research work are mentioned below:

3.1.4.1 Hot air oven

Hot air oven (Apollo Scientific Surgical Co., Bangalore.) it was used for sterilization of glassware.

3.1.4.2 Analytical weighing balance

A weighing balance supplied by Spark instruments, Bengaluru was used for weighing the major ingredients like cocoa powder, chakka, sapota pulp, sugar and SMP.

Sartorius Analytical weighing balance of 0.1 mg accuracy was used for weighing minor ingredients like citric acid and ascorbic acid.

3.1.4.3 Induction heater

The induction heater of Prestige company was used for the heating purposes during research work.

3.1.4.4 Incubators

Laboratory incubators (ALPHA, Bangalore) were used for manufacturing of lassi, yoghurt and shrikhand, and also used for microbial analysis of final products.

3.1.4.5 Spectrophotometer

ELICO-Spectrophotometer (Anthelie Light 2, France) was used for the determination of absorbance of Catechu extract during the study of heat stability of Catechu extract.

3.1.4.6 Colour measuring instruments

Computer: Dell, Core-i3, 4GB RAM

Scanner: Hewlett-Packard Scan jet 5370c (hp)

3.1.4.7 Planetary mixer

A bakery model planetary mixer supplied by Lalith Industries, Bengaluru was used for the homogeneous mixing of chakka, sugar, sapota pulp and Catechu extract during the preparation of shrikhand.

3.1.4.8 Sealing machine

Sealing machine supplied by Hitech Pack, Bengaluru was used for the sealing of LDPE pouches after filling of lassi and pasteurized flavoured milk for storage study.

3.1.4.9 pH meter

The electrode assembly of a digital pH meter (Digisun Electronics, Hyderabad, Model: DI 707) calibrated against standard buffer of pH 7.0 and 4.0 (Qualigens Fine Chemicals) was used for estimation pH of yoghurt and lassi.

3.1.4.10 Texture analyser

Firmness and consistency of yoghurt; and consistency & index of viscosity of lassi were measured by Texture Analyser (TA-XT plus, Stable Micro Systems, Surrey, UK).

3.1.4.11 Ostwald viscometer

The kinematic viscosity of flavoured milk samples were determined using **Ostwald** viscometer (Vensil Glassware, Chennai) The viscosity measurements were carried out at 25°C.

3.1.4.12 Autoclave

Autoclave (Appollo Scientific Surgical Co., Bengaluru) was used for the preparation for sterilised flavoured milk at 121°C for 15 min .

3.2. METHODOLOGY

3.2.1 Effect of type of extraction on catechu colour

Two extraction methods were tried: hot extraction and cold extraction. Known quantity of catechu heartwood was taken in a vessel and boiled in water for certain period of time. Then the wood pieces were filtered out and boiled further in fresh lot of water second time. In this manner, the wood pieces were extracted five times till all the colour got extracted from the pieces. The extent of colour extraction was measured by OD of solution at 500 nm. By this it is understood whether extraction of the colour is easier by hot extraction method. Similarly, known amount of catechu heartwood pieces were soaked in water for a longer period of time and extraction of colour into the medium observed. Alcohol was also tried as a medium of extraction.

3.2.2 Effect of various additives on catechu colour

The catechu extract was prepared as follows: Two per cent solution of colour concentrate of catechu was used. This extract of 10 ml was taken in test tubes and added with a little quantity of the following commonly used additives and any change in the colour of the extract was noted (betel leaf extract, calcium hydroxide, calcium

chloride and turmeric).. The present study being the first study on catechu colour, the objective here was to find out qualitatively whether any substance darkens or enhances the catechu colour. Any changes were recorded by digital imaging.

3.2.3 Stability of extracted catechu colour under various conditions

Ten ml of catechu extract was taken in test tubes and kept in an incubator at 37°C for 10 days. The various colour parameters RGB and L were measured by SAP (Scanner – Adobe Photoshop) method at the end of 10 days. These values were compared with the zero day values to know the effect storage at 37°C on colour intensity. Some tubes were left open exposed to atmospheric air and some tubes were filled to the brim and closed tightly with rubber stopper. The any effect of dissolved oxygen was neglected. Similarly some tubes closed with rubber stoppers were stored in dark. The RGB and L colour parameters at the end of 10 days storage at 37°C were measured by SAP method.

3.2.4 Effect of heat treatment on catechu colour

The catechu extract was taken in test tubes and subjected to thermization, boiling and sterilisation treatments. Any change in color was evaluated by measuring RGB and L by SAP method.

3.2.5 Effect of different processing treatments on catechu colour

Milk was added with catechu colour and subjected to thermization, boiling and sterilisation treatments. Colour parameters were measured by SAP method.

3.2.6 Antimicrobial property of catechu colour concentrate

The antimicrobial property of catechu colour concentrate (CCC) against *E.coli* was evaluated by agar diffusion technique.

3.2.7 Preparation catechu colour concentrate

Based on the heat stability and other tests, a method was optimised to prepare catechu colour concentrate (CCC).

3.2.8 Utilization of CCC as colouring agent in flavoured milk preparation

Four types of flavoured milks were prepared as shown in flow chart (Fig. 3.2): 1) Plain pasteurised flavoured milk with no CCC addition 2) Plain pasteurised flavoured milk with CCC addition as colouring agent 3) Sterilised flavoured milk with no CCC addition and 4) Sterilised flavoured milk with CCC addition as

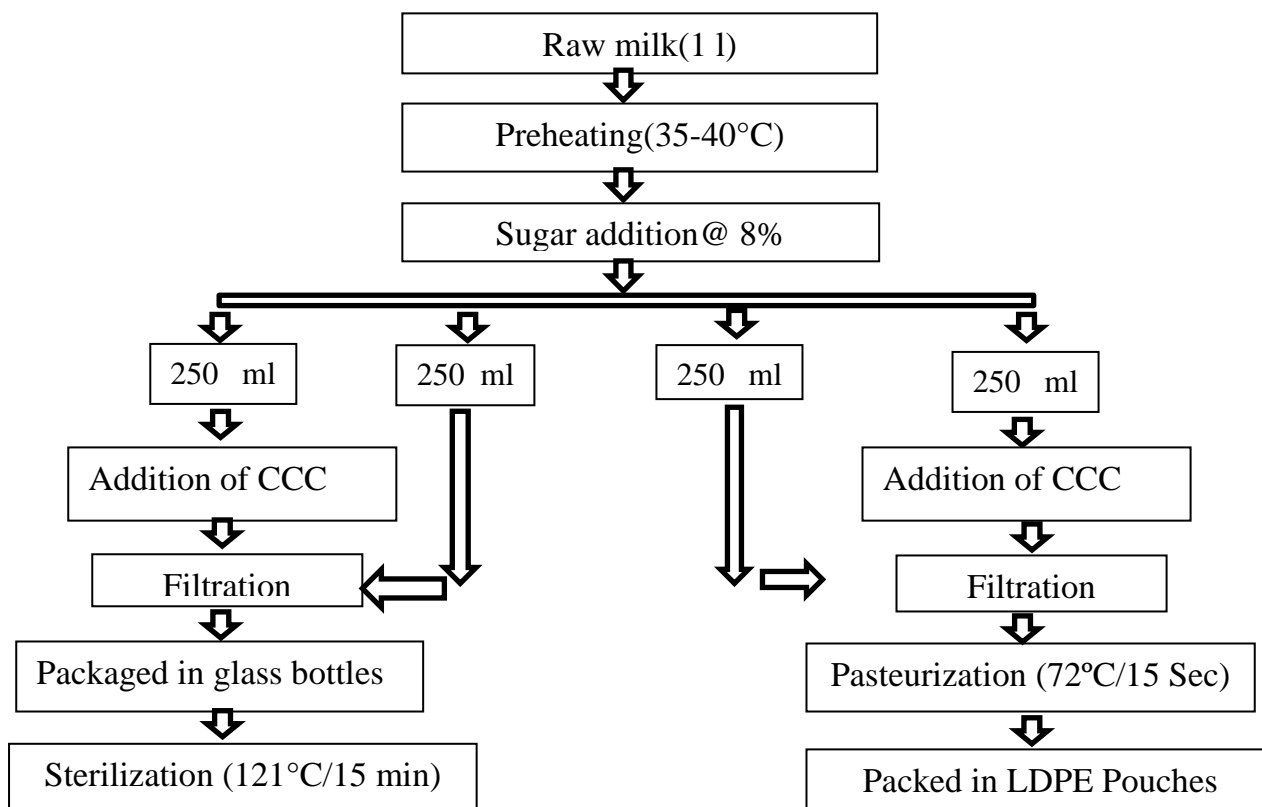


Fig 3.2: Flow chart for the preparation of various types of flavoured milks

colouring agent. CCC was added to milk before pasteurisation or sterilisation treatment. At the time of sugar addition, caramelised sugar was also added that gave caramel flavour in final product.

The effect of CCC on the flavoured milk was evaluated by sensory evaluation on Hedonic scale. The colour parameters viz. RGB and L were measured by SAP method. The kinematic viscosity was also determined using Ostwald viscometer.

3.2.9 Utilisation of CCC as colouring agent in shrikhand preparation

Shrikhand was prepared as per the flow chart given (Fig.3.3). CCC was incorporated at the time of kneading of chakka along with sugar.

The effect of CCC on shrikhand was evaluated by sensory evaluation on Hedonic scale. The colour parameters viz. RGB and L were measured by SAP method. The suitability of CCC on sapota pulp added shrikhand was also evaluated. The textural properties viz. Firmness, consistency, adhesive force and index of viscosity were determined by Texture Analyser.

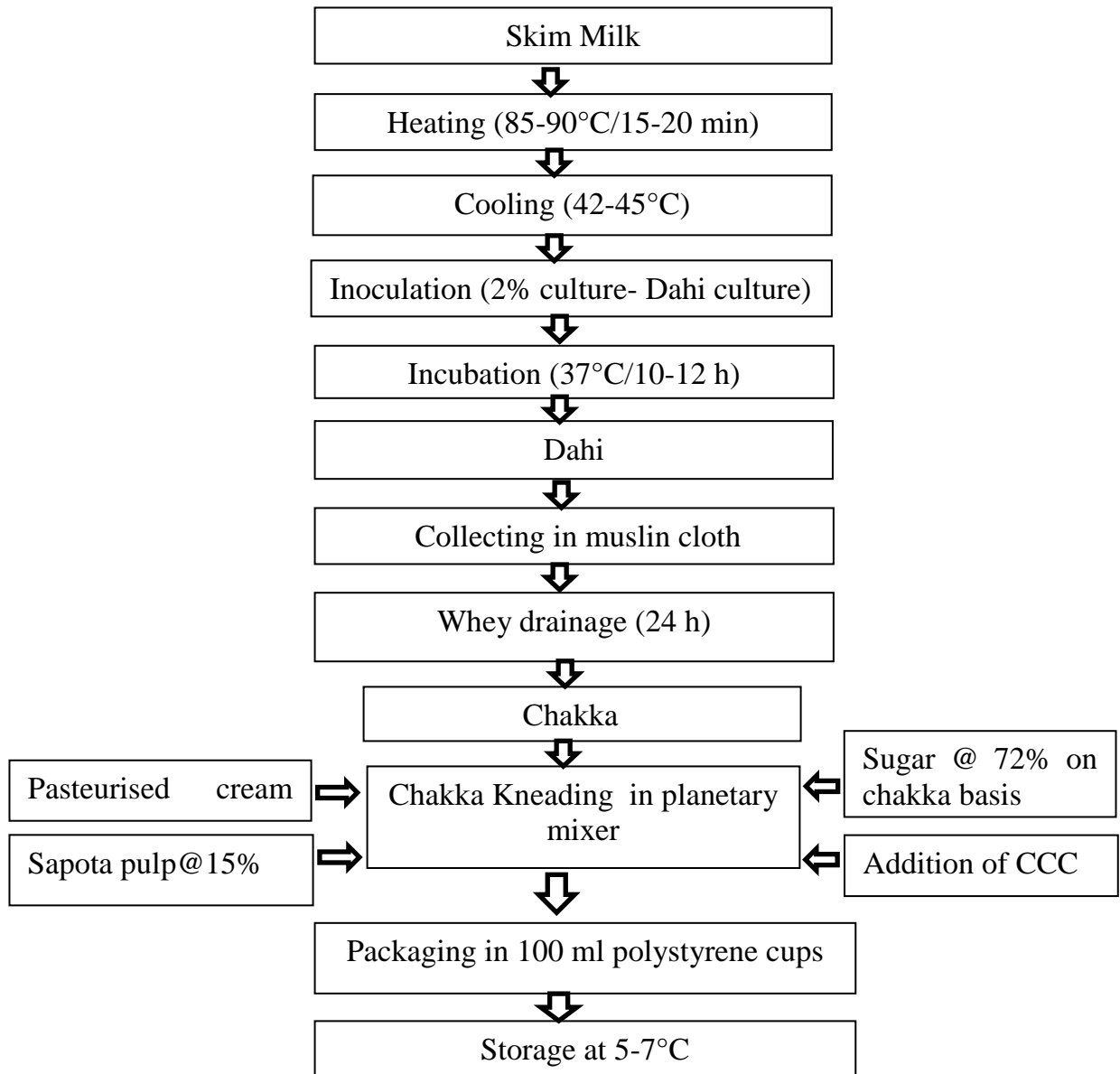
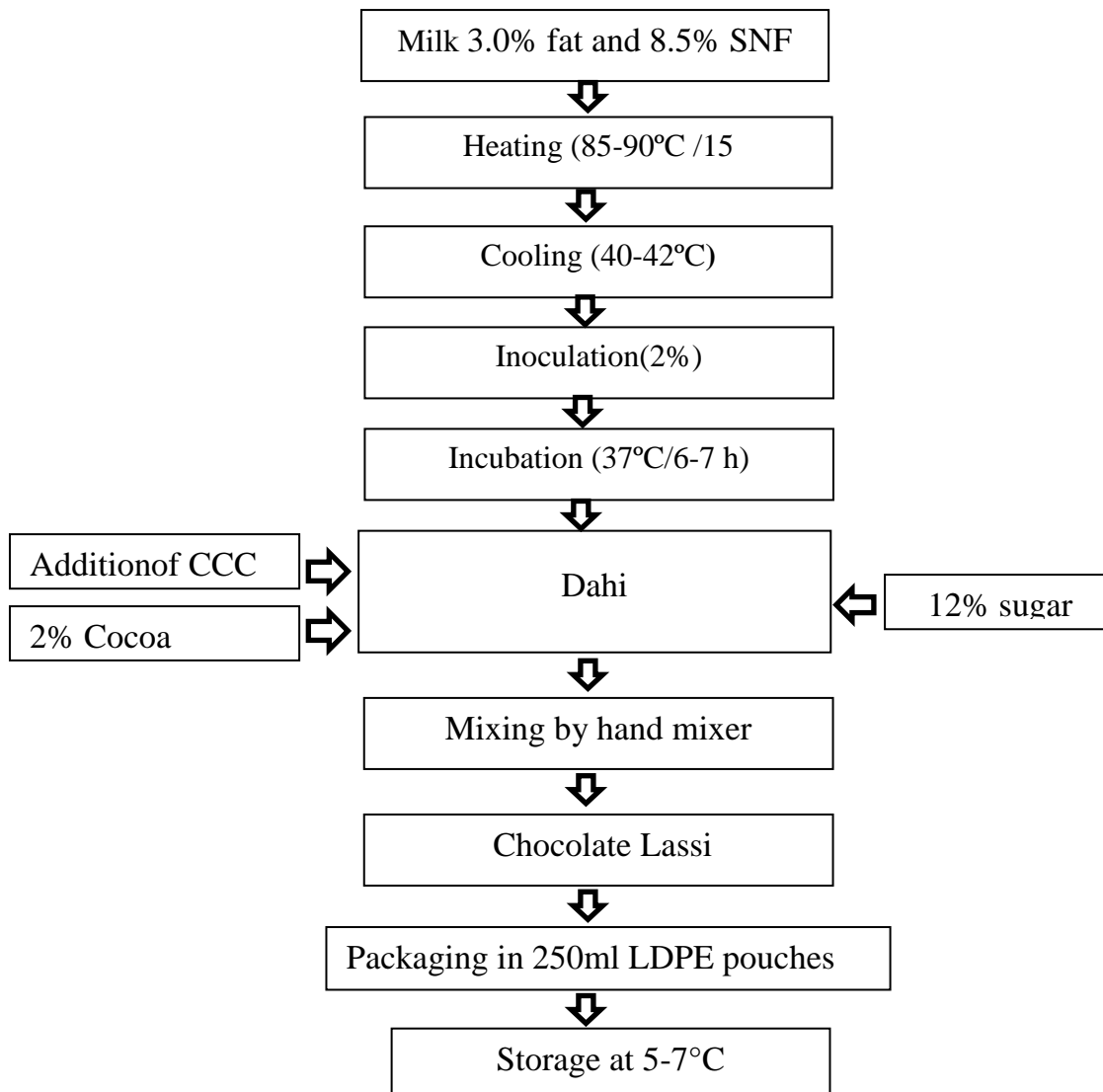


Fig.3.3: Flow chart for the preparation of shrikhand

3.2.10 Utilisation of CCC as colouring agent in plain lassi

Lassi was prepared by following the method shown in flow chart (Fig. 3.4). CCC was added at the time of mixing of dahi and sugar syrup. The effect of CCC on lassi was evaluated by sensory evaluation on Hedonic scale. The colour parameters viz. RGB and L were measured by SAP method. The suitability of CCC on cocoa flavoured lassi was also evaluated. The pH and viscosity measure of lassi was also determined to know the influence of added CCC.



* **Preparation of sugar syrup:** Sugar syrup was prepared by mixing water and sugar. Quantity of water was calculated @ 25% on dahi basis and sugar was calculated @ 10 parts of mixture of dahi and water. The sugar was dissolved in the calculated amount of water and heated at 90°C for 10 minutes, filtered and cooled to 37°C.

Fig. 3.4: Flow chart for the preparation of lassi

3.2.11 Utilisation of CCC as colouring agent in yoghurt preparation

Lassi was prepared by following the method shown in flow chart (Fig. 3.5). CCC was added to milk along with sugar and SMP before heat treatment.

The effect of CCC on set yoghurt was evaluated by sensory evaluation on Hedonic scale. The colour parameters viz. RGB and L were measured by SAP method. The textural properties viz. Firmness, consistency, adhesiveness and index of viscosity

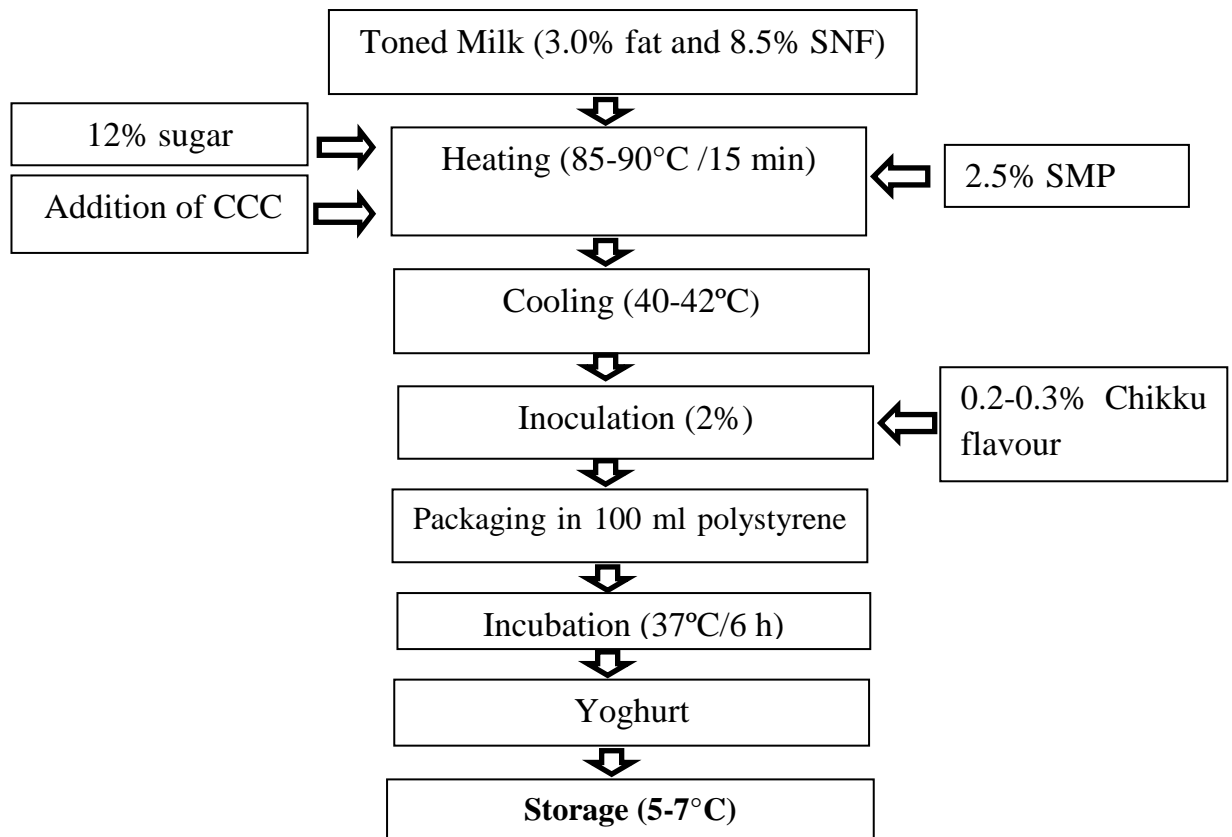


Fig. 3.5: Flow chart for the preparation of yoghurt

were determined using Texture Analyser. pH was also determined to know the influence of added CCC.

3.2.12 Stability of CCC colour in products during storage

Yoghurt in polystyrene cups were stored in a refrigerator ($7 \pm 1^\circ\text{C}$) for about 24 days, . lassi packed in LDPE pouches was stored for 13 days, shrikhand in polystyrene cups for 31 days and sterilised flavoured for 42 days (at ambient temperature). During storage the following parameters were analysed and recorded:

Yoghurt: R,G,B and L values and sensory parameters (CA, BT, Fl and OA)

Cocoa lassi: R,G,B and L values and sensory parameters (CA, BT, Fl and OA)

Sapota pulp shrikhand: R,G,B and L values and sensory parameters (CA, BT, Fl and OA)

Sterilised flavoured milk: R,G,B and L values and sensory parameters (CA, BT, Fl and OA)

3.4 METHODS OF ANALYSIS

3.4.1 Colour measurement

Colour/ reflectance of the samples was measured by two methods 1) Reflectance meter and 2) Scanner-Adobe Photoshop method.

3.4.1.1 Reflectance meter (Fig. 3.6)



Fig: 3.6: Reflectance meter

Colour in terms of reflectance was measured by the method described by Shaunak (2008). The instrument was put on and left for about 10 min for warming-up. The operating mode was switched to white mode. Standard black plate supplied by the manufacturer was placed against the bulb on the holder and the per cent reflectance on the dial scale was adjusted to zero by using the knob meant for the purpose. Similarly, using the standard white plate supplied by the manufacturer, the per cent reflectance on the dial scale was adjusted to 100% with the help of the specified knob. The sample (flavoured or shrikhand) was thoroughly mixed and filled into a clean small plastic cup (~40 mm diameter and ~40mm height) using a spatula and all mechanical and air gaps were removed by pressing with the spatula or gently tapping the petri plate. The glass side of the petri-plate was then placed against the bulb of reflectance meter. The per cent reflectance shown on the dial scale was noted. Measurements were made at 3-4 places on the plastic cup and average value was recorded as reflectance value. The same plastic cup was used throughout the study.

3.4.1.2 Scanner-Adobe Photoshop (SAP) method

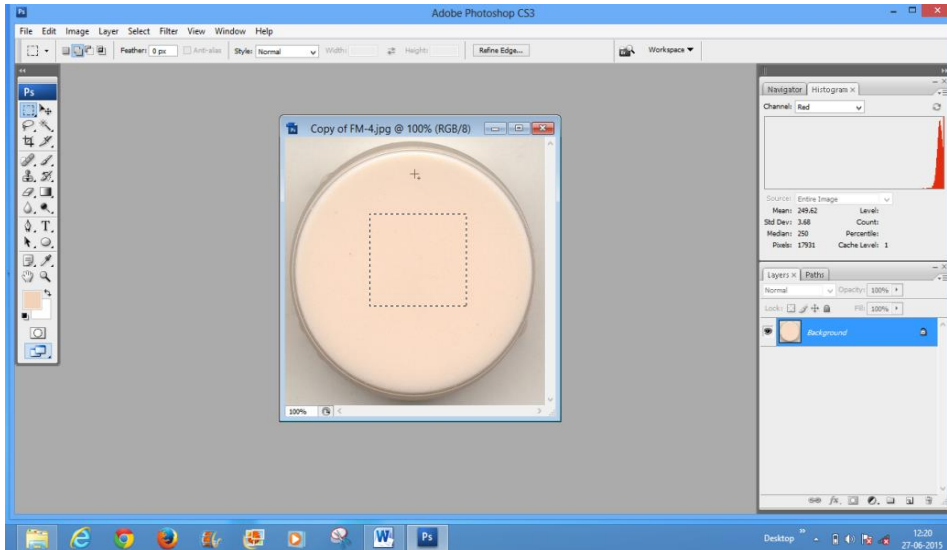
The colour of samples was measured by the method described by Vyawahare and Rao (2011). All the samples were tempered to about 30°C before measurement. The samples were prepared as follows: Lassi – Lassi samples were directly poured into scratch-less petri plate (~70 mm diameter) which has been thoroughly cleaned and dried earlier. Yoghurt and shrikhand– With the help of a spatula, yoghurt was scooped into petri plate and the surface evened out. Any air gaps were removed by spatula and by gentle tapping the plate. Flavoured milk – it was directly poured into petri plate without leaving any air bubbles. The petri dish containing the sample was placed on the bed of the scanner and was covered by scanner top. Scanning was performed and the image was saved as JPEG file. Scanning of the sample was performed under the following scanner parameters: resolution: 75 dpi, sharpness: medium; image quality: medium; background of the sample: white; thickness of sample: 1 cm. Colour analysis software namely Adobe Photoshop Version CS3 running under the Microsoft Windows XP environment was used to extract and analyse colour information from the scanned image. The scanned image in JPEG format was opened in Adobe Photoshop (Fig. 3.7a) and the following parameters were measured in RGB mode: Lightness (L), red (R) , green (G) and blue (B) (Fig. 3.7b).

3.4.2 Sensory evaluation

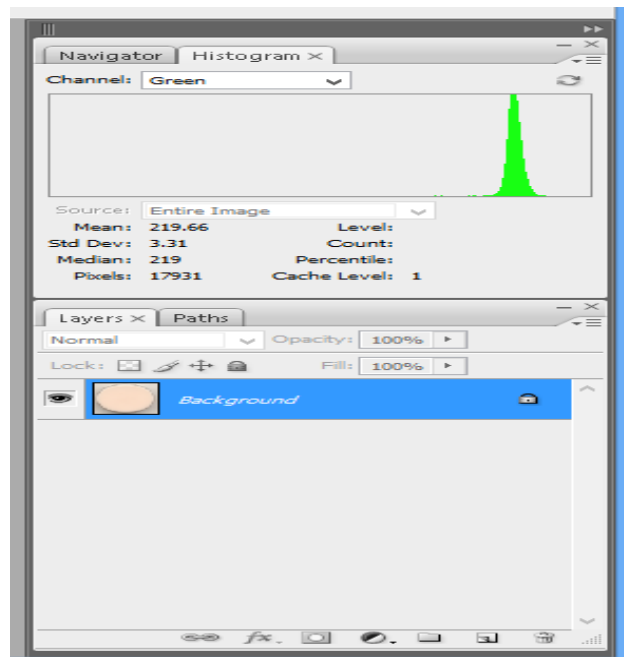
The lassi, yoghurt, shrikhand and flavoured milk samples were evaluated at refrigerated temperatures as these products are normally consumed at that temperature. The main objective of the sensory evaluation was to find out whether added CCC imparted any flavour to the product and whether its addition has any adverse or favourable influence on other sensory qualities of products.

Flavoured milk and lassi were transferred from their LDPE pouches into clean containers and served during sensory evaluation. Yoghurt and shrikhand were served in polystyrene cups in which they were packaged. The sensory evaluation sessions were conducted either in sensory evaluation laboratory or inside laboratory by interactions. The panel of judges consisted of semi-trained faculty members as well as post-graduates who showed keen interest and aptitude towards sensory evaluation.

The judges were asked to evaluate colour and appearance, body and texture, flavour, sweetness and overall acceptability of the samples on a 9-point Hedonic scale (9 – like extremely, 1 – dislike extremely) (Amerine *et al.*, 1965). Plain water was provided to rinse the mouth in between the samples. Sensory evaluation sessions were organized around 12 noon or 3-5 PM every time.



(a)



(b)

Fig. 3.7: Measurement of colour parameters by SAP method (a) JPEG format of scanned image in Adobe-Photoshop window (b) Measurement of colour parameters under RGB mode

3.4.3 Rheological analysis

3.4.3.1 Yoghurt and shrikhand

TA.XT plus Stable Micro System, England was used for measuring firmness, consistency, adhesiveness and index of viscosity of yoghurt; and firmness, consistency, adhesive force and index of viscosity of shrikhand. A p/25 probe was used during experiment. The samples were drawn from refrigerator and directly used for the test. The instrument test settings used were as follows. After loading the test settings, the yoghurt/ shrikhand cup was placed on the platform and on initiation, the probe moved down and penetrated the product up to a distance of 10 mm and retracted, generating a force – time curve (Fig.3.8). From the curve, the firmness (g), consistency (gases), adhesiveness or index of viscosity (gases) and adhesive force (g) were derived. These were later changed to Newton's or N. Sec.

| Mode | Measure force |
|-----------------|----------------------|
| Options | Return to Start |
| Pre-test speed | 1.0 mm/s |
| Test speed | 10.0 mm/s |
| Distance | 10.0 mm |
| Trigger type | Auto, 5 g |
| Tare mode | Auto |
| Advance options | On |

3.4.3.2 Flavoured milk

The kinematic viscosity of flavoured sample was determined using a glass capillary viscometer (Ostwald viscometer). The viscosity measurements were carried out at about 25°C.

The capillary viscometer was cleaned thoroughly with chromic acid, rinsed with distilled water 4-5 times and dried by cold dust free air. A fixed volume of distilled water (12 ml) was transferred with the help of a pipette into the viscometer through wider arm of the instrument. A soft rubber tubing was attached to the narrower arm of the viscometer containing the marked bulb and distilled water was drawn up over the upper mark. The opening of rubber tube was closed with fingers and the pressure

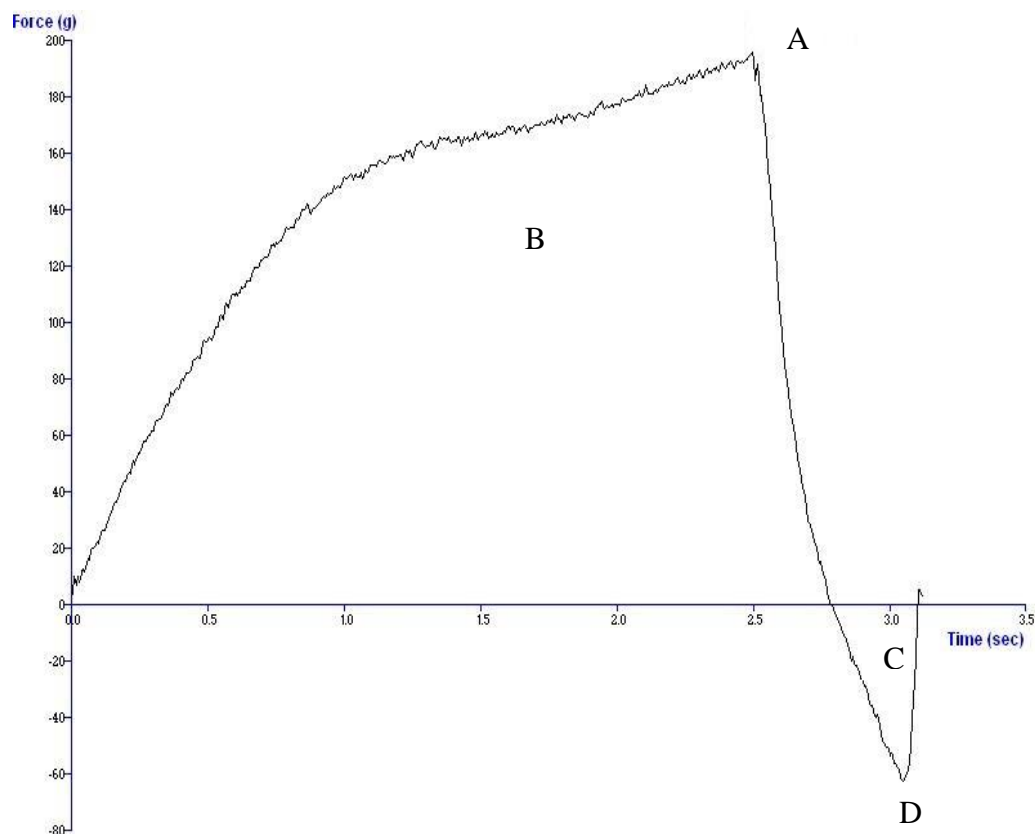


Fig. 3.8: Typical force – time curve of yoghurt and shrikhand

A – Maximum force on positive peak (firmness, g); B – Area of positive peak (consistency, g.sec); C – Area of negative peak (Adhesiveness or index of viscosity, g.sec); Maximum force on negative peak (adhesive force, g)

released to allow the water to flow down. Stop watch was started when the meniscus of water just crosses the upper mark and stopped when it crosses the lower meniscus. The time elapsed was recorded This process was repeated at least 5 times and average time was calculated.

The viscometer was emptied and rinsed with tempered sample of flavoured milk. Then 12 ml of flavoured milk was drawn into the viscometer as mentioned and rest of the procedure was same as described above. The density of water as well as specific gravity bottles was determined using specific gravity bottle. The kinematic viscosity was calculated as below:

$$v = k \times t$$

v – Kinetic viscosity, k – Instrument constant*, t – Average time, sec

(*Instrument constant was determined using distilled water as standard liquid)

3.4.3.3 Lassi

Viscosity measure is an indirect measurement of viscosity of lassi. This was done by measuring the flow time of lassi through a 75 mm glass funnel. A glass funnel was taken and marked an upper level. The end of the stem was connected to a fairly rigid plastic tube with a facility for instant opening and closing by means of clip with sufficient spring tension. The plastic tube was closed and the funnel was filled with lassi up to the mark. Then the plastic tube was opened and stop watch started and when the flow ceased, the watch was stopped. The time elapsed was recorded and expressed as viscosity measure in terms of seconds. The higher the time elapsed, the higher is the viscosity and vice versa.

3.4.4 pH

Yoghurt cup was taken out from refrigerator and tempered to about 20°C. The electrode assembly of a digital pH meter (Digisun Electronics, Hyderabad, Model: DI 707) was directly dipped into yoghurt and the reading on the display panel was recorded after it became stable. Similarly, pH of lassi was measured by taking 50-60 ml sample in 100 ml beaker and by proceeding as above.

3.4.5 Antimicrobial activity of catechu extract

Antimicrobial property of catechu extract was determined by agar diffusion technique (Lakshmi *et al.*, 2011), The test organism used was E.coli grown in BHI Broth medium. About 0.25 ml of active E.coli culture was dispensed in sterile periplate and 10 ml of nutrient agar was poured into the plate and mixed well. The agar was allowed to firmly set by keeping the plates at 4°C for 2 h. Then, with the help of sterile 'punch', 2-3 'wells' were made into which catechu colour concentrate was introduced at four levels viz. 50, 100, 150 and 200 µl. The plates were then kept at 4°C for 3 h for diffusion purpose. Then the plates were incubated at 37°C for 24 h without inversion. At the end of incubation, the plates were observed for any zone of inhibition and if found the zones were measured for their diameter with the help of a scale and expressed in mm (Fig. 3.9).

3.4.6 Statistical analysis

All the experiments were performed in one way randomised block design. Data obtained during the present project work were subjected to statistical analysis by one



Fig. 3.9: Inhibition zones of CCC obtained by agar diffusion technique

way ANOVA using SPSS (version 16.0). Where required paired t-test was also performed by MS-Excel package (version 2010).

Chapter- 4

Results and Discussion

4.0 RESULTS AND DISCUSSION

This Chapter deals with the results obtained during present investigation on utilization of Catechu heartwood colour in flavoured milk, shrikhand, yoghurt and lassi. The study included effect of catechu colour on physico-chemical characteristics of products and the colour stability during storage. The study was also conducted on extraction of colour from catechu heartwood and its properties. The results obtained in the study have been presented and discussed in this chapter.

4.1 STUDIES ON EXTRACTION OF CATECHU HEARTWOOD COLOUR AND ITS PROPERTIES

Catechu plant has a hard interior wood which possesses a reddish brown colour. Most of this colour is water soluble, so can be extracted in water medium. However, there are reports of extraction of many natural colours from plant source using different media other than water like ethanol, methanol etc. Moreover, the extent of colour extraction depends on factors like temperature, pH etc. Hence, water and ethanol were tried as medium of extraction and colour quality studied.

4.1.1 Effect of type of extraction on catechu colour

Catechu heartwood was extracted in water as well as other medium like alcohol. Colour extraction was maximum by heat extraction and very less in cold extraction. Hence, all further trials were conducted using hot extraction. Catechu colour is water soluble, slightly soluble in alcohol and insoluble in oils.

4.1.2 Effect of various additives on catechu colour

The colour quality of natural colours depends on the composition of the medium of extraction. Presence of additives might alter their colour quality as well as intensity. Hence, effect of various additives was studied.

4.1.2.1 Effect of betel leaf extract and calcium hydroxide on catechu colour

It is common practice to use catechu powder in paan which is a betel leaf preparation. It is believed that catechu powder together with betel leaf and lime combinedly develops a red colour in mouth when chewed. Since, paan contains betel leaf, lime and catechu powder combinedly and gives bright red on tongue, lime and betel leaf

extracts were added to catechu colour extract and seen for any red colour development. But surprisingly use of betel leaf extract resulted in light yellow colour, which slowly faded off to no colour on storage for 4 days at ambient temperature (Fig.4.1A). So far, colour development on chewing of pan has not been explained though mention has been made in some sources (Lakshmi *et al.*, 2011a; Patel, 2011). The extract added with only lime first turned dull, light brown and on storage for 4 days at ambient temperature got discoloured. However, settling of colour particles was observed at the bottom (Fig. 4.1A). Even on thorough mixing the colour was not restored. Betel leaf extract (betel leaves brought from market were washed in running tap water, cut into pieces and ground in pestle and mortar with minimum amount of water, filtered through multi-layered muslin cloth and the extract collected) alone was added to catechu heartwood extract and seen for any change in colour. It was observed that there was no improvement in colour by addition of betel leaf extract, rather the colour became light and dull (Fig.4.1B). Similar were the observations when calcium hydroxide alone was used along with catechu extract (Fig. 4.1C). Based on these observations, whether colour development in the mouth as a result of chewing of paan has to do with any enzyme action has to be explored.

4.1.2.2 Effect of calcium chloride on catechu colour

On addition of calcium chloride, there was no improvement in the colour of the extract (Fig. 4.1D).

4.1.2.3 Effect of acidification on catechu colour

On acidification with HCl, the extract turned from dark reddish brown to light yellowish brown (Fig. 4.1E).

4.1.2.4 Effect of alkalisation on catechu colour

On addition of NaOH, the extract turned darker indicating at alkaline pH the colour becomes dark (Fig. 4.1F).

4.1.2.5 Effect of turmeric on catechu colour

On addition of turmeric, there was no change in the colour, rather it turned slightly turbid (Fig. 4.1G).

4.1.2.6 Effect of turmeric and calcium hydroxide on catechu colour

On addition of turmeric along with lime, the extract turned reddish yellow, however there was settling of colour particles (Fig. 4.1H).

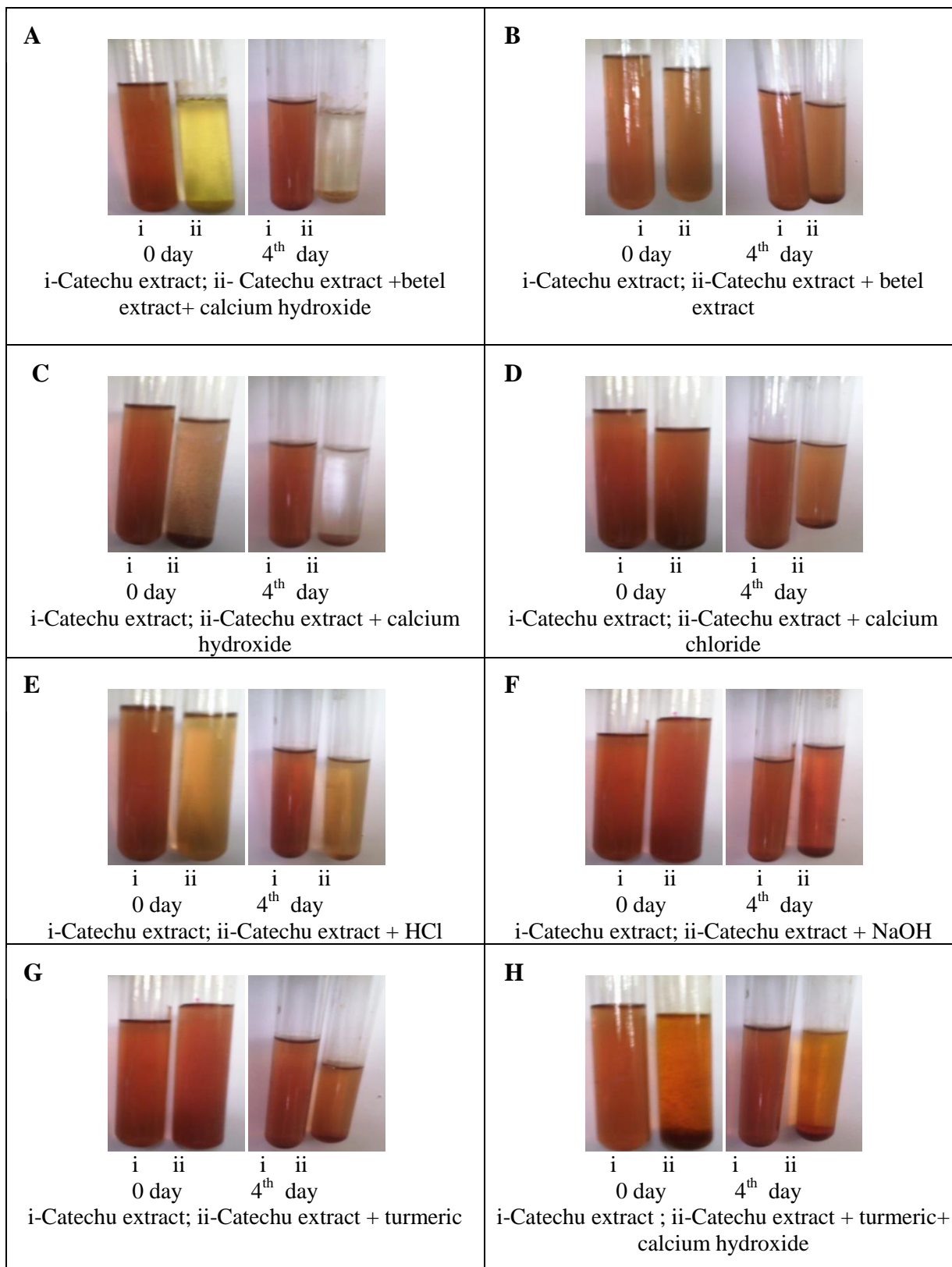


Fig. 4.1: Effect of various additives on catechu colour

4.1.3 Stability of extracted catechu colour under various conditions

The stability of the extracted colour is very important if it has to be utilised in dairy products. Hence, colour stability was studied. Two per cent solution of concentrated catechu colour was used for the stability tests.

4.1.3.1 At 37°C storage

It may be observed from the table that there were changes in RGB and L values of the extract during storage of 10 days at ambient temperature. The L value slightly decreased indicating that the extract became slightly darker. The recreated colours using RG and B values are shown in Table-4.1.

4.1.3.2 Exposure to atmospheric air

In presence of atmospheric air and by storage at room temperature for 10 days, colour became dark, but in the absence of atmospheric air, the colour further darkened as indicated by lower L values (Table-4.2 a and b).

4.1.3.3 Light

It was observed that the catechu colour became darker in presence of light than in absence of light as indicated by L values (Table-4.3 a and b).

Table-4.1: Effect of storage at 37°C on colour of catechu extract







| Days | R | G | B | L | Recreated colour |
|------|--------|--------|-------|--------|---|
| 0 | 198.83 | 149.04 | 97.06 | 158.13 |  |
| 10 | 202.23 | 144.87 | 51.01 | 151.71 |  |

Table-4.2 : Effect of exposure to air on the colour of catechu extract

(a) Exposed to atmospheric air

| Days | R | G | B | L | Recreated colour |
|------|--------|--------|-------|--------|---|
| 0 | 198.83 | 149.04 | 97.06 | 158.13 |  |
| 10 | 208.79 | 140.39 | 35 | 149.32 |  |

(b) Air tight closure

| Days | R | G | B | L | Recreated colour |
|------|--------|--------|-------|--------|---|
| 0 | 198.83 | 149.04 | 97.06 | 158.13 |  |
| 10 | 192.35 | 109.25 | 9.8 | 123.26 |  |

4.1.4 Effect of heat treatment on catechu colour

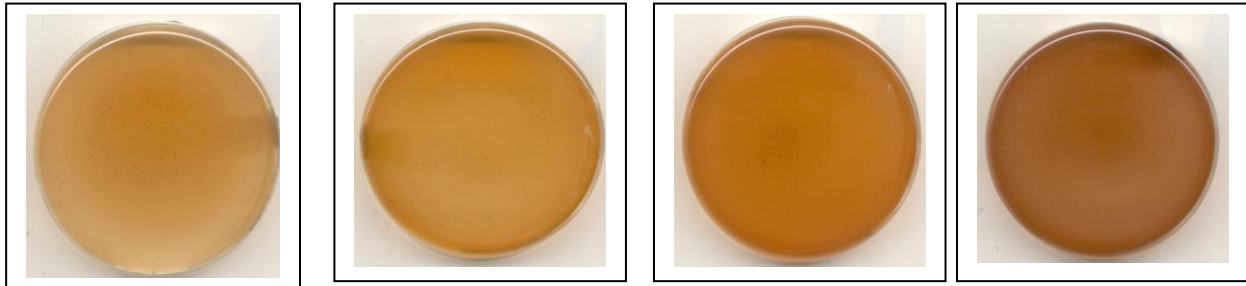
In order to determine the stability of catechu colour to different heat treatments, the colour was extracted from catechu heartwood by hot extraction method and subjected to thermization (60°C), boiling and sterilisation (~121°C) treatments. Initially, the extracted colour showed brown colour (Fig. 4.2 –A). It became slightly darker after thermised treatment (60°C). After boiling treatment, the solution became further dark (Fig. 4. 2-c) and darkest after sterilisation treatment (d). The R,G,B and L values are presented in Table – 4.4. The R,G,B and L values of fresh extract were 194.09, 138.67, 68.37 and 147.46 respectively which significantly changed because of heat treatments (Fig. 4.3).

The fact that the solutions became darker is indicated by L values which decreased to 142.06, 121 and 105.94 respectively by thermization, boiling and sterilisation treatments. From these results, it may be inferred that the intensity of catechu colour increased by heat treatment, which seems to be a unique property for a natural pigment. Many of the natural pigments like betanins, carotenes, chlorophyll, anthocyanin's etc. are heat sensitive (Rahman, 2007) and lose their colour on heating, but catechu seems to be highly heat stable. It was reported that tea epicatechins are remarkably stable to heat in acidic environment: at pH 5, only about 15% is degraded after seven hours in boiling water (therefore, adding lemon juice to brewed tea does not cause any reduction in their content) (Anonymous, 2015 a).

4.1.5 Effect of different processing treatments on catechu colour

The catechu extract was added to milk @ 1% and subjected to thermization, boiling and sterilisation temperatures. Immediately after the addition of the extract, the milk looked brown (Table-4.5) whose L value was 200.63. On thermization, there was no change in L value (201.44), but on sterilisation, there was slight increase in L value

(204.67) indicating milk became light brown. Boiling of milk also, there no effect on both sample but slightly luminosity of coloured sample increased from 200 to 204. This shows that processing has little effect on the colour heat stability. The decrease in colour may be attributed to effect of milk proteins.



(a) Fresh extract (b) Thermised extract (c) Boiled extract (d) Sterilised extract

Fig. 4.2: Colour of catechu extract as affected by various heat treatments

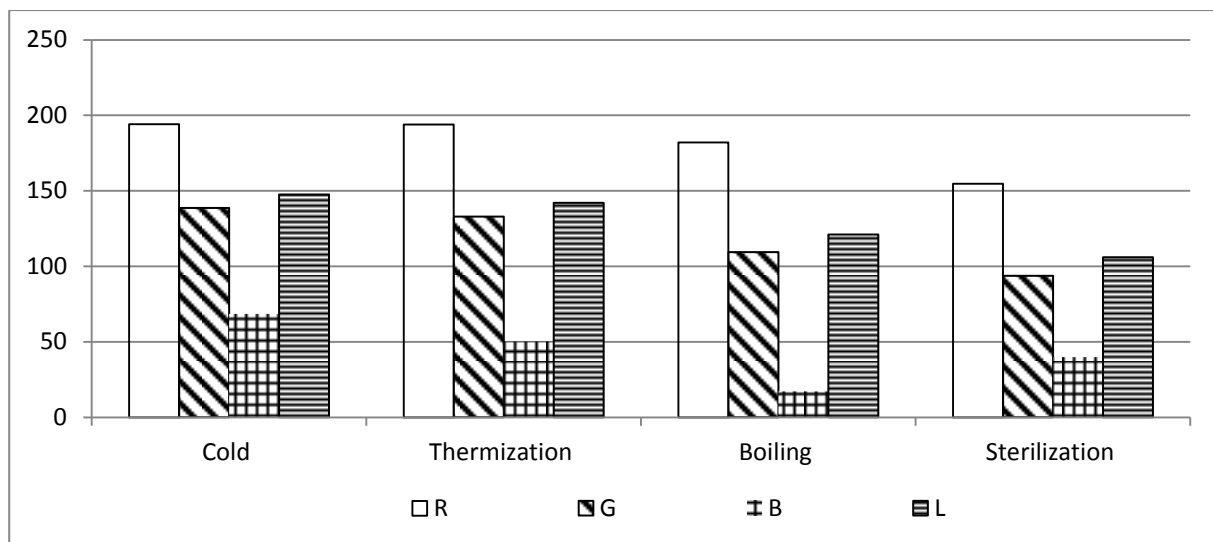


Fig. 4.3: Instrumental colour parameters of catechu extract as influenced by various heat treatments

Table-4.3: Effect of exposure to light on the colour of catechu extract

(a) Presence of light

| Days | R | G | B | L | Recreated colour |
|------|--------|--------|-------|--------|------------------|
| 0 | 198.83 | 149.04 | 97.06 | 158.13 | |
| 10 | 195.62 | 123.84 | 34.41 | 135.49 | |

(b) Absence of light







| Days | R | G | B | L | Recreated colour |
|------|--------|--------|-------|--------|---|
| 0 | 198.83 | 149.04 | 97.06 | 158.13 |  |
| 10 | 201.14 | 130.27 | 48.6 | 142.47 |  |

Table-4.4: Instrumental colour parameters of catechu extract as influenced by various heat treatments

| Treatment | R | G | B | L | Recreated colour |
|---------------|--------|--------|-------|--------|---|
| Cold (20° C) | 194.09 | 138.67 | 68.37 | 147.46 |  |
| Thermization | 194 | 132.9 | 50.25 | 142.06 |  |
| Boiling | 182.08 | 109.29 | 17.18 | 121 |  |
| Sterilization | 154.68 | 93.68 | 39.83 | 105.94 |  |

4.1.6 Antimicrobial property of catechu colour concentrate

The catechu extract was prepared by heat extraction method and evaluated for any antimicrobial properties by agar diffusion method (section 3.4.5). The size of zone of inhibition indicated the antimicrobial property, the higher the diameter, the more is the antimicrobial property.

Different amounts of catechu extract were taken in agar wells, namely, 50, 100, 150 and 200 µl which produced inhibition zones of diameter 8, 10, 16 and 27 mm respectively (Table-4.6). This indicated that catechu extract has exhibited some anti – *E. coli* property. The diameter of the zone increased with increasing amount of the extract. This has confirmed the anti-*E. coli* property of catechu extract. Catechu contains catechin and eppicatechin which are known to be good antioxidants (Anonymous, 2015b), hence it might have displayed antimicrobial property. There are several reports confirming the antimicrobial property of phytochemicals (Ramadan *et al.*, 1972; Galli *et al.*, 1985; Wilkins and Board, 1989; Tassou *et al.*, 2004). However, antimicrobial activity demonstrated in vitro is not necessarily a good indication of practical value in food preservation. The active compounds are often bound with food

Table-4.5: Effect of various processing treatments on catechu colour

(a)

| Product | At 20° C | | | | Recreated colour |
|--------------------------------|----------|--------|--------|--------|------------------|
| | R | G | B | L | |
| Plain milk (Control) | 254.52 | 254.63 | 243.64 | 252.43 | |
| Milk added with catechu colour | 223.74 | 194.51 | 171.17 | 200.63 | |

(b)

| Product | Thermization (60° C) | | | | Recreated colour |
|--------------------------------|------------------------|--------|--------|--------|------------------|
| | R | G | B | L | |
| Plain milk (Control) | 254.44 | 254.69 | 235.93 | 252.62 | |
| Milk added with catechu colour | 225.69 | 194.91 | 171.10 | 201.44 | |

(c)

| Product | Boiling | | | | Recreated colour |
|--------------------------------|---------|--------|--------|--------|------------------|
| | R | G | B | L | |
| Plain milk (Control) | 254.68 | 254.67 | 231.12 | 252.06 | |
| Milk added with catechu colour | 225.96 | 199.23 | 176.50 | 204.67 | |

(d)

| Product | Sterilisation (121° C) | | | | Recreated colour |
|--------------------------------|------------------------|--------|--------|--------|------------------|
| | R | G | B | L | |
| Plain milk (Control) | 244.83 | 229.77 | 194.02 | 230.23 | |
| Milk added with catechu colour | 226.69 | 199.48 | 173.09 | 204.66 | |

Table-4.6: Antimicrobial activity of aqueous catechu extract *on *E. coli*

| Quantity(µl) | Zone of inhibition(diameter-mm) |
|--------------|---------------------------------|
| 50 | 8 |
| 100 | 10 |
| 150 | 16 |
| 200 | 27 |

* CCC with about 10% TS

components (e.g. proteins, fats, sugars, salts). Therefore, only a proportion of the total dose added to a food remains free to exert antibacterial activity (Tassou *et al.*, 2004). Growth of *Escherichia coli* was inhibited by oregano essential oil (EO) in broth cultures (Skandamis *et al.*, 2001).. However, no reports exist on antimicrobial property of catechu extract against *E.coli*.

4.2 METHOD OF PREPARATION OF CATCHU COLOUR CONCENTRATE

The flow chart of optimised method of preparation of catechu colour concentrate is given in Fig. 4.4. About 100 g of catechu heartwood pieces (4-5 cm) were washed under tap water to remove adhering dust. They were then boiled in 500 ml water for about 20 min. The catechu pieces were removed from the solution by filtration and again boiled in a fresh 500 ml lot of water. In such a manner, 6-7 extractions were collected. All the extracts obtained were pooled in a container and heat concentrated to a final volume of about 25 ml. This was called catechu colour concentrate (CCC) which contained about 10% TS.

4.3 UTILIZATION OF CATECHU COLOUR CONCENTRATE (CCC) IN SELECTED DAIRY PRODUCTS

4.3.1 Optimisation of CCC in selected dairy products

Utilization of CCC as colouring agent in flavoured milk preparation

As mentioned earlier, Catechu heartwood gives reddish brown colour, but not smell and aroma. The prepared catechu colour concentrate (CCC) was used in pasteurised as well as sterilised flavoured milk as colouring agent and results are reported.

4.3.1.1 Effect of CCC on sensory quality of flavoured milk

When the Catechu heartwood colour was used for colouring purpose, the colour and appearance of the FM was enhanced, mainly at 0.8% level because it imparted desirable colour to the product (Table-4.7).

The color and appearance (CA) score of control FM was 7.42 which increased with increasing addition of CCC. However, the color and appearance (CA) score increased up to 0.8% of Catechu heartwood colour utilization (score 8.09). But thereafter, the CA score significantly reduced up to 1.6% Catechu heartwood colour (score 7.65).

But it was noted that the scores of CA remained in acceptable limit. The use of 0.8% CCC imparted brown colour to FM which found most acceptance with the judges.

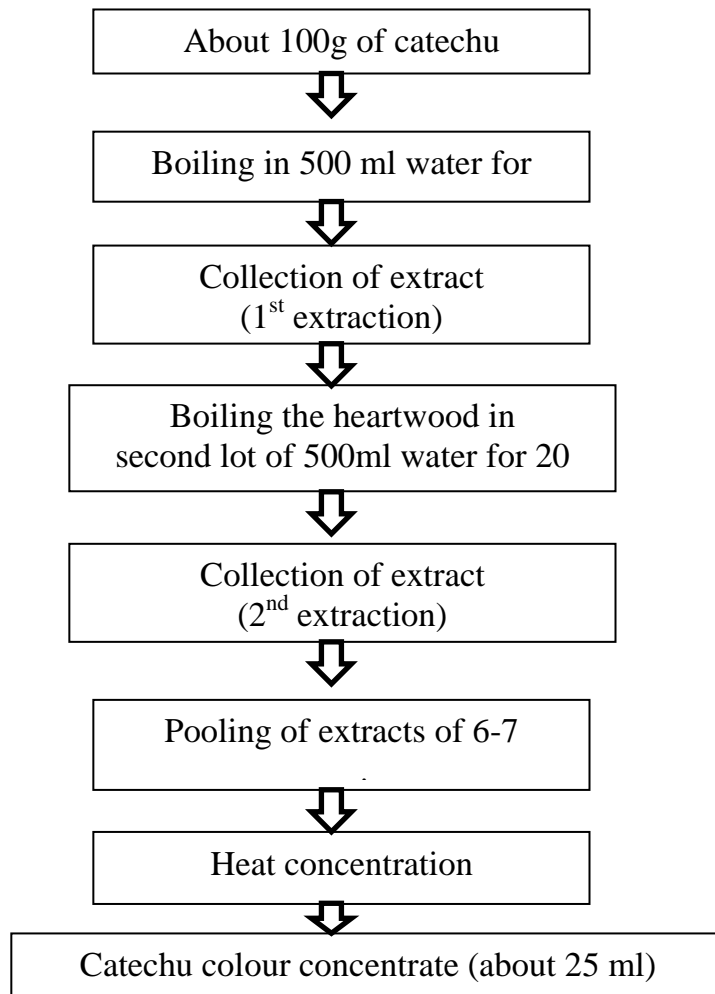


Fig. 4.4: Flow chart for method of preparation of catechu colour concentrate (CCC)

Regarding Body and Texture (BT), it may be noticed from the results presented in Table-4.7 that it remained almost unaffected up to 0.8% level of CCC (scores 7.78 – 7.94), but 1.6% CCC addition showed decreased score of 7.81. However, this aspect has not been confirmed by any supportive tests like proteolysis estimation. The flavour parameter was not significantly changed by CCC. The flavour score of the control sample without addition of CCC was 7.69, whereas that for 0.8% CCC addition was 7.71 and for 1.2% addition it was 7.90 which statistically was not significant compared to control score. This shows that CCC addition did impart any flavour to FM. The Overall Acceptability (OA) score for control sample was 7.42

Table- 4.7: Effect of CCC level on sensory acceptance score of flavoured milk

| CCC level, % | Colour appearance | & Body texture | & Flavour | Overall acceptance |
|--------------|-------------------------|------------------------|------------------------|-------------------------|
| 0 | 7.43±0.19 ^a | 7.78±0.21 ^a | 7.69±0.11 ^a | 7.42±0.21 ^a |
| 0.4 | 7.63±0.22 ^a | 7.86±0.14 ^a | 7.80±0.17 ^a | 7.65±0.17 ^{ab} |
| 0.8 | 8.09±0.18 ^b | 7.94±0.05 ^a | 7.71±0.45 ^a | 8.10±0.11 ^c |
| 1.2 | 7.79±0.30 ^{ab} | 7.87±0.10 ^a | 7.90±0.16 ^a | 7.85±0.21 ^{bc} |
| 1.6 | 7.65±0.46 ^a | 7.81±0.17 ^a | 7.85±0.14 ^a | 7.65±0.37 ^{ab} |

Note: Values with different superscripts in a row are significantly different ($P \leq 0.05$); CCC – Catechu colour concentrate






which significantly enhanced to 8.1 for 0.8% addition of CCC. The effect of addition of CCC was significant on CA and thereby OA of flavoured milk (ANOVA, Ap-1, Table-4.7a).

4.3.1.2 Effect of CCC level on instrumental colour parameters of flavoured milk

The effect of addition of CCC on colour of FM was also checked by measuring instrumental colour parameters [(Table-4.8 and ANOVA, Ap-2 (Table-4.8a)]. The plain pasteurised FM had R,G,B and L values of 254.45, 254.67, 240.69 and 252.84 respectively. These values decreased on addition of CCC with least values being in FM with 1.6% CCC (R-215.04, G- 180.53, B- 157.32 and L-188.53. The decreasing L- values showed that the FM became darker with addition of CCC. This trend was also reflected in the recreated colour blocks given in Table- 8. ANOVA (Ap-2, Table-8a) showed that the decrease in all the colour parameters was statistically significant ($P < 0.05$).

Table-4.9 shows the comparative sensory scores of plain FM and sterilised flavoured milk with and without CCC. The CA scores of plain pasteurised milk was 7.18 which significantly increased to 7.396 on sterilisation. The plain FM with added CCC scored 7.67 which on sterilisation was 7.759 both of which were not significantly different indicating that there was no effect of sterilisation on CA of CCC added FM.

Table-4.8: Effect of CCC level on instrumental colour parameters of flavoured milk

| CCC level, % | R | G | B | L | Recreated colour |
|--------------|--------------------------|--------------------------|--------------------------|--------------------------|---|
| 0 | 254.45±0.01 ^e | 254.67±0.06 ^e | 240.69±3.38 ^e | 252.84±0.65 ^b |  |
| 0.4 | 237.06±0.06 ^d | 215.92±3.79 ^d | 191.32±4.18 ^d | 194.25±51.0 ^a |  |
| 0.8 | 228.15±0.96 ^c | 199.97±1.39 ^c | 176.77±1.72 ^c | 205.69±1.31 ^a |  |
| 1.2 | 223.32±0.69 ^b | 192.40±0.57 ^b | 169.27±0.80 ^b | 199.02±0.46 ^a |  |
| 1.6 | 215.04±1.61 ^a | 180.53±1.10 ^a | 157.32±2.40 ^a | 188.53±1.91 ^a |  |

Note: Values with different superscripts in a column significantly differ at P<0.05; CCC – Catechu colour concentrate

Similarly, the BT score of plain pasteurised milk was 7.38 and that of sterilised one was 7.51 which were statistically similar. However, the BT score significantly on sterilisation of colour containing FM 7.38 and 7.65). The flavour score of plain FM was 7.255 and that of colour added FM was 7.52, but there was no significant difference between them, indicating that CCC had no influence on flavour of FM. However, there was a significant increase in flavour on sterilisation both in plain as well as CCC containing FM (scores 7.51 and 7.77), which is because of desirable cooked flavour imparted by the process (Israr *et al.*, 2014) Overall it was noted that the acceptance of FM significantly enhanced because addition of CCC as well as sterilisation. ANOVA also reflected these observations (Ap-3, Table -4.9a).

4.3.1.3 Effect of CCC and heat treatment on instrumental colour parameters of flavoured milk

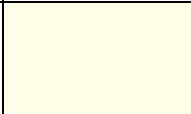



Table-4.10 shows the effect of added CCC and sterilisation treatment on colour parameters of FM. The RGB and L values of plain FM were 254.624, 254.65, 232.21 and 252.14 respectively which reduced to 251.236, 234.62, 198.64 and 248.22 on sterilisation. Out of these green and blue significantly decreased while decrease in red and L was not significant. The change in reflectance determined by Reflectance meter was also not significant (90.125 and 88.5%). However, on colour addition there was

Table-4.9: Effect of CCC level on sensory scores of plain and sterilised flavoured milks

| Treatment | Colour & appearance | Body & texture | Flavour | Overall acceptance |
|------------------------|---------------------|-------------------|-------------------|--------------------|
| CCC + sterilization | 7.76 ^b | 7.65 ^b | 7.77 ^c | 7.76 ^d |
| Plain + sterilization | 7.40 ^c | 7.51 ^a | 7.43 ^b | 7.38 ^b |
| Plain + pasteurisation | 7.18 ^a | 7.38 ^a | 7.26 ^a | 7.19 ^a |
| CCC + pasteurisation | 7.67 ^b | 7.47 ^a | 7.52 ^a | 7.57 ^c |

Note: Values with different superscripts in a column significantly differ at P<0.05; CCC – Catechu colour concentrate

Table- 4.10: Effect of CCC and heat treatment on instrumental colour parameters of flavoured milk

| Sample description | R | G | B | L | Reflectance, % | Recreated colour |
|-----------------------------|---------------------|---------------------|---------------------|---------------------|--------------------|---|
| Plain + pasteurisation | 254.62 ^b | 254.65 ^c | 232.21 ^d | 252.14 ^b | 90.13 ^b |  |
| Plain+ sterilization | 251.23 ^b | 234.62 ^b | 198.64 ^c | 248.22 ^b | 88.50 ^b |  |
| CCC (0.8%) + pasteurisation | 243.74 ^a | 209.63 ^a | 186.75 ^a | 217.43 ^a | 73.31 ^a |  |
| CCC (0.8%) + sterilisation | 245.11 ^a | 212.28 ^a | 182.89 ^a | 218.84 ^a | 74.56 ^a |  |

Note: Values with different superscripts in a column significantly differ at P<0.05; CCC – Catechu colour concentrate

significant decrease in all the attributes including % reflectance. But when the color added FM was sterilised, there was no significant change in the RGLB and % reflectance values (243.738, 209.63, 186.75, 217.43 and 73.312 before sterilisation; 245.109, 212.28, 182.89, 218.84 and 74.562 after sterilisation treatment) (Table-4.10). ANOVA however indicated that there was a significant changes in all colour

Table-4.11: Effect of CCC on viscosity of flavoured milk

| Treatment | Kinematic viscosity of treated milk (cS) |
|----------------------|--|
| CCC + sterilisation | 2.42±0.62 ^b |
| Plain+sterilization | 2.45±0.67 ^b |
| Plain+pasteurisation | 2.14±0.47 ^a |
| CCC + pasteurisation | 2.14±0.46 ^a |

Note: Values with different superscripts in a column significantly differ at P<0.05; CCC – Catechu colour concentrate

parameters on colour addition (Ap-4, Table-4.10a). Lewis and Deeth (2009) reported that retort sterilisation of milk leads to brown colour caused by Maillard reactions.

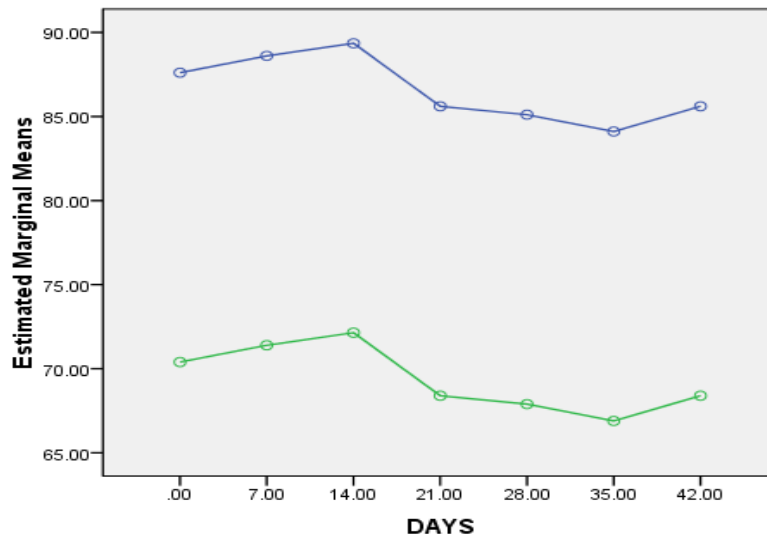
4.3.1.4 Effect of CCC on viscosity of flavoured milk

Kinematic viscosity of FM was determined and the results are presented in Table-4.11. The viscosity values of plain pasteurised, plain sterilised, CCC added and CCC added & sterilised were 2.14, 2.45, 2.14 and 2.42 cS, respectively. These results indicate that on sterilisation the viscosity significantly increased i.e. the milk becomes slightly viscous, but on colour addition there was no change in the viscosity value. This however significantly increased when CCC added FM was sterilised. In both cases, the effect of CCC was not significant (P>0.05). ANOVA indicated significant effect of treatments (Ap-5, Table-4.11a). Since the quantity of CCC added was only to an extent of 0.8%, it had no influence on the viscosity of FM, however on sterilisation it is known that milk becomes slightly more viscous (Ismail *et al.*, 1973) which may be attributed to protein – protein interactions (Law *et al.*, 1994) and changes in salt balance. The insignificant effect on viscosity between the plain sterilised and CCC added sterilised milks shows that CCC had no influence on viscosity even on sterilisation.

4.3.1.5 Effect of storage on colour stability of CCC added sterilised flavoured milk

The CCC added FM was filled in 200 ml retort able bottles, sterilised at 121°C for 15 min and stored at RT. The stability of the colour was recorded. This study is required because the colour should be stable during storage in retail market. The plain sterilised milk had a reflectance of about 87% on zero day which first increased

and then decreased during storage (Fig. 4.5). The initial % reflectance of CCC containing sterilised milk was about 73%, which followed similar trend as above during storage. This indicates that the CCC was not affected during storage. The changes observed



Upper line-plain sterilized, Bottom line-coloured sterilized

Fig. 4.5: Effect of storage on reflectance of plain and sterilised flavoured milks

were attributed to the inherent changes taking place in sterilised milk during storage especially Maillard browning. Though Maillard reactions are initiated during heat processing, they continue during storage though resulting formation of different products (Reineccius, 2005).

Changes in kinematic viscosity of FM during storage are presented in Table 4.12. It was observed that the viscosity decreased in both plain sterilised milk as well as CCC added sterilised milk. It shows that the effect was same in both the cases as t-test showed no significance in both the data (Table-4.12). Johannes and van Boekel (2003) reported that though increase in viscosity of sterilised milk is commonly reported, decrease in viscosity is also common in high treated milks related to protein denaturations. This report supports the finding in the present study that viscosity decreased in initially and then increased.

Utilisation of CCC as colouring agent in shrikhand preparation

4.3.1.6 Effect of CCC on sensory scores of shrikhand

Table-4.12: Effect of storage on kinematic viscosity of plain and sterilised flavoured milk

| Days | Plain sterilised flavoured milk (cS) | CCC added and sterilised flavoured milk (cS) |
|---------------|--------------------------------------|--|
| 7 | 1.876 | 2.08 |
| 14 | 1.996 | 1.99 |
| 21 | 1.94 | 1.96 |
| 28 | 1.94 | 1.92 |
| 35 | 2.07 | 2.06 |
| 42 | 2.01 | 2.02 |
| <i>t-stat</i> | - 0.95 ^{NS} | |

NS – Not significant (P>0.05); CCC – Catechu colour concentrate

Table-4.13: Effect of CCC on sensory scores of shrikhand

| CCC level, % | Colour & appearance | Body & texture | Flavour | Overall acceptance |
|--------------|-------------------------|------------------------|-------------------------|------------------------|
| 0 | 7.59±0.11 ^{ab} | 7.76±0.13 ^a | 7.57±0.10 ^a | 7.67±0.09 ^a |
| 0.5 | 7.56±0.11 ^{ab} | 7.68±0.13 ^a | 7.62±0.08 ^a | 7.51±0.14 ^a |
| 1 | 7.68±0.19 ^{ab} | 7.68±0.12 ^a | 7.69±0.14 ^a | 7.68±0.18 ^a |
| 1.5 | 7.87±0.19 ^b | 7.69±0.12 ^a | 7.72±0.10 ^{ab} | 7.97±0.12 ^b |
| 2 | 7.63±0.31 ^{ab} | 7.65±0.16 ^a | 7.60±0.20 ^a | 7.65±0.21 ^a |
| 2.5 | 7.47±0.32 ^a | 7.55±0.20 ^a | 7.50±0.16 ^a | 7.46±0.18 ^a |

Note: Values with different superscripts in a column significantly differ at P<0.05; CCC – Catechu colour concentrate

Shrikhand is a fermented product which when not added with saffron, is white in colour. However, natural colours like catechu may be added not only to enhance its consumer appeal but also to match any fruit addition like sapota pulp or addition of cocoa. Hence, CCC addition was tried in shrikhand. During shrikhand making, CCC was added to chakka at various levels (0 – 2.5%). It can be seen that the CA scores of control shrikhand was 7.59 which changed to 7.56, 7.68, 7.87, 7.63 and 7.47 respectively by addition of 0.5, 1.0, 1.5, 2.0 and 2.5% addition of CCC (Table-4.13). It has been shown that addition of CCC to chakka during shrikhand preparation significantly enhanced the acceptability scores (vide ANOVA in Ap-6, Table-4.13a). There was no statistical difference among almost all levels in all attributes, but 1.5% rate of addition of CCC had significantly higher CA, flavour and OA scores. Though the CCC did not contribute to flavour, the pleasant colour imparted by CCC might

Table-4.14: Effect of CCC addition on instrumental colour parameters of shrikhand

| CCC level, % | R | G | B | L | Reflectance | Recreated colour |
|--------------|------------------------|------------------------|------------------------|-------------------------|------------------------|------------------|
| 0 | 254.3±0.2 ^b | 251.1±3.7 ^b | 207.7±8.3 ^a | 247.2±3.1 ^e | 94.2±2.1 ^e | |
| 0.5 | 250.3±1.3 ^b | 228.7±4.1 ^b | 186.9±6.9 ^a | 230.5±3.2 ^d | 87.7±1.0 ^d | |
| 1 | 249.1±1.7 ^b | 224.6±3.8 ^b | 182.4±8.4 ^a | 227.2±2.9 ^{cd} | 84.7±2.9 ^{cd} | |
| 1.5 | 248.2±2.5 ^a | 219.4±3.4 ^b | 176.1±6.4 ^b | 223.1±2.6 ^{bc} | 82.5±3.0 ^{bc} | |
| 2 | 245.4±3.2 ^a | 211.9±6.1 ^a | 166.8±8.3 ^b | 216.8±5.2 ^a | 79.0±2.1 ^{ab} | |
| 2.5 | 248.2±3.0 ^a | 213.1±6.7 ^a | 165.2±7.9 ^b | 218.2±5.5 ^{ab} | 75.8±3.5 ^a | |

CCC – Catechu colour concentrate; ; Note: Values with different superscripts in a column significantly differ at P<0.05

have influenced judges' perception of flavour. Colour is known to have influence on flavour quality as well (Spence *et al.*, 2010).

4.3.1.7 Effect of CCC addition on instrumental colour parameters of shrikhand

The initial RGBL and Reflectance values of plain shrikhand were 254.3, 251.1, 207.7, 247.2 and 94.2% respectively. There was decrease in all these values on addition of CCC. These values gradually decreased significantly to 248.2, 213.1, 165.2, 218.2 and 75.8 on addition of CCC @2.5% on chakka basis. The recreated colour blocks given in Table-4.14 also reflected this trend. ANOVA (Ap-7, Table-4.14a) also indicated significant effect of CCC on colour parameters of shrikhand.

4.3.1.8 Effect of CCC on textural properties of shrikhand

The effect if any of CCC on the instrumentally measured textural properties of shrikhand was evaluated. The CCC added was up to an extent of 2.5% which is very less and may not be expected to alter the textural properties, Yet, the dilution aspect and its impact on proteins may not be ruled out. Hence, the properties were determined.

Table- 4.15: Effect of CCC on textural properties of shrikhand

| CCC level, % | Firmness(N) | Consistency (N.Sec) | Adhesive force (N) | Index of viscosity(NS ⁻¹) |
|--------------|------------------------|-------------------------|------------------------|---------------------------------------|
| 0 | 1.42±0.58 ^a | 8.30±4.88 ^a | 1.26±0.49 ^a | 0.54±0.27 ^a |
| 0.5 | 1.34±0.59 ^a | 8.32±5.59 ^a | 1.16±0.57 ^a | 0.77±0.48 ^a |
| 1 | 1.27±0.44 ^a | 7.11±3.048 ^a | 1.03±0.37 ^a | 0.68±0.36 ^a |
| 1.5 | 1.17±0.58 ^a | 7.06±5.54 ^a | 1.02±0.69 ^a | 0.68±0.53 ^a |
| 2 | 0.99±0.35 ^a | 5.85±3.75 ^a | 0.86±0.45 ^a | 0.60±0.40 ^a |
| 2.5 | 1.09±0.45 ^a | 4.86±4.40 ^a | 0.85±0.41 ^a | 0.42±0.49 ^a |

Note: Values with similar superscripts in a column do not differ significantly (P>0.05; CCC – Catechu colour concentrate

The firmness values varied from 1.42 N in control to 1.09 N in 2.5% CCC added shrikhand. There seems to be decrease in firmness value, but statistical analysis revealed no significant difference (ANOVA, Ap-8,Table-4.15a). Similarly, the consistency, adhesive force and index of viscosity values ranged from 4.86 – 8.30, 0.85 – 1.26 and 0.42 – 0.77 respectively (Table-4.15). Though there was an apparent decrease or increase in all these attributes by the addition of CCC, the decrease / increase was not statistically significant (P>0.05) (Table-4.15a). Shrikhand is semi solid in nature and addition of up to 2.5% CCC might not have made any impact on the textural attributes. It may also be attributed to significant variations in the instrumental values obtained as indicated by SD values. The textural properties of shrikhand depends on number of factors like moisture content of chakka, sugar content used and acidity. (Patel and Chakraborty, 1988). The typical consistency of shrikhand is influenced to a great extent by the moisture, fat and sugar levels in the product. The last two factors also have an influence on flavour (Anonymous, 2015c).

4.3.1.9 Effect of CCC on sensory scores of sapota pulp added shrikhand

The CCC was added @ 1.5% to shrikhand containing 5, 10, 15 and 20% sapota pulp. At these pulp levels, the CA scores were 7.93, 7.96, 8.00 and 7.85 respectively (Table-4.16) was As sapota pulp increased shrikhand colour increased. At 20% level,

Table- 4.16: Effect of CCC on sensory scores of sapota pulp added shrikhand

| 1.5% CCC + Sapota pulp, % | Colour appearance | & Body & texture | Flavour | Overall acceptance |
|---------------------------|------------------------|------------------------|------------------------|------------------------|
| 5 | 7.93±0.12 ^b | 7.86±0.09 ^b | 7.79±0.21 ^b | 7.82±0.27 ^a |
| 10 | 7.96±0.18 ^b | 7.90±0.06 ^b | 7.89±0.21 ^b | 7.90±0.27 ^a |
| 15 | 8.00±0.16 ^b | 7.91±0.13 ^b | 7.98±0.11 ^b | 8.03±0.06 ^b |
| 20 | 7.85±0.22 ^a | 7.80±0.08 ^a | 7.67±0.28 ^a | 7.65±0.26 ^a |
| 20 (without catechu) | 7.56±0.25 ^a | 7.69±0.17 ^a | 7.38±0.17 ^a | 7.43±0.10 ^a |

Note: Values with different superscripts in a column significantly differ at P<0.05; CCC – Catechu colour concentrate

the CA score was slightly less because of darker colour with or without added colour as indicated by scores of 7.85 and 7.56 which were statistically similar. Similar observations were noted with BT and flavour attributes. As far as OA was concerned, all the combinations were well acceptable, however, shrikhand containing 15% pulp with 1.5% added colour was the most acceptable. Addition of colour to 20% pulp containing product was of no use. ANOVA presented in Ap-9, Table-4.16a showed that the addition of colour had significant influence on the sensory attributes of shrikhand containing sapota pulp.

4.3.1.10 Effect of CCC on instrumental textural properties of sapota pulp added shrikhand

Catechu colour showed no significant effect on the textural attributes of shrikhand because amount of colour added was too small to influence the textural properties (Table-4.17). ANOVA (Ap-10, Table-4.17a) showed no significant effect of added colour.

4.3.1.11 Effect of CCC and sapota on instrumental colour parameters of shrikhand

As the sapota level increased, the RGB and L values decreased (Table-4.18). Without colour the whitish shrikhand had R,G,B and L were 254.32, 251.12, 207.68 and 247.25 respectively. These decreased to 238.36, 205.10, 158.45 and 209.91 when

Table-4.17: Effect of CCC on instrumental textural properties of sapota pulp added shrikhand

| 1.5% CCC+Sapota pulp, % | Firmness(N) | Consistency (N.Sec) | Adhesive Force (N) | Index of viscosity (NS ⁻¹) |
|-------------------------|------------------------|------------------------|------------------------|--|
| 5 | 1.30±0.16 ^a | 8.68±1.64 ^a | 1.06±0.21 ^a | 0.81±0.18 ^a |
| 10 | 1.37±0.37 ^a | 9.37±2.42 ^a | 1.13±0.35 ^a | 0.76±0.13 ^a |
| 15 | 1.01±0.10 ^a | 6.79±1.03 ^a | 0.89±0.07 ^a | 0.62±0.09 ^a |
| 20 | 1.00±0.10 ^a | 7.02±0.96 ^a | 0.86±0.12 ^a | 0.64±0.02 ^a |
| 20 (without catechu) | 1.23±0.16 ^a | 8.88±2.21 ^a | 1.10±0.31 ^a | 0.86±0.20 ^a |

Note: Values with similar superscripts in a column do not differ significantly (P>0.05; CCC – Catechu colour concentrate

Table-4.18: Effect of CCC and sapota on instrumental colour parameters of shrikhand

| 1.5% CCC + sapota pulp, % ? | R | G | B | L | Recreated colour |
|------------------------------|--------------------------|--------------------------|--------------------------|-------------|------------------|
| 0(without CCC & sapota pulp) | 254.32±0.22 ^b | 251.12±3.67 | 207.68±8.35 | 247.25±3.11 | |
| 20(without CCC) | 249.80±0.55 ^b | 227.78±0.38 ^b | 179.26±0.60 ^b | 228.57±0.87 | |
| 5 | 242.06±1.62 ^a | 210.38±1.20 ^b | 165.78±4.03 ^b | 214.70±0.56 | |
| 10 | 241.06±4.37 ^a | 207.42±1.40 ^a | 162.21±1.30 ^a | 212.10±2.35 | |
| 15 | 242.49±0.43 ^a | 211.16±3.22 ^b | 165.38±5.92 ^b | 215.48±2.58 | |
| 20 | 238.36±4.43 ^a | 205.10±2.09 ^a | 158.45±1.03 ^a | 209.91±2.40 | |

Note: Values with different superscripts in a column significantly differ at P<0.05; CCC – Catechu colour concentrate

sapota level increased to 20%. The recreated colour also showed a gradual darkening of colour by increasing sapota level. ANOVA (Ap-11, Table-4.18a) showed significant effect of sapota pulp with added CCC. Shambharkar *et al.* (2011) used sapota pulp in shrikhand. In shrikhand several fruit pulps have been tried by other workers. However, sapota pulp being brown in colour is well suited for addition of catechu extract.

Utilisation of CCC as colouring agent in plain lassi

4.3.1.12 Effect of CCC on sensory scores of lassi

CCC was added to plain lassi @ 0.5, 1.0, 1.5 and 2.0% which imparted brown colour to lassi. It may be observed from (Table –4.19) that as CCC addition increased, the CA acceptance score also increased from 7.36 (0.5% level) to 7.87 (1.5% level). The CA score of 1% and thereafter was not significantly different from each other. There was no influence of CCC on BT score as shown by statistically non-significance (ANOVA, Ap-12, Table-4.19a). ANOVA showed significant effect of CCC on flavour, however actual increased value was too less for consideration (Table-4.19). From OA scores it is clear that 1.5% CCC scored significantly more than other levels.

4.3.1.13 Effect of CCC on instrumental colour parameters of lassi

On addition of catechu colour, the lassi attained darker colour (brown). The RGB and L values decreased on increase in CCC level. Initial values of RGB and L values were 254.9, 254.8, 232.8 and 252.4 (Table-4.20). These values decreased to 240.1, 203.2, 159.1 and 209.4, respectively. These values were statistically different only at 2% CCC. ANOVA indicated statistically significant effect of CCC on instrumental colour parameters of lassi (Ap-13, Table-4.20a).

4.3.1.14 Effect of CCC on pH of lassi

pH of lassi varies depending on the extent of dilution employed. George *et al.* (2012) reported pH of lassi as 4.56, while Sutariya and Rao (2014) gave a value of 4.39. Any additive used in lassi may alter its pH and thereby its quality. Hence, the effect of CCC on pH of lassi was studied. It was observed that pH values of lassi added with 0, 0.5, 1.0, 1.5 and 2.0% CCC were 4.79, 4.80, 4.80, 4.81 and 4.80 respectively (Table-21) clearly indicating no effect of the CCC on the pH values ($P > 0.05$) (ANOVAS,

Table-4.19: Effect of CCC on sensory scores of lassi

| CCC level, % | Colour & appearance | Body & texture | Flavour | Overall acceptance |
|--------------|------------------------|------------------------|------------------------|------------------------|
| 0 | 7.36±0.12 ^a | 7.71±0.10 ^a | 7.56±0.11 ^a | 7.54±0.14 ^a |
| 0.5 | 7.46±0.16 ^a | 7.73±0.60 ^a | 7.63±0.11 ^a | 7.55±0.08 ^a |
| 1.0 | 7.72±0.11 ^b | 7.73±0.08 ^a | 7.67±0.13 ^a | 7.67±0.10 ^a |
| 1.5 | 7.87±0.25 ^b | 7.76±0.07 ^a | 7.74±0.12 ^a | 7.92±0.13 ^b |
| 2.0 | 7.65±0.18 ^b | 7.76±0.03 ^a | 7.74±0.11 ^a | 7.78±0.10 ^b |

Note: Values with different superscripts in a column significantly differ at P<0.05; CCC – Catechu colour concentrate

Table-4.20: Effect of CCC on instrumental colour parameters of lassi

| CCC level, % | R | G | B | L | Recreated colour |
|--------------|-------------------------|-------------------------|--------------------------|-------------------------|------------------|
| 0 | 254.9±0.16 ^c | 254.8±0.19 ^e | 232.8±10.41 ^e | 252.4±1.23 ^e | |
| 0.5 | 245.3±1.34 ^b | 223.3±2.71 ^d | 188.9±3.44 ^d | 226.1±2.37 ^d | |
| 1.0 | 243.8±3.37 ^b | 215.1±1.05 ^c | 176.2±0.33 ^c | 219.4±1.59 ^c | |
| 1.5 | 243.7±2.77 ^b | 209.3±1.68 ^b | 167.2±1.56 ^b | 214.1±1.91 ^b | |
| 2.0 | 240.1±2.53 ^a | 203.2±2.48 ^a | 159.1±2.11 ^a | 209.4±2.34 ^a | |

Note: Values with different superscripts in a column significantly differ at P<0.05; CCC – Catechu colour concentrate

Ap-14, Table-4.21a). The pH of lassi depends on the pH of curd from which it is made and dilution made for lassi preparation. But in the present study, curd was directly converted into lassi without any dilution. The consistency and viscosity was measured indirectly by passing it through a funnel (section 3.4.3.2) and expressed in seconds. The elapsed time of control lassi and CCC added lassi varied from 14.08 –

4.3.1.15 Effect of CCC on viscosity measure of lassi

16.08 sec (Table-4.22) and the effect of CCC was not significant (P>0.05) (ANOVA, Ap-15, Table-4.22a). Use of CCC at such low levels as used in the present study is not expected to influence viscosity of lassi unless there are any interactions among

Table-4.21: Effect of CCC level on pH of lassi

| CCC level, % | pH |
|--------------|-------------------------|
| 0 | 4.79±0.053 ^a |
| 0.5 | 4.80±0.054 ^a |
| 1.0 | 4.80±0.049 ^a |
| 1.5 | 4.81±0.041 ^a |
| 2.0 | 4.80±0.049 ^a |

Note: Values with similar superscripts do not differ significantly (P>0.05; CCC – Catechu colour concentrate

Table- 4.22: Effect of CCC level on viscosity measure of lassi

| CCC level, % | NS ⁻¹ |
|--------------|-------------------------|
| 0 | 14.08±2.69 ^a |
| 0.5 | 16.08±1.17 ^a |
| 1.0 | 15.92±1.26 ^a |
| 1.5 | 14.50±2.53 ^a |
| 2.0 | 14.17±3.11 ^a |

Note: Values with similar superscripts do not differ significantly (P>0.05; CCC – Catechu colour concentrate

the constituents. However, (Sutariya and Rao, 2014) observed significant variations in viscosity of lassi by addition of lemongrass distillates.

4.3.1.16 Effect of CCC on sensory scores of cocoa lassi

Before using CCC , cocoa level was optimised in lassi. Cocoa was added @ 0.5, 1.0, 1.5, 2.0 and 2.5% and effect on sensory score studied. The CA scores were 7.41, 7.49, 7.65, 7.87, 8.13 and 7.75 for lassi contain cocoa @ 0, 1.0, 1.5, 2.0 and 2.5% respectively. The maximum scores were for 1.5% CCC with 2.0% cocoa levels (Table-4.23). The influence of cocoa level on BT score of the lassi was not significant (Ap-16, Table-4.23a).

Whereas the flavour scores increased with cocoa levels up to 2% level and thereafter decreased, but not significantly. Maximum flavour score was recorded by 2.0 and 2.5% cocoa level with 1.5% CCC. Similar observation was made with OA scores. Thus it was observed that a cocoa level of 2% was used for further trial.

4.3.1.17 Effect of CCC on instrumental colour parameters of cocoa lassi

Table – 4.23: Effect of CCC on sensory scores of cocoa lassi

| Cocoa level, % | Colour & appearance | Body & texture | Flavour | Overall acceptance |
|----------------|-------------------------|----------------|-------------------------|------------------------|
| 0 | 7.41±0.13 ^a | 7.54±0.29 | 7.32±0.16 ^a | 7.34±0.06 ^a |
| 0.5 | 7.49±0.08 ^{ab} | 7.65±0.28 | 7.46±0.18 ^a | 7.51±0.11 ^b |
| 1.0 | 7.65±0.07 ^{bc} | 7.69±0.22 | 7.61±0.14 ^{bc} | 7.65±0.09 ^b |
| 1.5 | 7.87±0.17 ^{cd} | 7.82±0.12 | 7.84±0.15 ^{bd} | 7.90±0.10 ^c |
| 2.0 | 8.13±0.24 ^{de} | 7.98±0.08 | 8.16±0.09 ^b | 8.15±0.06 ^d |
| 2.5 | 7.75±0.13 ^e | 8.14±0.47 | 8.05±0.14 ^b | 8.06±0.17 ^d |

Note: Values with different superscripts in a column significantly differ at P<0.05; CCC – Catechu colour concentrate

The initial RGB and L values of lassi added with different levels of cocoa were 234.0, 205.2, 167.9 and 209.6. These values gradually decreased on addition of cocoa resulting in darkening of colour (Table-4.24). The influence of cocoa on colour parameters was significant (P<0.05). There was no statistically significant difference between 1.5 and 2% levels with respect to RG and L values. ANOVA (Ap-17, Table-4.24a) showed significant effect of cocoa on colour parameters of Cocoa lassi. The recreated colour boxes also indicate this trend.

4.3.1.18 Effect of CCC on sensory quality of cocoa lassi

CCC @ 1.5% was added to 2% cocoa containing lassi and sensory quality studied (Table-4.25). The CA scores were 7.76 and 8.35 before and after addition of CCC which significantly enhanced colour quality. Whereas, the effect on BT was not much significant as indicated by scores of 7.88 and 8.00 respectively. There was an increase in flavour quality of the lassi as observed by scores of 7.81 and 8.16. Though CCC did not contribute to flavour, the increase in flavour score might be attributed to enhanced colour quality. For similar reason, the OA score also significantly enhanced by CCC addition. This was reflected in RGB and L values (Table-4.26). The addition of CCC to chocolate lassi decreased the colour parameters including Luminosity (196.41 and 189.05).

Table-4.24: Effect of CCC on instrumental colour parameters of cocoa lassi

| Cocoa level, % | R | G | B | L | Recreated colour |
|----------------|-------------------------|-------------------------|--------------------------|-------------------------|------------------|
| 0 | 234.0±6.5 ^d | 205.2±7.21 ^e | 167.9±6.47 ^e | 209.6±6.92 ^d | |
| 0.5 | 224.9±2.27 ^c | 195.2±1.35 ^d | 166.8±12.42 ^d | 200.1±1.61 ^c | |
| 1.0 | 220.0±4.82 ^b | 188.7±4.23 ^c | 161.9±16.24 ^c | 194.4±4.35 ^c | |
| 1.5 | 213.2±6.86 ^b | 180.1±5.04 ^b | 146.1±3.13 ^b | 186.4±5.23 ^b | |
| 2.0 | 210.7±9.05 ^b | 176.8±8.51 ^b | 144.0±6.38 ^a | 182.0±9.28 ^b | |
| 2.5 | 195.9±8.30 ^a | 162.4±6.31 ^a | 131.2±1.11 ^a | 168.8±6.60 ^a | |

Note: Values with different superscripts in a column significantly differ at P<0.05; CCC – Catechu colour concentrate

Table-4.25: Effect of CCC on sensory quality of cocoa lassi

| Cocoa and CCC level | Colour appearance | Body & texture | Flavour | Overall acceptance |
|-------------------------------|-------------------|----------------|-----------|--------------------|
| 2% Cocoa+0% catechu extract | 7.76±0.133 | 7.88±0.096 | 7.81±0.16 | 7.81±0.13 |
| 2% Cocoa+1.5% catechu extract | 8.35±0.137 | 8.00±0.133 | 8.16±0.18 | 8.25±0.15 |

CCC – Catechu colour concentrate

Utilisation of CCC as colouring agent in yoghurt preparation

CCC was added to yoghurt mix and incubated (Section 3.2.10). The pH values are given in Table-4.27. It was observed that there was no significant change in pH of

Table-4.26: Effect of cocoa on instrumental colour parameters of cocoa lassi

| Cocoa and CCC level | R | G | B | L |
|-------------------------------|-------------|--------------|--------------|-------------|
| 2% Cocoa+0% catechu extract | 194.61±5.06 | 191.14±5.36 | 162.38±4.67 | 196.41±5.34 |
| 2% Cocoa+1.5% catechu extract | 211.00±4.28 | 160.56±28.69 | 139.28±2.926 | 189.05±3.79 |

CCC – Catechu colour concentrate

Table-4.27: Effect of CCC on pH of yoghurt

| CCC level, % | pH |
|--------------|------------------------|
| 0 | 4.66±0.21 ^a |
| 0.5 | 4.66±0.20 ^a |
| 1 | 4.65±0.20 ^a |
| 1.5 | 4.64±0.19 ^a |
| 2 | 4.64±0.17 ^a |
| 2.5 | 4.56±0.13 ^a |

Note: Values with similar superscripts do not differ significantly (P>0.05); CCC – Catechu colour concentrate

yoghurt (ANOVA, Ap-18, Table-4.27a). This shows that the CCC had no influence on the fermentation during yoghurt preparation. Natural colours like anthocyanin have been used in yoghurt preparation by other workers (Wallace and Glusti, 2008), however no effect on fermentation rate was reported. Though the natural colours are reported to possess good antioxidant property and antibacterial property, the rate of addition was not enough to retard the growth of LAB. Moreover, the catechu extract is not reported to possess anti-bacterial effect against LAB, though it is reported to be effective against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*. bacteria (Lakshmi *et al.*, 2011b).

4.3.1.19 Effect of CCC on sensory scores of yoghurt

Table-4.28: Effect of CCC on sensory scores of yoghurt

| CCC level, % | Colour appearance | Body texture | Flavour | Overall acceptance |
|--------------|-------------------------|------------------------|------------------------|--------------------------------------|
| 0 | 7.55±0.26 ^{ab} | 7.80±0.11 ^a | 7.75±0.29 ^a | 7.59±0.25 ^a |
| 0.5 | 7.44±0.12 ^a | 7.71±0.17 ^a | 7.70±0.18 ^a | 7.45 ^a ±0.02 ^a |
| 1 | 7.60±0.09 ^{ab} | 7.70±0.18 ^a | 7.70±0.19 ^a | 7.63 ^a ±0.08 ^a |
| 1.5 | 7.76±0.15 ^{bc} | 7.75±0.13 ^a | 7.72±0.16 ^a | 7.78 ^a ±0.10 ^a |
| 2 | 8.00±0.16 ^c | 7.74±0.14 ^a | 7.79±0.15 ^a | 8.02±0.14 ^b |
| 2.5 | 7.95±0.24 ^c | 7.62±0.28 ^a | 7.69±0.24 ^a | 7.89 ^b ±0.22 ^b |

Note: Values with different superscripts in a column significantly differ at P<0.05; CCC – Catechu colour concentrate

The yoghurt prepared with different levels of CCC were subjected sensory evaluation in order to determine best acceptable level. The yoghurt added with CCC was brown in colour which was liked by judges. As CCC increased, the yoghurt became browner. The CA scores were 7.55, 7.44, 7.60, 7.76, 8.00 and 7.95 respectively for CCC additions of 0, 0.5, 1.0, 1.5, 2.0 and 2.5% (Table-4.28). It may be noticed that up to addition of 1.5%, though score increased it was statistically not significant. However, 2% addition scored significantly scored higher indicating that at this level, CCC imparted a good acceptable brown colour. At 2.5% addition, there was no further change in the CA score. Further, it was observed that CCC had no significant impact on BT and flavour scores of yoghurt. BT scores ranged from 7.62-7.80 and flavour scores from 7.69-7.75. Overall it was observed that the acceptability remained same up to 1.5% addition of CCC, but thereafter at 2% addition significant improvement was noticed. The OA score increased from an initial value of 7.59 to 8.02. Use of CCC at 2.5% showed no further improvement in OA score. ANOVA (Ap-19, Table-4.28a) indicated statistically significant effect of CCC on CA and OA score, but insignificant effect on BT and flavour attributes.

4.3.1.20 Effect of CCC on instrumental textural properties of yoghurt

Table-29 shows instrumental textural properties of yoghurt prepared with the addition of CCC at levels ranging from 0 – 2.5%. It may be seen that the yoghurt without CCC had firmness, consistency, adhesiveness and index of viscosity 1.28 N, 8.91 N.sec,

Table-4.29: Effect of CCC on instrumental textural properties of yoghurt

| CCC level, % | Firmness(N) | Consistency (N.Sec) | Adhesiveness (N.) | Index of viscosity (NS ⁻¹) |
|-----------------|------------------------|-------------------------|------------------------|---|
| 0 | 1.28±0.44 ^a | 8.91±3.76 ^b | 0.29±0.18 ^a | 0.19±0.15 ^a |
| 0.5 | 1.34±0.61 ^a | 10.32±5.17 ^b | 0.39±0.17 ^a | 0.23±0.11 ^a |
| 1 | 1.08±0.40 ^a | 7.72±2.97 ^{ab} | 0.60±0.45 ^a | 0.41±0.33 ^a |
| 1.5 | 0.97±0.40 ^a | 7.18±2.73 ^a | 0.54±0.28 ^a | 0.32±0.17 ^a |
| 2 | 1.08±0.47 ^a | 7.47±3.16 ^{ab} | 0.49±0.33 ^a | 0.29±0.19 ^a |
| 2.5 | 1.14±0.56 ^a | 9.57±5.14 ^b | 0.52±0.28 ^a | 0.28±0.14 ^a |

Note: Values with different superscripts in a column significantly differ at P<0.05; CCC – Catechu colour concentrate

0.29 N and 0.19 NS⁻¹, respectively. There were changes in these values, however, showed no significant changes except for adhesiveness and index of viscosity. ANOVA (Ap-20, Table-4.29a) indicated statistical significance of CCC on textural properties of yoghurt.

4.3.2 Stability of CCC colour in products during storage

From the previous sections, it was concluded that CCC imparted good CA to freshly prepared dairy products like FM, lassi, shrikhand and yoghurt. However since these products are stored under retail conditions till consumers pick them for consumption, the colour added to them has to remain stable, at least till the product's best before date lasts. Hence, colour stability was checked under normal storage temperature as well as duration. The colour stability was evaluated subjectively as well as by measuring instrumental colour parameters.

Cocoa lassi

4.3.2.1 Effect of storage on instrumental colour parameters of CCC coloured cocoa lassi

The colour added chocolate lassi was stored for 13 days and its colour parameters measured at 2-3 day interval. The results are tabulated in Table-4.30. The initial R,G,B and L values were 219.24, 191.25, 163.32 and 196.47 respectively for lassi with 2% cocoa and 215.63, 179.01, 141.35 and 185.69 for cocoa lassi with added

Table-4.30: Effect of storage on instrumental colour parameters of CCC coloured cocoa lassi

| Days | Product | R | G | B | L | Recreated colour |
|------|---------|--------|--------|--------|--------|------------------|
| 1 | Control | 219.24 | 191.25 | 163.32 | 196.47 | |
| | Sample | 215.63 | 179.01 | 141.35 | 185.69 | |
| 4 | Control | 211.61 | 183.62 | 155.89 | 188.86 | |
| | Sample | 205.74 | 170.58 | 136.05 | 177.18 | |
| 6 | Control | 224.27 | 194.59 | 165.31 | 200.16 | |
| | Sample | 209.31 | 174.35 | 138.97 | 180.8 | |
| 9 | Control | 218.2 | 188.47 | 159.34 | 194.07 | |
| | Sample | 208.04 | 172.72 | 136.83 | 179.22 | |
| 11 | Control | 212.44 | 185.38 | 156.82 | 190.44 | |
| | Sample | 206.25 | 172.26 | 137.21 | 178.25 | |
| 13 | Control | 222.65 | 194.33 | 165.69 | 199.76 | |
| | Sample | 211.81 | 174.71 | 139.65 | 181.78 | |

Control: cocoa @2%, Sample: cocoa @ 2%+1.5%CCC; CCC – Catechu colour concentrate

catechu colour @1.5% (Table-30). By addition of catechu colour, the lassi's L value decreased i.e. became slightly darker. On storage for 13 days there were no much changes in colour values of the lassi indicating stability of the added colour. The recreated colours presented also support more or less the same observation made by L values.

4.3.2.2 Effect of storage on sensory scores of CCC coloured cocoa lassi

The chocolate lassi added with catechu colour was stored at refrigerated condition and observed for any colour changes as well sensory quality. The sensory scores are presented in Fig. 4.6. By addition of catechu colour, the CA score of cocoa lassi (8.0) increased to 8.5 (Fig. 4.6a). Though cocoa powder is mainly flavouring agent, it also contributes to colour. However, use of catechu enhances the colour appeal. The colour is compatible to chocolate containing product like chocolate lassi. The BT scores for control and catechu containing samples were 8.04 and 8.08 respectively (Fig. 4.6b); flavour scores were 8.22 and 8.42 (Fig. 4.6c); and OA scores were 8.12

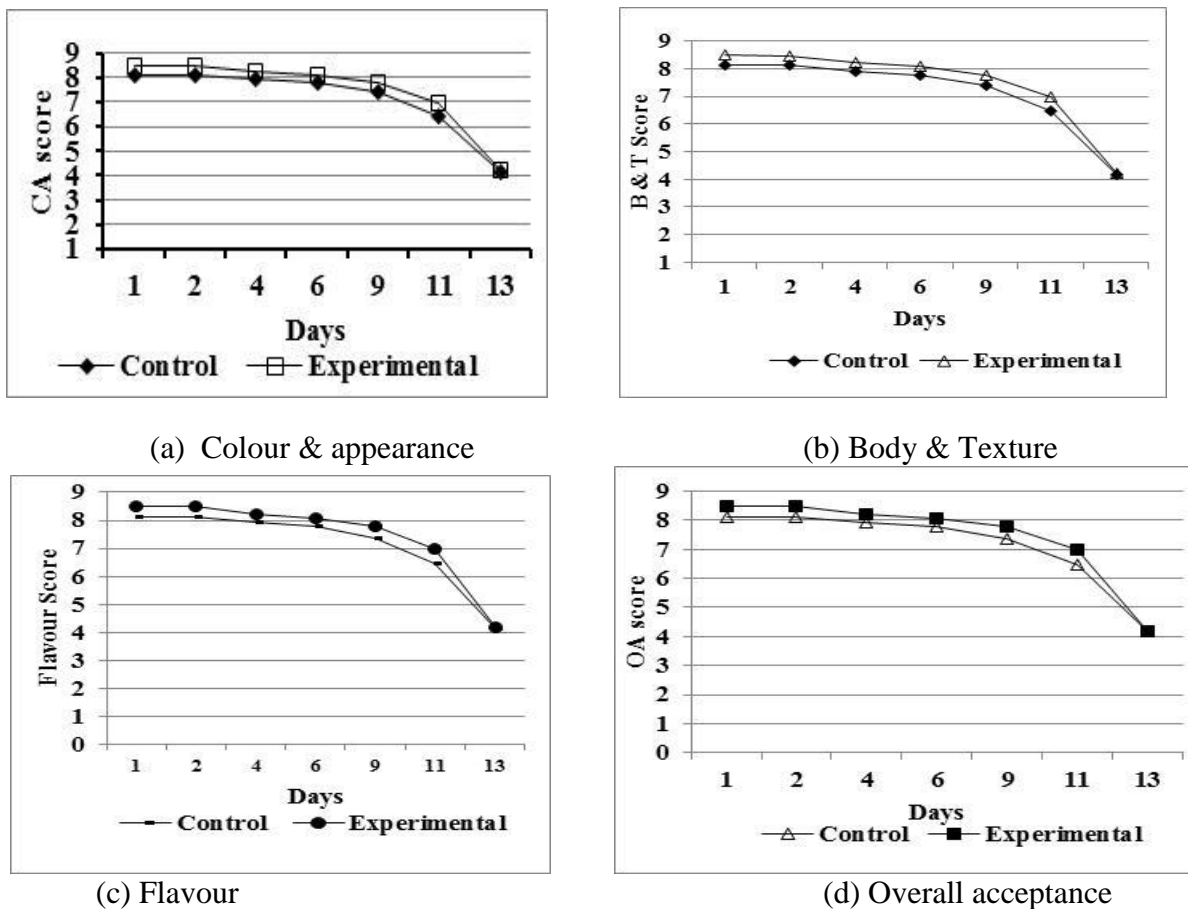


Fig. 4.6: Effect of storage on sensory scores of CCC coloured cocoa lassi

and 8.49 (Fig. 4.6d). The catechu added lassi scored higher for flavour score and thereby higher OA score. Though catechu did not contribute to any flavour, the colour developed by it might have enhanced flavour quality of the product. During storage, the CA scores in general decreased which may be attributed to slight particle separation. However, the colour more or less remained stable during storage. The OA scores remained in acceptable range till 9 days (8.08 and 7.38) and thereafter showed whey separation and yeasty defect.

Shrikhand

4.3.2.3 Effect of storage on instrumental colour parameters of CCC coloured sapota shrikhand

Shrikhand was prepared by incorporation of 15% sapota pulp and 1.5 % catechu extract and the colour parameters were monitored during storage under refrigerated condition (5°C). The initial R,G,B and L value of control were 232.34, 215.29, 181.44

and 216.55 respectively. On addition of catechu colour, these values changed to 219.67, 189.39, 151.98 and 194.22. The L value indicated that the product became darker on addition of the colour which can be discerned by recreated colour shown in Table- 4.31.

Table-4.31: Effect of storage on instrumental colour parameters of CCC coloured sapota shrikhand

| Days | Sample | R | G | B | L | Recreated colour |
|------|---------|--------|--------|--------|--------|------------------|
| 1 | Control | 232.34 | 215.29 | 181.44 | 216.55 | |
| | Sample | 219.67 | 189.39 | 151.98 | 194.22 | |
| 3 | Control | 232.33 | 214.37 | 180.79 | 215.94 | |
| | Sample | 218.19 | 187.88 | 149.74 | 192.64 | |
| 5 | Control | 239.82 | 221.14 | 186.41 | 222.8 | |
| | Sample | 227.19 | 195.05 | 157.6 | 200.42 | |
| 8 | Control | 233.94 | 216.16 | 182.01 | 217.61 | |
| | Sample | 218.94 | 188.24 | 151.04 | 193.21 | |
| 10 | Control | 223.05 | 200.55 | 165.62 | 204.58 | |
| | Sample | 215.84 | 184.13 | 148.4 | 189.54 | |
| 12 | Control | 229.93 | 209.84 | 178.31 | 212.5 | |
| | Sample | 215.47 | 185.62 | 149.61 | 190.58 | |
| 14 | Control | 225.04 | 207.06 | 175 | 208.8 | |
| | Sample | 218.73 | 187.15 | 149.1 | 192.29 | |
| 17 | Control | 229.08 | 210.16 | 176.84 | 212.04 | |
| | Sample | 217.03 | 186.3 | 149.12 | 191.22 | |
| 22 | Control | 231.18 | 212.09 | 178.64 | 214.14 | |
| | Sample | 214.56 | 182.73 | 142.32 | 188.14 | |
| 26 | Control | 230.50 | 211.06 | 177.07 | 213.70 | |
| | Sample | 214.30 | 182.00 | 141.00 | 186.43 | |
| 31 | Control | 229.60 | 210.15 | 176.54 | 212.33 | |
| | Sample | 213.42 | 181.46 | 140.61 | 186.00 | |

Control:15% sapota, Sample:15% sapota+1.5% CCC; CCC – Catechu colour concentrate

During storage, these values changed leading to minor changes in overall colour in both the samples. It may be seen that in control sample, there was not much change in overall shade of colour (L value 216.55 on zero day and 214 on 13th day), in catechu added sample there was slight decrease in L-value from 194.22 on zero day to 188.14 on 13th day, indicating the product became slightly darker. This is because catechu colour has the property of becoming darker on storage as shown in earlier results (section 3.4.1.2).

During storage, these values changed leading to minor changes in overall colour in both the samples. It may be seen that in control sample, there was not much change in overall shade of colour (L value 216.55 on zero day and 214 on 13th day), in catechu added sample there was slight decrease in L-value from 194.22 on zero day to 188.14 on 13th day, indicating the product became slightly darker. This is because catechu colour has the property of becoming darker on storage as shown in earlier results (section 3.4.1.2).

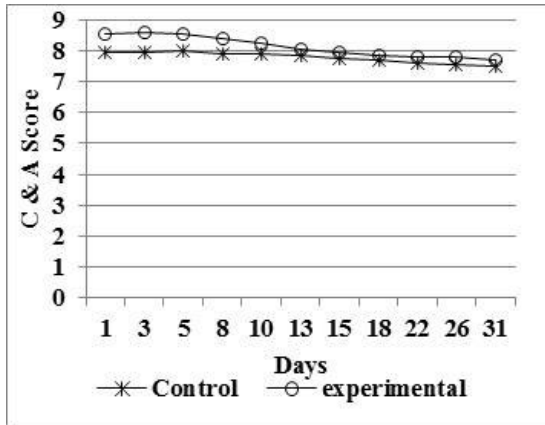
4.3.2.4 Effect of storage on sensory scores of CCC coloured sapota shrikhand

The catechu added shrikhand was subjected to sensory evaluation during storage and scores tabulated in Fig. 4.7. The CA, flavour and OA scores of sapota containing shrikhand were 7.9, 8.36 and 7.96 respectively, whereas these scores were 8.54, 8.44 and 8.54 for catechu added sapota shrikhand (Fig. 4.7a,b,c and d). It shows that by catechu addition, the CA and OA scores increased significantly because of brown colour imparted by catechu extract. It may be observed that the sensory scores decreased during storage. There was not much decrease in CA scores, however, flavour and OA scores decreased considerably. The scores at the end of 26th day were 7.68, 7.3, 7.52 and 7.52 for CA, BT, flavour and OA of control sample respectively; these scores for catechu containing sample were: 7.92, 7.34, 7.64 and 7.68 (Fig. 4.7). These results indicate that all the samples kept well till 26 days.

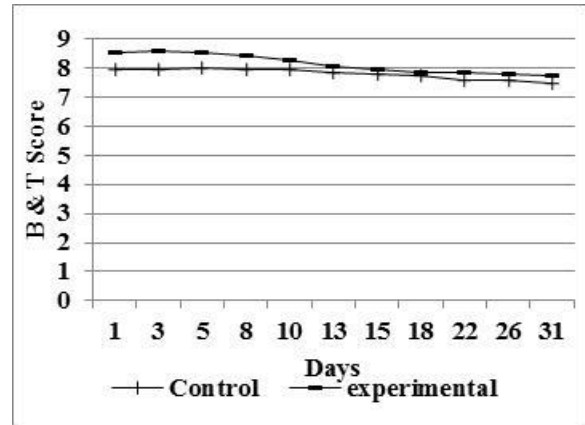
Yoghurt

4.3.2.5 Effect of storage on instrumental colour parameters of CCC coloured yoghurt

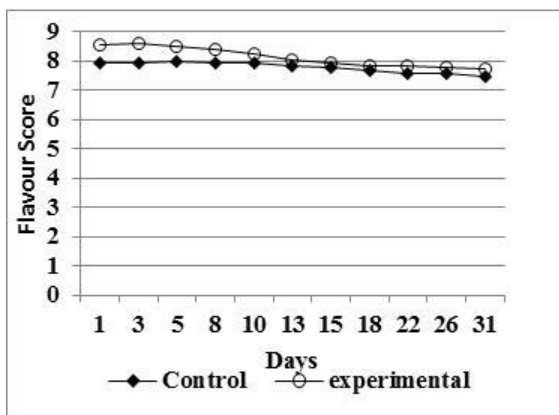
The CCC was used @ 2% in yoghurt and its storage stability checked by measuring RGB and L values which are presented in Table-32. The initial R,G,B and L values of



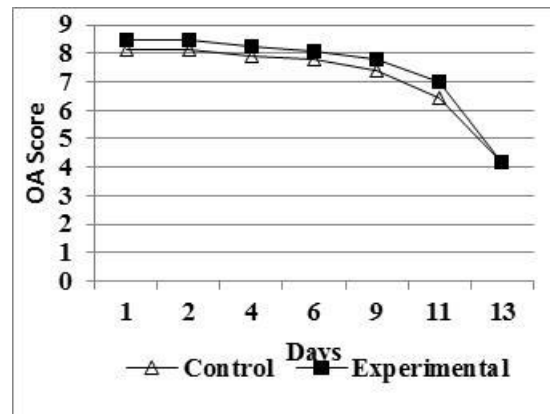
(a) Colour & appearance



(b) Body & Texture



(c) Flavour



(d) Overall acceptance

Fig. 4.7: Effect of storage on sensory scores of CCC coloured sapota shrikhand

CCC added yoghurt were 222.71, 190.76, 155.74 and 196.35 respectively and the recreated colour obtained from RGB values is also given in Table-32 (brown colour as against creamy white in control). These values changed during storage indicating some alterations in the colour of yoghurt. This may be attributed to interactive changes taking place among constituents of yoghurt rather than actual changes in CCC colour. The RGB and L values of CCC added yoghurt at the end of storage period of 24 days were 218.20, 186.55, 152.44 and 191.48 respectively. These values are not much different from those of initial values indicating that there as little change in the initial brown colour.

4.3.2.6 Effect of storage on sensory scores of CCC coloured yoghurt

This was also revealed by the recreated colour blocks given in Table-4.32. The results indicate good stability of CCC colour in yoghurt during storage. The CCC also

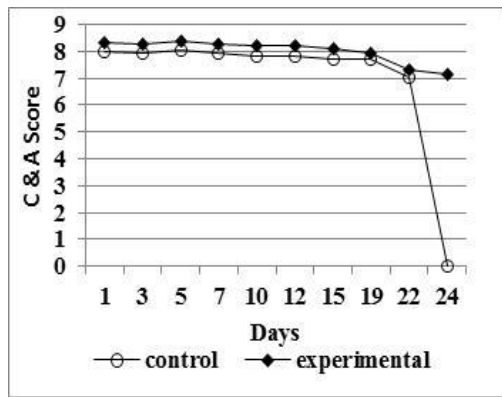
Table-4.32: Effect of storage on instrumental colour parameters of CCC coloured yoghurt

| Days | Product | R | G | B | L | Recreated colour |
|------|---------|--------|--------|--------|--------|------------------|
| 1 | Control | 254.74 | 254.76 | 226.23 | 251.66 | |
| | Sample | 222.71 | 190.76 | 155.74 | 196.35 | |
| 3 | Control | 254.67 | 254.53 | 223.61 | 251.19 | |
| | Sample | 227.75 | 195.35 | 161.08 | 201.17 | |
| 5 | Control | 251.08 | 246.06 | 212.21 | 243.73 | |
| | Sample | 212.79 | 183.43 | 149.99 | 188.43 | |
| 10 | Control | 254.89 | 254.82 | 230.54 | 251.96 | |
| | Sample | 217.99 | 186.27 | 152.31 | 191.91 | |
| 12 | Control | 254.68 | 254.63 | 222.87 | 251.77 | |
| | Sample | 218.85 | 186.97 | 151.97 | 192.54 | |
| 15 | Control | 252.97 | 248.38 | 208.69 | 245.27 | |
| | Sample | 220.56 | 189.35 | 156.96 | 196.02 | |
| 18 | Control | 254.67 | 254.67 | 220.14 | 250.86 | |
| | Sample | 217.19 | 186.16 | 152.99 | 191.68 | |
| 22 | Control | 253.21 | 250.54 | 215.15 | 247.56 | |
| | Sample | 218.65 | 187.12 | 154.46 | 194.2 | |
| 24 | Control | 252.70 | 250.64 | 215.48 | 248.15 | |
| | Sample | 218.20 | 186.55 | 152.44 | 191.48 | |

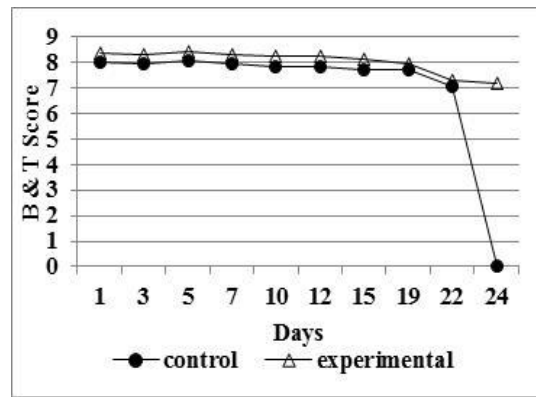
Control: 0 % CCC, Sample:2%CCC; CCC – Catechu colour concentrate

remains unaffected by the fermentation during yoghurt preparation as well as during storage. It is known that fermentation continues to take place during storage of yoghurt, hence its pH and acidity decreases (Jordano *et al.*, 1988; Saint-Eve *et al.*, 2008).

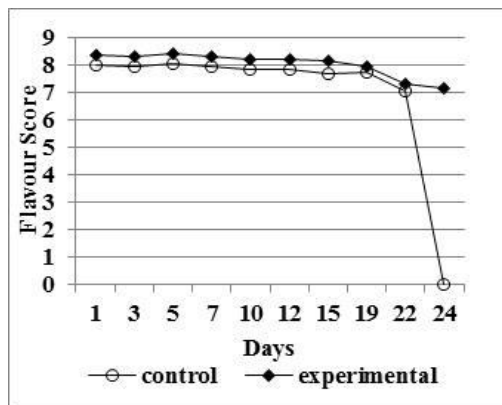
The changes in CCC containing yoghurt during storage were studied mainly with respect to colour and appearance. The initial CA, BT, FL and OA scores of control yoghurt were 7.64, 8.04, 8.08 and 7.98 respectively, whereas these values for CCC yoghurt were 8.20, 8.04, 8.26 and 8.34 indicating better initial acceptance (Fig.8 a,b,c



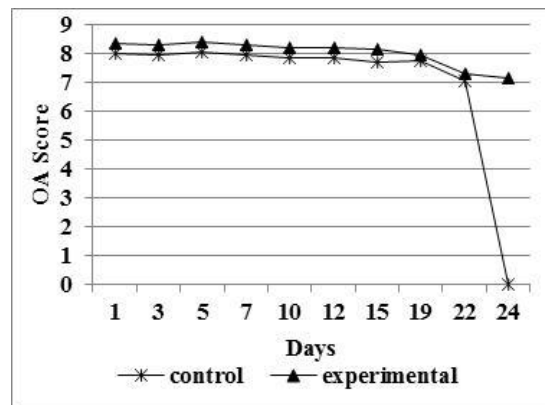
(a) Colour & appearance



(b) Body & Texture



(c) Flavour



(d) Overall acceptance

Fig. 4.8: Effect of storage on sensory scores of CCC coloured yoghurt

and d). These values gradually decreased during storage both in control as well as CCC yoghurt samples. The CA scores remained fairly same (8.2 – 8.16) up to a storage period of 18 days and thereafter slightly reduced to 7.9 and 7.1 on 22nd and 24th day respectively (Fig. 4.8a). However, this decrease may be attributed to changes in appearance rather than colour intensity. The BT score of control decreased from 8.04 on zero day to 7.52 on 22nd day, whereas the score of control sample reduced from 8.04 to 7.82 (Fig. 4.8b). The flavour and OA scores also decreased in a similar ranges. Bansode *et al.* (2012) also reported a shelf life of 8-16 days at 4°C for yoghurt which support observation in the present study.

Flavoured milk

4.3.2.7 Effect of storage on instrumental colour parameters of sterilised flavoured milk

Table-4.33: Effect of storage on instrumental colour parameters of sterilised flavoured milk

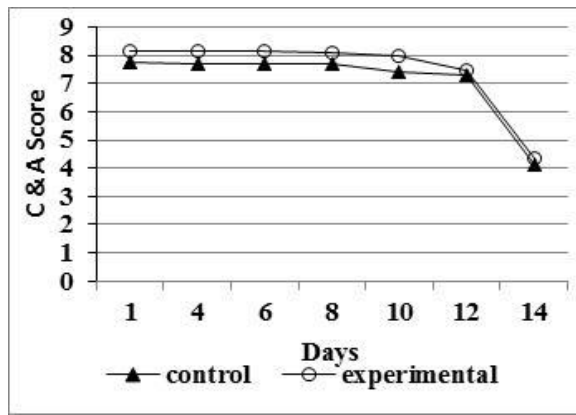
| Days | Product | R | G | B | L | Recreated colour |
|------|---------|--------|--------|--------|--------|------------------|
| 1 | Control | 251.84 | 236.10 | 199.29 | 236.68 | |
| | Sample | 249.14 | 219.21 | 194.16 | 225.34 | |
| 7 | Control | 252.00 | 240.00 | 210.00 | 240.55 | |
| | Sample | 229.73 | 192.86 | 173.17 | 201.63 | |
| 14 | Control | 254.71 | 254.62 | 225.94 | 251.52 | |
| | Sample | 245.35 | 213.86 | 187.61 | 220.30 | |
| 21 | Control | 254.77 | 254.72 | 228.20 | 251.82 | |
| | Sample | 239.83 | 207.71 | 184.37 | 214.67 | |
| 28 | Control | 254.33 | 246.00 | 212.29 | 244.72 | |
| | Sample | 240.92 | 209.89 | 185.71 | 216.43 | |
| 35 | Control | 254.77 | 254.65 | 228.64 | 251.83 | |
| | Sample | 251.00 | 215.82 | 188.79 | 223.26 | |
| 42 | Control | 254.39 | 254.73 | 240.75 | 253.19 | |
| | Sample | 240.00 | 208.50 | 185.00 | 215.00 | |

Control: plain sterilised flavoured milk, Sample: coloured sterilised flavoured milk containing 0.8% CCC

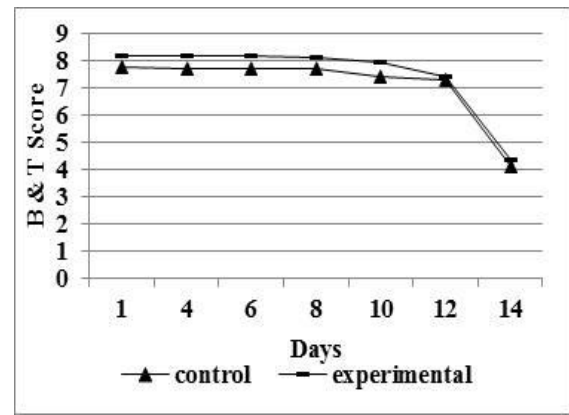
Catechu added sterilised FM was stored in retort able bottles at room temperature (27-30°C) and changes in colour parameters were recorded. The initial R,G,B and L values of control sample were 251.84, 236.10, 199.29 and 236.68 respectively on zero day. These values for catechu added sample were 249.14, 219.21, 194.16 and 225.34 (Table-4.33). The colour added samples were initially darker (Section 4.3.2.6). In case of control, the colour gradually faded during storage as indicated by decreasing L values (Section 4.3.2.6). However, in case of colour added sample, the L value decreased and the sample slightly became darker. This trend indicated that the added colour not only remained unaffected but sterilisation treatment but also increased during storage. The L value in case of colour added sample decreased from 225.34 on zero day to 215 on 42th day. Whereas that for control sample increased from 236.68 to 253.19. The increase in colour of catechu added sample is supported by earlier reported result of its heat stability (section 4.1.4).

4.3.2.8 Effect of storage on sensory scores of CCC coloured flavoured milk

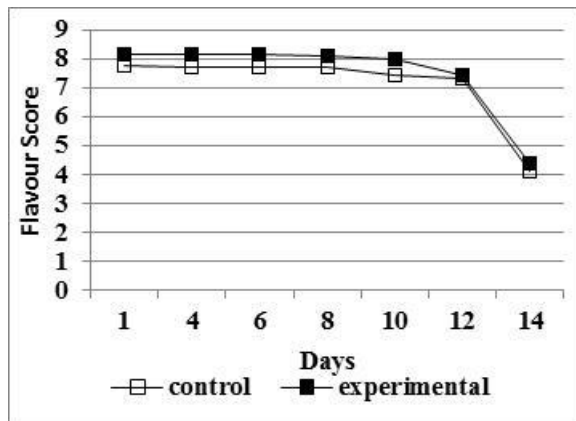
The pasteurised flavoured milk with or without catechu colour was packaged in LDPE pouches and stored under refrigerated condition. Changes in colour and



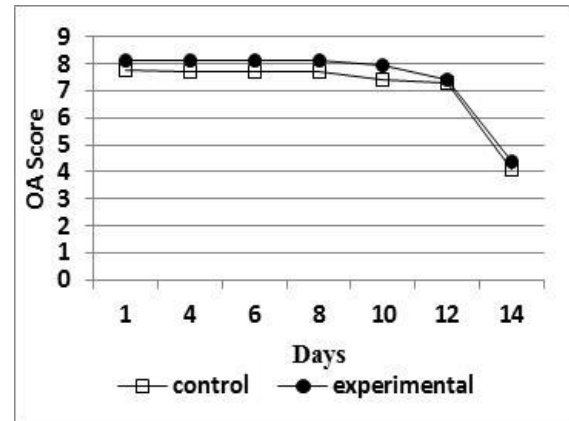
(a) Colour & appearance



(b) Body & Texture



(c) Flavour



(d) Overall acceptance

Fig. 4.9: Effect of storage on sensory scores of CCC coloured flavoured milk

sensory properties were observed. The initial CA score of control flavoured milk (FM) was 7.9 which enhanced to 8.35 on addition of catechu colour. During storage, in both cases the CA scores decreased (Fig. 4.9a). At the end of 14 days of storage, the scores were 6.68 and 6.76 respectively. This is possibly because of separated fat particles appearing on the surface as milk used was not homogenised. The BT scores also significantly reduced during storage which probably was influenced by changes in flavour characteristics. The initial BT scores were 7.80 in control and 7.85 in experimental sample both of which decreased to 6.70 and 6.82 respectively (Fig. 4.9b) which appeared to be similar. Flavour score for control was 7.76 on zero day and the same for catechu FM was 8.06 which decreased to 4.60 and 4.64 at the end of 14 day storage (Fig. 4.9c). This is because of development slight sourness and off flavour in both control and experimental samples. OA scores were 7.76 and 8.15 on zero day for control and catechu containing FM which decreased to 4.10 and 4.36 at

the end of storage (Fig. 4.9d). From the results it is understood that there was no effect of catechu colour on the shelf life of FM.

Chapter- 5

Summary and Conclusion

5.0 SUMMARY AND CONCLUSION

- 5.1 Colour is an important sensory attribute by which consumer first forms an idea of a product's quality. A good colour always consolidates the flavour attribute of food products, and in the same sense it can be said that it might mask any minor drawbacks in flavour quality as well as nutritional quality. Most of the flavours used in food products are synthetic because of their intensity and stability towards processing conditions. However, they carry little beneficial value as far as consumer's health is concerned; rather they are perceived to be harmful in the long run. Several attempts were made to extract colour from natural sources such as plant sources and utilise them in foods. The success of these efforts was constrained by the stability problems of natural colours. Yet, the efforts of finding newer sources of natural colours are continuing. Several woods possess natural colours that can be extracted by different methods. However, no extracted from wood are being used in food stuffs though they are widely used in non-food industries like tanning, textile etc. In this project, colour extracted from heartwood of catechu plant (*Acacia catechu*) has been tried in various dairy products. This being the first work on catechu colour in dairy products, stability aspects of the extracted colour were also studied.
- 5.2 Two types of extractions were tried for colour extraction from catechu heartwood: hot extraction which involved heating catechu heartwood pieces in water, and cold extraction which involved soaking of catechu heartwood pieces in water at ambient temperatures. The stability of catechu extract towards various additives, pH, temperature and heat treatments was also studied. A catechu colour concentrate (CCC) was prepared and utilised in various dairy products and storage stability tested. The colour intensity and stability were measured by reflectance meter, Scanner-Adobe Photoshop (SAP) method and sensory evaluation.
- 5.3 Hot extraction was found to be more suitable for colour extraction from the catechu heartwood. It was dark brown in colour. Several additives were tried to

enhance the brown colour intensity of the extract, but none of them was found favourable, rather they showed adverse effect on the catechu colour.

- 5.4 In order to study heat stability of catechu colour, the extract was subjected to various heat treatments. It was found that catechu colour on heat treatment like thermization, pasteurisation and sterilisation showed darkening trend as indicated by lower lightness values measured by SAP method. This shows a good heat stability of catechu colour to heat treatments.
- 5.5. Acidification or alkalisation of medium showed a significant effect on the colour. At low pH, the extract showed yellowish colour, whereas under alkaline conditions, it displayed light brown colour. At about neutral pH, the catechu extract showed a desirable darker brown colour.
- 5.6 A catechu colour concentrate (CCC) was prepared by extraction of catechu heart wood 6-7 times in water and heat concentrating the pooled extract to a minimum volume to obtain CCC. This was utilised as colouring agent in flavoured milk, lassi, shrikhand and yoghurt, and the colour quality and stability were evaluated by measuring RGL values by SAP method as well as sensory evaluation.
- 5.7 When the catechu heartwood colour was used for colouring purpose, the colour and appearance of the flavoured (FM) was enhanced, because it imparted desirable colour to the product without affecting flavour quality. The color and appearance (CA) score of FM increased with increasing addition of CCC, but at 0.8% the CA score was maximum. The L values decreased which was of advantage when used in sterilised flavoured milk. By sterilisation, the viscosity of flavoured milk increased but there was no effect of added CCC to that of flavoured milk. The added CCC colour was also not affected by storage of sterilised flavoured milk as indicated by RGB and L values.
- 5.8 It has been shown that addition of CCC to chakka during shrikhand preparation significantly enhanced the acceptability scores; 1.5% rate of addition of CCC resulted in significantly higher CA, flavour and OA scores of shrikhand. The initial RGL and reflectance values of plain shrikhand were 254.3, 251.1, 207.7, 247.2 and 94.2%, respectively. There was decrease in all these values on addition of CCC. These values further gradually decreased significantly to 248.2, 213.1,

165.2, 218.2 and 75.8 on addition of CCC @ 2.5% on chakka basis. There was no influence of catechu colour on the textural attributes of shrikhand. Use of CCC @ 1.5% level was also beneficial in giving desirable colour to sapota pulp added shrikhand without any adverse influence on flavour and textural attributes.

5.9 CCC was added to plain lassi @ 0.5, 1.0, 1.5 and 2.0% which imparted brown colour to lassi. It was observed that as CCC addition increased, the CA acceptance score also increased from 7.36 (0.5% level) to 7.87 (1.5% level). The RGB and L values decreased on increase in CCC level. Initial values of RGB and L values were 254.9, 254.8, 232.8 and 252.4, respectively. These values decreased to 240.1, 203.2, 159.1 and 209.4 on addition of 2% CCC. These values were statistically different only at 2% CCC. There was no effect of CCC on pH and viscosity measure of lassi.

5.10 In case of yoghurt, it was noticed that up to addition of 1.5% level of CCC, the CA score increased, but it was statistically not significant. However, 2% addition scored significantly higher indicating that at this level, CCC imparted a good acceptable brown colour to yoghurt. The OA score increased from an initial value of 7.59 to 8.02. Use of CCC at 2.5% showed no further improvement in OA score. There were changes in the colour values; however, these were not significant.

5.11 The colour added chocolate lassi was stored for 13 days and its colour parameters measured at 2-3 day interval. By addition of catechu colour, the lassie's L value decreased i.e. it became slightly darker. On storage for 13 days there were no much changes in colour values of the lassi indicating stability of the added colour. The sensory evaluation for CA also showed the same trend.

5.12 Shrikhand was prepared by incorporation of 15% sapota pulp and 1.5 % catechu extract and the colour parameters were monitored during storage under refrigerated condition (5°C). The initial R, G, B and L value of control were 232.34, 215.29, 181.44 and 216.55, respectively. On addition of catechu colour, these values changed to 219.67, 189.39, 151.98 and 194.22. There was not much decrease in CA scores, however, flavour and OA scores decreased considerably. The scores at the end of 26th day were 7.68, 7.3, 7.52 and 7.52 for CA, BT,

flavour and OA of control sample, respectively; these scores for catechu containing sample were: 7.92, 7.34, 7.64 and 7.68. These results indicate that all the samples kept well till 26 days without any change in colour intensity.

5.13 The results also indicated good stability of CCC colour in yoghurt during storage. The CCC also remained unaffected by the fermentation of milk during yoghurt preparation as well as during storage. These observations were based on the CA scores during storage.

5.14 Catechu added sterilised FM was stored in retortable bottles at ~30°C and changes in colour parameters were recorded. The initial R, G, B and L values of control sample were 251.84, 236.10, 199.29 and 236.68, respectively on zero day. These values for catechu added sample were 249.14, 219.21, 194.16 and 225.34 indicating slight darker colour in stored product and implying good storage stability of added catechu colour.

It was concluded that water was the best medium for extraction of colour from catechu heartwood and hot extraction method yielded maximum colour extraction. The catechu colour had a good stability to heat treatments; hence its colour concentrate could be prepared by heat concentration. The CCC produced a good brown colour in dairy products and it was stable to fermentation and low pH. The CCC colour also showed a good stability during storage of these products. Further scope exists for refining of the CCC to obtain a pure colour which is more stable, so can be widely employed by the dairy industry.

Bibliography

REFERENCES

- Agroforestry data base available at <http://www.worldagroforestrycentre.org/SEA/Products/AFDbases/AF/asp/SpeciesInfo.asp?SpID=21>.
- Ahmad, W.Y.W., Rahim, R., Ahmad, M.R., Kadir, M.I.A. and Misnon, M.I.(2011) The application of *Gluta aptera* Wood (Rengas) as natural dye on silk and cotton fabrics. *Univ. J. Environ. Res. Technol.*, **1**(4): 545-551.
- Ainsworth, M.C. and Hepworth, T.C. (1916) Inks: their composition and manufacture, 2nd Edn., Charles Griffin & Company, Ltd., London.
- Ali, S., Hussain, T. and Nawaz, R. (2009) Optimization of alkaline extraction of natural dye from henna leaves and its dyeing on cotton by exhaust method. *J. Clean. Prod.*, **17**:61-66.
- Amerine, M.A., Pangborn, R.M. and Roessler, E.B. (1965) Principles of sensory evaluation of foods, Academic Press, New York.
- Anitha Roy, Geetha, R.V. and Lakshmi, T. (2011). In vitro evaluation of anti mycotic activity of heartwood extract of *Acacia Catechu* Willd. *J. Pharma. Res.*, **4** (7)
- Anonymous (2015a) Catechins: definition, structure, green tea, black tea, cocoa. www.tuscany-diet.net/2014/02/.../catechins-definition-structure-green-tea...
- Anonymous (2015b) <http://www.phytotech.in/www3/html/conv>.
- Anonymous (2015c) ecourses.ndri.res.in/moodle/mod/resource/view.php?id=4619.
- Bansode, V.V., Pawar, V.D., Satpute, P.T., Zakiruddin, M. Jadhao, A.S. and Machewad, G.M. (2012) Studies on effect of initial pH, storage temperature and preservatives on shelf life of yoghurt. *Indian J. Dairy Sci.*, **65**(6): 456-460.
- Bechtold, T., Ali, A.M. and Mussak, R. (2006) Natural dyes for textile dyeing: a comparison of methods to assess the quality of Canadian golden rod plant material. *Dyes and Pigments*, **75**:287- 293.

- Braca, A., Tommasi, N. D., Bari, L. D., Pizza, C., Politi, M. and Morelli, I. (2000) Antioxidant principles from *Bauhinia terapotensis*. *J.Nat. Prod.*, **64**: 892–895.
- Cannon, J. and Margaret Cannon, M. (1994) Dye plants and dyeing. The Herbert Press, Ltd., London.
- Carlo, J. (2002) The history and use of colored Inks. In: “The broad spectrum: studies in the materials, techniques, and conservation of color on paper.” (Stratis, H.K. and Salveson, B.B., eds), Archetype Publications, Ltd. London, pp. 108-117.
- Deshpande, V.H. and Patil, A.D. (1981) Flavonoids of *Acacia catechu* heartwood. *Indian J. Chem.*, **20**: 628.
- Galli, A., Franzetti, L. and Briguglio, D. (1985) Antimicrobial properties in vitro of essential oils and extract of spices used for food. *Ind. Aliment.*, **24**: 463–466.
- Gaur, R.D. (2008) Traditional dye yielding plants of Uttarakhand. *Natural Prod. Rad.*, **7**(2):154-165.
- Gayathri Devi, Anitha John, R., Sreekala Devi, V. and Prabhakaran, A. (2011) Pharmacognostical studies on *Acacia catechu* willd and identification of antioxidant principles. *Int. J. Pharm. Pharmaceut. Sci.*, **3** (2):
- Geetha, R.V., Anitha Roy and , Lakshmi, T. (2011a) In vitro evaluation of anti-bacterial activity of heart wood extract of *acacia catechu willd* on enteric pathogens. *Int. J. Pharma. Sci. Rev. Res.*, **9**(2):
- Geetha, R.V., Anitha Roy and Lakshmi, T. (2011b) In vitro evaluation of anti-bacterial activity of heartwood extract of *Acacia catechu* on oral microbes. *Int. J. Current Res Rev.*, **3**(6): 630-640.
- George, V., Arora, S., Sharma, V., Wadhwa, B. K. And Singh, A. K. (2012) Stability, physico-chemical, microbial and sensory properties of sweetener/sweetener blends in lassi during storage. *Food Bioproc. Technol.*, **5**(1), 323-330.
- Gulzar A., Manjul P.S., Anitha S., Upendra K., Yatendra K. (2010) Preliminary phytochemical and antimicrobial screening of leaves extract of *Acacia catechu* Willd. *J. Pharm. Res.*, **3**(11): 2583-2584.
- Hammeke, Erin. "Logwood dye on paper." (2004).

- Hazra, B., Rhitajit Sarkar, Santanu Biswas and Nripendranath Mandal (2010) The Antioxidant, iron chelating and DNA protective properties of 70% methanolic extract of 'Katha' (Heartwood extract of *Acacia catechu*). *J. Compl. Integr. Med.*, **7** (1).
- Isabelle, P., Tuomos, M.T. and Waltmo, M. H. (2003) *Enterococcus faecalis* the root canal survivor and star in post treatment disease. *Endodontic Topics*, **6**: 135-159.
- Ismail, A.A. and Deeb, S.A. (1973) Effect of heat processing, storing and homogenization on the viscosity, opacity and stability of cow and buffalo milk. Dairying section; Faculty of Agriculture, Alexandria University, Alexandria (Egypt).
- Ismail, S. and Asad, M. (2009) Immunomodulatory activity of *Acacia catechu*. *Indian J. Physiol. Pharmacol.*, **53**(1): 215-220.
- Israr ul Haq, Khaskheli, M., Salman, M. and Marri, M.Y. (2014) Effect of heat treatment on sensory characteristics and shelf life of skim milk. *African J. Food Sci.*, **8**(2):75-79.
- Jarald, E., Siddheshwar, B. J. and Dharam, C. J. (2009) Biochemical study on the hypoglycaemic effects of extract and fraction of *Acacia catechu* wild. in alloxan-induced diabetic rats. *Int. J. Diab. Metabol.*, **17**:63-69.
- Jayasekhar, P., Mohanan, P.V. and Rathinam, K.(1997) Hepatoprotective activity of ethyl acetate extract of *Acacia catechu*. *Indian J. Pharmacol.*, **29**:426-8.
- Jha, V. (2002) Indigenous colours in Mithila (north Bihar) – a historical perspective. *Indian J. Hist. Sci.*, **37**(1): 37-55.
- Johannes, A. N. and van Boekel, M.A.J.S. (2003) Protein Stability in Sterilised Milk and Milk Products. In: “Advanced Dairy Chemistry—1 Proteins” (Fox, P.F. and McSweeney, P.L.H., eds.), Kluwer Academic, London., pp 947-974
- Jordano, R., Martinez, P., Ganido, M.D. and Jodiral, B. (1988) Changes of acidity and pH in commercial yoghurt. Cited from Dairy Sci. Abstr., **50**: 4161.
- Karwani, G., Singhvi, I., Gupta, S., Kapadiya, N. and Sisodia, S. S. (2011) Anti-secretory and antiulcer activity of *Acacia Catechu* against indomethacin plus pyloric ligation Induced gastric ulcers in rats. *J. Cell Tissue Res.*, **11**(1) 2567 – 2571.

- Khan, M .I., Ahmad, A., Yusuf, M., Shahid, M., Manzoor, N. and Mohammad, F. (2011) Assessment of antimicrobial activity of *Catechu* and its dyed substrate. *J. Cleaner Prod.*, **19**(12): 1385-1394.
- Khare, C.P. (2004) Indian herbal remedies rational western therapy, ayurvedic and other traditional usage, Botany. Springer-verlog Berlin Heidelberg New York. ISBN-3-540-01026-2
- Kim, D. S., Baek, N. I., Oh, S. R., Jung, K. Y., Lee, I. S. and Lee, H. K. (1997) NMR assignment of brazilin. *Phytochem.*, **46**: 177–178.
- Kulkarni, S.S., Gokhale, A.V., Bodake, U.M. and Pathade, G.R. (2011) Cotton dyeing with natural dye extracted from Pomegranate peel. *Univ. J. Environ. Res. Technol.*, **1**(2): 135-139.
- Lakshmi, T., Geetha, R.V. and Anitha Roy (2011a) In vitro evaluation of anti bacterial activity of *Acacia catechu willd* heartwood extract. *Int. J. Pharma Biosci.*, **2**(1): 235-240.
- Lakshmi,T., Geetha, R.V. and Anitha Roy (2011b) *In vitro* evaluation and of antibacterial activity of heartwood extract of *Acacia catechu wild*. *Int. J. Pharma Biosci.*, **2**(2):188-192.
- Law, A.J.R., Horne, D.S., Banks, J.M. and Leaver, J. (1994) Heat-induced changes in the whey proteins and caseins. *Milchwissenschaft*, **49**: 3: 125.
- Lemmens, R.H.M.J. and Wulijarni-Spetjptoed (1991) Dye and tannin producing plants: plant resources of south-east Asia. No. 3. Pudoc Wageningen. Netherlands.
- Lewis, M.J. and Deeth, H.C. (2009) Heat treatment of milk. In: “Milk processing and quality management”, (Tamime, A.Y., ed.), John Wiley and Sons, London, U.K., p. 183.
- Lioe, H.N., Adawiyah, D.R. and Anggraeni, R. (2012) Isolation and characterization of the major natural dyestuff component of Brazilwood (*Caesalpinia sappan L.*). *Int. Food Res. J.*, **19**(2): 537-54.
- Madhava Naidu and Sowbhagya, H.B. (2012) Food colours, technological advances in food colours. *Chem. Ind. Dig.*, March 2012.

- Mahanta, D. and Tiwari, S.C. (2005) Natural dye yielding plants and indigenous knowledge on dye preparation in Arunachal Pradesh, northeast India, *Current Sci.*, **88** (9): 1474-1480.
- Manhita, A., Ferreira, T. and Candeias, A. (2011) Extracting natural dyes from wool- an evaluation of extraction methods. *Analyt. Bioanalyt. Chem.*, **400**: 1501-1514.
- Mayer, F. and Cook, A. (1943) The chemistry of natural coloring matters: the constitutions, properties and biological relations of the important natural pigments. Reinhold Publishing Corporation, New York.
- Monga, J., Chauhan, C.S. and Sharma, M. (2011) Human epithelial carcinoma cytotoxicity and inhibition of DMBA/TPA induced squamous cell carcinoma in Balb/c mice by *Acacia catechu* heartwood. DOI: 10.1111/j.2042-7158.2011.01354.x.
- Montazer, M. and Parvinzadeh, M. (2007) Dyeing of wool with Marigold and its properties. *Fibers and Polymer*, **8**(2): 181-185.
- Nagaraja, T.G., Sarang, S.V. and Jambhale, D.C. (2008) Evaluation of anti-mycotic activity of *Acacia catechu* Willd. and Anti-mycotic activity of *Acacia catechu*. *J. Biopesticides*, **1**(2):197 - 198.
- Nagarajan, N.S., Muruges, N., Thirupathy, K.P., Radha, N. and Murali, A. (2005) Antidiabetic and antihyperlipidemic effects of *Cleome feline*. *Fitoterapia*, **76**: 310-315.
- Naik, G. H., K. I., Priyadarsini, J. G., Satav, M. M., Banavalikar, D. P., Sohoni, M., Biyani, K. and Mohan, H. (2003) Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. *Phytochem.* **63**(1): 97-104.
- Nalamolu, K.R. and Srinivasu, N. (2006) Antidiabetic and renoprotective effects of chloroform extract of *Terminalia chebula* seeds in Streptozotocin induced diabetic rats. BMC Complement Altern Med online publication 13 November 2006; doi: 10.1186/1472-6882-6-17.

- Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. and Simons, A. (2009) Agroforestry Database: a tree reference and selection guide version 4.0 (<http://www.worldagroforestry.org/af/treedb/>).
- Patel, J., Kumar, V. and Bhatt, S. (2009) Antimicrobial screening and phytochemical analysis of the resin part of *Acacia catechu*. *Pharma. Biol. J.*, **47**(1): 34-37.
- Patel, N.K. (2011) Natural dye based sindoor. *Life Sci. Leaflets*, **11**: 355-361.
- Patel, R. S. and Chakraborty, B. K. (1988) Shrikhand-- a review. *Indian J. Dairy Sci.*, **41**(1): 109-115.
- Patil, S., Jolly, C.I. and Narayanan, S. (2003) Free radical scavenging activity of *Acacia catechu* and *Rotula aquatica*: implications in cancer therapy. *Indian Drugs*, **40** (6): 328-332.
- Phuong, M.L., Ali, B.A., Aziz, E., Abdellatif, S., Yahia C. and Pierre, S.H. (2004) The petroleum ether extract of *Nigella sativa* exerts lipid lowering and insulin-sensitizing action in the rats. *J. Ethnopharmacol.*, **94**: 251-259.
- Pingale, S.S. (2010) Hepatoprotection by *Acacia catechu* in CCL4 induced liver dysfunction. *Int. J. Pharmaceut. Sci. Rev. Res.*, **5**(1): 220-22.
- Rage, N., Dahanukar, S. and Karandikar, S.M. (1984) Hepatoprotective effect of cyanidanol against carbon tetrachloride induced liver damage. *Indian Drugs*, **22**: 556-60.
- Rahman, S.M. (2007) Handbook of food preservation, 2nd Edition, CRC Press, Taylor & Francis Group, Boca Raton, USA., p.600.
- Ramadan, F.M., El-Zanfaly, R.T., El-Wakeil, F.A. and Allian, A.M. (1972) On the antibacterial effects of some essential oils I. Use of agar diffusion method. *Chem. Mikrobiol. Technol. Lebensm.*, **2**: 51-5.
- Ray, D., Sharatchandra, K.H. and Thokchom, I.S. (2006) Antipyretic, antidiarrhoeal, hypoglycaemic and hepatoprotective activities of ethyl acetate extract of *Acacia catechu* Willd. in albino rats. *Indian J. Pharmacol.*, **38**(6): 408-413.
- Reineccius, G. (2005) Flavour chemistry and technology, 2nd Edition, Taylor & Francis, Boca Raton, USA., p.183.

- Reitman, S. and Frankel, S. (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American J. Clin. Pathol.*, **28**:56-63.
- Sai Sreenivas, K.N., Rajkumar, S.N., Rishi Kumar Puri and Ankitha Anto, C. (2015)
- Saint-Eve, A., Levy, C., Le Moigne.M., Ducret, V. and Souchon, I. (2008) Quality changes in yoghurt during storage in different packaging materials. *J. Food Technol.*, **2**(10):285-293.
- Samanta, A.K., Konar, A. and Chakraborti, S. (2010) Dyeing jute with tesu extract: Part 1- Effects of different mordants and dyeing process variables. *Indian J. Fibres Text. Res.*, **36**: 63-73.
- Sanjay, G.A.K., Tiku, G.S., Dhiraj, V. and Ashwini, B. (2011) Antioxidant activity and protective effect against plasmid DNA strand scission of leaf, ark, and heartwood extracts from *Acacia catechu*. DOI: 10.1111/j.1750-3841.2011.02284.x.
- Senthilkannan, M.S. (2010) Natural dyes: are they really environmental friendly?. Consumer Testing Lab Services, SGS India (P) Limited.
- Shambharkar, A. D., Shelke, R. R., Gubbawar, S. G. And Bharad, P. M. (2011) Utilization of sapota pulp in the preparation of Shrikhand. *Food Sci. Res. J.*, **2**(2):183-187.
- Shaunak, P.R. (2008) Studies on effect of non-conventional sweeteners on quality of milk chocolate. M.Tech DT Thesis submitted to National Dairy Research Institute (Deemed University), Bangalore.
- Shrivastava, A. and Dedhia, E.M. (2006) Extraction and dyeing methods for natural dyes. In: "Natural dyes: scope and challenge", The Maharaja Sayajirao University of Baroda, Vadodara, India., pp. 67-80.
- Skandamis, P., Koutsoumanis, K., Fasseas, K. and Nychas, G.J.E. (2001) Evaluation of the inhibitory effect of oregano essential oil on *Escherichia coli* in broth culture with or without EDTA, using viable counts, turbidity and impedance. *Italian J. Food Sci. Technol.*, **13**: 65–75.

- Spence, C., Levitan, C.A., Shankar, M.A. and Zampini, M. (2010) Does Food Color Influence Taste and Flavor Perception in Humans? *Chemosensory Perception*, **3**(1): 68-84.
- Srinivasan, R., Govindarasu, G.S., Sakthivel, K., Krishnamurthy, M., Ramiya, B., Mariappan, K., Muchukathan, G. and Chinnavenkataraman, G. (2012) In vitro antimicrobial activity of *Caesalpania sappan* L). *Asian Pacific J. Trop. Biomed.*, S136 - S139.
- Sutariya, H. and Rao, K. J. (2014) Utilisation of lemon grass distillate in the preparation of lassi. *Indian J. Dairy Sci.*, **67**(1): 20-26.
- Tassou, C.C., Nychas, G.J.E. and Skandamis, P.N. (2004) Herbs and spices and antimicrobials. In: "Handbook of herbs and spices" (Peter, K.V., ed.), Woodhead Publishing Ltd., Cambridge, England, p. 35-42.
- Tawde, K.V., Gacche, R.N. and Pund, M.M. (2012) Evaluation of selected Indian traditional folk medicinal plants against *Mycobacterium tuberculosis* with antioxidant and cytotoxicity study. *Asian Pacific J. Trop. Dis.*, **2**(Suppl.2): S685-S691.
- Use of pathimugam (*Caesalpania sappan* Linn) in Sandesh as natural colouring agent. Abstract presented in Dairy Industry Conference, Kolkata.
- Vaishali, V.A., Sangeeta, S.M., Mandar, A., Kishore, M.P. (2002) Antioxidant and trace element potential of Chyavanprash and some Ayurvedic preparations. *Indian J. Trad. Knowl.*, **2**: 215-223.
- Valte, V., Thokchom, I. S., Joychandra Singh, O., Babycha, L. And Aruna, S. (2012) Protective effect of ethyl acetate extract of *Acacia catechu* in carbon tetrachloride induced hepatotoxicity. *Indian Med. Gaz.*, **50**: 159-162.
- Vankar, P.S., Shanker, R., Dixit, S., Mahanta, D. And Tiwari, S.C. (2008) Sonicator dyeing of modified cotton, wool and silk with *Mahonia napaulensis* DC and identification of the colorant in Mahonia. *J. Ind. Crops Prod.*, **27**: 371-379.
- Vyawahare, A.S. and Jayaraj Rao, K. (2011) Application of computer vision systems in colour evaluation of kunda: a heat desiccated dairy product. *Int. J. Dairy Sci.*, **6**(4): 253-266.

- Wallace, C. and M.M. Giusti, M.M. (2008) Determination of color, pigment, and phenolic stability in yogurt systems colored with nonacylated anthocyanins from *Berberis boliviana* L. as compared to other natural/synthetic colorants. *J. Food Sci.*, 73(4): C241-C248.
- Wan Yunus Wan Ahmad, Razis Rahim, Mohd Rozi Ahmad, Muhammad Ismail Abdul Kadir, Mohd Iqbal Misnon (2011) The Application of *Gluta Aptera* Wood (Rengas) as Natural Dye on Silk and Cotton Fabrics. *Universal Journal of Environmental Research and Technology*, 1(4): 545-551.
- WHO (1985) 5th Programme Report, Programme for control of diarrhoeal diseases, Geneva. WHO Bulletin, **63**: 557-772.
- Wilkins, K.M. and Board, R.G. (1989) Natural antimicrobial systems. In: Mechanisms of action of food preservation procedures (Gould, G.W., ed.), pp. Elsevier, London, 285–362.
- Wounter, J. and Verhecken, A. (1989) Study conservation, **34**: 189-200.
- Yimam, M., Zhao, Y., Ma, W., Jia, Q., Do, S.G. and Shin, J.H. (2010) 90-Day oral toxicity study of UP446, a combination of defined extracts of *Scutellariabaicalensis* and *Acacia catechu* in rats. *Food Chem. Toxicol.*, **48**(5): 1202 – 1209.
- Yong, K.J., Kim, I.H. and Nam, S.W. (1999) Combination dyeing of silk fabrics with Dansam and Sappan wood. *J. Korean Soci. Dyes Finishers*, 2013 – *Koreascience*, **11**: 9.

Annexure

APPENDIX

Ap-1 (Table-4.7a): ANOVA for effect of CCC level on sensory acceptance score of flavoured milk

| Source | df | Color & appearance | | Body and texture | | Flavour | | Overall acceptability | |
|--------------------|----|--------------------|---------|------------------|---------------------|---------|--------------------|-----------------------|---------|
| | | MSS | F-value | MSS | F-value | MSS | F-value | MSS | F-value |
| Between CCC levels | 4 | 0.228 | 2.814* | 0.031 | 0.662 ^{NS} | 0.014 | .543 ^{NS} | 0.257 | 4.88* |
| Error | 15 | 0.085 | - | 0.051 | - | 0.022 | - | 0.053 | - |
| Total | 19 | 2.22 | - | 0.382 | - | 0.979 | - | 1.818 | - |

*Significant at 5% level; NS: Non significant; CCC – Catechu Colour Concentrate

Ap-2 (Table-4.8a): ANOVA for effect of CCC level on instrumental colour parameters of flavoured milk

| Source | df | Red | | Green | | Blue | | Luminosity | |
|--------------------|----|--------|---------|--------|---------|---------|---------|------------|---------|
| | | MSS | F-value | MSS | F-value | MSS | F-value | MSS | F-value |
| Between CCC levels | 4 | 906.88 | 890.24 | 906.88 | 890* | 4200.84 | 551.11* | 2664.84 | 5.10* |
| Error | 15 | 1.019 | - | 1.019 | - | 7.622 | - | 522.23 | - |
| Total | 19 | 3642.8 | - | 3642.8 | - | 1691.71 | - | 1.82 | - |

* Significant at 5% level ; NS: Non significant; CCC - Catechu Colour Concentrate

Ap-3 (Table-4.9a): ANOVA for effect of CCC level on sensory scores of plain and sterilised flavoured milks

| Source | df | Color & appearance | | Body and texture | | Flavour | | Overall Acceptability | |
|--------------------|----|--------------------|---------|------------------|---------|---------|---------|-----------------------|---------|
| | | MSS | F-value | MSS | F-value | MSS | F-value | MSS | F-Value |
| Between CCC levels | 3 | 0.557 | 20.82* | 0.107 | 4.054* | 0.362 | 9.586* | 0.507 | 15.32* |
| Error | 28 | 0.027 | - | 0.011 | - | 0.038 | - | 0.033 | - |
| Total | 31 | 2.419 | - | 1.058 | - | 2.14 | - | 2.449 | - |

* Significant at 5% level ; NS: Non significant; CCC - Catechu Colour Concentrate

Ap-4 (Table-4.10a): ANOVA for Effect of CCC and heat treatment on instrumental colour parameters of flavoured milk

| Source | df | Red | | Green | | Blue | | Luminosity | |
|--------------------|-----|---------|---------|----------|---------|----------|---------|------------|---------|
| | | MSS | F-value | MSS | F-value | MSS | F-value | MSS | F-value |
| Between CCC levels | 3 | 210.827 | 11.61* | 3568.85 | 38.51* | 4016.5 | 49.41* | 2136.74 | 35.36* |
| Error | 28 | 18.165 | - | 92.68 | - | 81.285 | - | 60.43 | - |
| Total | 131 | 1141.1 | - | 13301.57 | - | 14325.49 | - | 8102.14 | - |

* Significant at 5% level ; NS: Non significant; CCC - Catechu Colour Concentrate

Ap-5 (Table-4.11a): ANOVA for effect of CCC on viscosity of flavoured milk

| Source | df | MSS | F-value |
|------------------|----|-------|---------------------|
| Between products | 3 | 0.238 | 0.737 ^{NS} |
| Error | 28 | 0.322 | - |
| Total | 31 | - | - |

NS- Not significant (P>0.05)

Ap-6 (Table-4.13a): ANOVA for effect of CCC on sensory scores of shrihand

| Source | df | Color & appearance | | Body and texture | | Flavour | | Overall acceptability | |
|--------------------|----|--------------------|---------|------------------|---------|---------|---------|-----------------------|---------|
| | | MSS | F-value | MSS | F-value | MSS | F-value | MSS | F-value |
| Between CCC levels | 5 | 0.074 | 1.47* | 0.16 | 7.74* | 0.028 | 1.46* | 0.128 | 5.15* |
| Error | 18 | 0.05 | - | 0.022 | - | 0.19 | - | 0.025 | - |
| Total | 23 | 1.28 | - | 0.475 | - | 0.48 | - | 1.088 | - |

* Significant at 5% level ; NS: Non significant

Ap-7 (Table-4.14a): ANOVA for effect of CCC addition on instrumental colour parameters of shrihand

| Source | df | Red | | Green | | Blue | | Luminosity | | reflectance | |
|--------------------|----|--------|---------|---------|---------|---------|---------|------------|---------|-------------|---------|
| | | MSS | F-value | MSS | F-value | MSS | F-value | MSS | F-value | MSS | F-value |
| Between CCC levels | 5 | 35.288 | 6.81* | 832.35 | 35.67* | 979.67 | 16.35* | 494.32 | 31.7* | 170.36 | 26.34* |
| Error | 18 | 5.186 | - | 23.33 | - | 59.93 | - | 15.594 | - | 6.47 | - |
| Total | 23 | 269.78 | - | 4581.76 | - | 5977.73 | - | 2752.3 | - | 968.24 | - |

* Significant at 5% level ; NS: Non significant; CCC - Catechu Colour Concentrate

Ap-8 (Table-4.15a): ANOVA for effect of CCC on textural properties of shrihand

| Source | df | Firmness | | Consistency (| | Adhesive force | | Index of viscosity | |
|--------------------|----|----------|------------|---------------|------------|----------------|------------|--------------------|------------|
| | | MSS | F-value | MSS | F-value | MSS | F-value | MSS | F-value |
| Between CCC levels | 5 | 0.13 | 0.49 NS | 7.46 | 0.35 NS | 0.11 | 0.41 NS | 0.06 | 0.90 NS |
| Error | 18 | 0.257 | - | 21.41 | - | 0.258 | - | 0.188 | - |
| Total | 23 | 5.253 | - | 422.73 | - | 5.173 | - | 3.682 | - |

Significant at 5% level ; NS: Non significant; CCC - Catechu Colour Concentrate

Ap-9 (Table-4.16a): ANOVA for effect of CCC on sensory scores of sapota pulp added shrikhand

| Source | df | Color & appearance | | Body and texture | | Flavour | | Overall acceptability | |
|--------------------|----|--------------------|---------|------------------|---------|---------|---------|-----------------------|---------|
| | | MSS | F-Value | MSS | F-value | MSS | F-value | MSS | F-value |
| Between CCC levels | 4 | 0.12 | 3.30* | 0.035 | 2.84* | 0.221 | 5.25* | 0.23 | 4.89* |
| Error | 15 | 0.37 | - | 0.012 | - | 0.042 | - | 0.46 | - |
| Total | 19 | 1.05 | - | 0.32 | - | 1.51 | - | 1.59 | - |

* Significant at 5% level ; NS: Non significant

Ap-10 (Table-4.17a): ANOVA for effect of CCC on instrumental textural properties of sapota pulp added shrikhand

| Source | df | Firmness | | Consistency | | Adhesive force | | Index of viscosity | |
|--------------------|----|----------|--------------------|-------------|--------------------|----------------|--------------------|--------------------|-------------------------------|
| | | MSS | F-value | MSS | F-value | MSS | F-value | MSS | F-value |
| Between CCC levels | 4 | 0.11 | 2.61 ^{NS} | 5.32 | 0.20 ^{NS} | 0.066 | 1.18 ^{NS} | 0.041 | 2.08 ^{N_s} |
| Error | 15 | 0.043 | - | 3.08 | - | 0.056 | - | 0.02 | - |
| Total | 19 | 1.08 | - | 67.55 | - | 1.108 | - | 0.46 | - |

* Significant at 5% level ; NS: Non significant; CCC - Catechu Colour Concentrate

Ap-11 (Table-4.18a): ANOVA for effect of CCC and sapota on instrumental colour parameters of shrikhand

| Source | df | Red | | Green | | Blue | | Luminosity | |
|-----------------------|----|--------|---------|---------|---------|---------|---------|------------|---------|
| | | MSS | F-value | MSS | F-value | MSS | F-value | MSS | F-value |
| Between Sapota levels | 5 | 35.29 | 6.81* | 832.36 | 35.67* | 979.67 | 16.35* | 494.32 | 31.7* |
| Error | 18 | 5.19 | - | 23.33 | - | 59.93 | - | 15.59 | - |
| Total | 23 | 269.79 | - | 4581.76 | - | 5977.13 | - | 2752.3 | - |

* Significant at 5% level ; NS: Non significant; CCC - Catechu Colour Concentrate

Ap-12 (Table-4.19a): ANOVA for effect of CCC on sensory scores of lassi

| Source | df | Color & appearance | | Body and texture | | Flavour | | Overall acceptability | |
|--------------------|----|--------------------|---------|------------------|--------------------|---------|---------|-----------------------|---------|
| | | MSS | F-Value | MSS | F-value | MSS | F-Value | MSS | F-value |
| Between CCC levels | 4 | 0.17 | 5.63* | 0.002 | 0.36 ^{NS} | 0.221 | 5.25* | 0.23 | 4.89* |
| Error | 15 | 0.03 | - | 0.005 | - | 0.042 | - | 0.46 | - |
| Total | 19 | 1.12 | - | 0.083 | - | 1.51 | - | 1.59 | - |

* Significant at 5% level ; NS: Non significant; CCC - Catechu Colour Concentrate

Ap-13 (Table-4.20a): ANOVA for effect of CCC on instrumental colour parameters of lassi

| Source | df | Red | | Green | | Blue | | Luminosity | |
|--------------------|----|--------|---------|---------|---------|----------|---------|------------|---------|
| | | MSS | F-value | MSS | F-value | MSS | F-Value | MSS | F-value |
| Between CCC levels | 4 | 123.96 | 22.72* | 1637.92 | 469.03* | 3362.58 | 132.16* | 1127.40 | 299.68* |
| Error | 15 | 5.45 | - | 3.49 | - | 25.44 | - | 3.76 | - |
| Total | 19 | 577.69 | - | 6604.03 | - | 13831.89 | - | 4566.02 | - |

Ap-14 (Table-4.21a): ANOVA for effect of CCC on pH of lassi

| Source | df | MSS | F-value |
|--------------------|----|-------|---------------------|
| Between CCC levels | 4 | 0.000 | 0.044 ^{NS} |
| Error | 15 | 0.002 | - |
| Total | 19 | - | - |

NS- Not significant (P>0.05)

Ap-15 (Table-4.22a): ANOVA for effect of CCC on viscosity measure of lassi

| Source | df | MSS | F-value |
|--------------------|----|-------|---------------------|
| Between CCC levels | 4 | 3.784 | 0.721 ^{NS} |
| Error | 15 | 0.002 | - |
| Total | 19 | 5.246 | - |

* Significant at 5% level ; NS: Non significant; CCC –Catechu colour concentrate

Ap-16 (Table-4.23a): ANOVA for effect of CCC on sensory scores of cocoa lassi

| Source | df | Color & appearance | | Body and texture | | Flavour | | Overall acceptability | |
|--------------------|----|--------------------|---------|------------------|--------------------|---------|---------|-----------------------|---------|
| | | MSS | F-Value | MSS | F-value | MSS | F-value | MSS | F-value |
| Between CCC levels | 5 | 0.316 | 15.08* | 0.20 | 2.70 ^{NS} | 0.41 | 19.35* | 0.41 | 36.12* |
| Error | 18 | 0.021 | - | 0.074 | - | 0.02 | - | 0.011 | - |
| Total | 23 | 1.96 | - | 2.34 | - | 2.46 | - | 2.25 | - |

* Significant at 5% level ; NS: Non significant; CCC - Catechu Colour Concentrate

Ap-17 (Table-4.24a): ANOVA for effect of CCC on instrumental colour parameters of cocoa lassi

| Source | df | Red | | Green | | Blue | | Luminosity | |
|------------------------|----|---------|---------|---------|---------|---------|---------|------------|---------|
| | | MSS | F-value | MSS | F-value | MSS | F-value | MSS | F-Value |
| Between Catechu Colour | 5 | 685.83 | 15.36* | 899.89 | 25.78* | 876.67 | 10.11* | 823.30 | 21.82* |
| Error | 18 | 44.56 | - | 34.91 | - | 86.67 | - | 37.74 | - |
| Total | 23 | 4233.02 | - | 5127.84 | - | 5943.47 | - | 4795.73 | - |

* Significant at 5% level ; NS: Non significant; CCC - Catechu Colour Concentrate

Ap-18 (Table-4.27a): ANOVA for effect of CCC on pH of yoghurt

| Source | df | MSS | F-value |
|--------------------|----|-------|---------------------|
| Between CCC levels | 5 | 0.005 | 0.145 ^{NS} |
| Error | 17 | 0.035 | - |
| Total | 22 | - | - |

NS: non significant; CCC - Catechu Colour Concentrate

Ap19 (Table-4.28a): ANOVA for effect of CCC on sensory scores of yoghurt

| Source | df | Color & appearance | | Body and texture | | Flavour | | Overall Acceptability | |
|--------------------|----|--------------------|---------|------------------|--------------------|---------|---------------------|-----------------------|---------|
| | | MSS | F-Value | MSS | F-value | MSS | F-Value | MSS | F-value |
| Between CCC levels | 5 | 0.204 | 6.245* | 0.015 | 0.48 ^{NS} | 0.005 | 0.125 ^{NS} | 0.175 | 7.24* |
| Error | 18 | 0.033 | - | 0.32 | - | 0.043 | - | 0.024 | - |
| Total | 23 | 1.61 | - | 0.642 | - | 0.803 | - | 1.312 | - |

* Significant at 5% level ; NS: Non significant; CCC - Catechu Colour Concentrate

Ap-20 (Table-4.29a): ANOVA for effect of CCC on instrumental textural properties of yoghurt

| Source | df | Firmness | | Consistency | | Adhesive force | | Index of viscosity | |
|--------------------|----|----------|--------------------|-------------|--------------------|----------------|---------------------|--------------------|--------------------|
| | | MSS | F-value | MSS | F-value | MSS | F-Value | MSS | F-value |
| Between CCC levels | 5 | 0.076 | 0.32 ^{NS} | 28.90 | 1.89 ^{NS} | 0.053 | 0.595 ^{NS} | 0.023 | 0.61 ^{NS} |
| Error | 18 | 0.236 | - | 15.32 | - | 0.088 | - | 0.037 | - |
| Total | 23 | 4.62 | - | 420.28 | - | 1.855 | - | 0.779 | - |

NS: non significant; CCC - Catechu Colour Concentrate

Ap-21 (/Fig.4.6): Effect of storage on sensory scores of CCC coloured cocoa lassi

| Day of storage | CA | | BT | | Fl | | OA | |
|----------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| | Control | Sample | Control | Sample | Control | Sample | Control | Sample |
| 1 | 8.00± 0.31 | 8.5± 0.29 | 8.04± 0.09 | 8.08± 0.11 | 8.22± 0.52 | 8.42± 0.51 | 8.12± 0.28 | 8.49± 0.40 |
| 2 | 7.96± 0.29 | 8.56± 0.29 | 8.04± 0.09 | 8.08± 0.11 | 8.22± 0.52 | 8.36± 0.47 | 8.12± 0.28 | 8.48± 0.39 |
| 4 | 7.72± 0.59 | 7.72± 0.59 | 8.04± 0.09 | 8.08± 0.11 | 7.92± 0.24 | 8.06± 0.40 | 7.92± 0.29 | 8.22± 0.28 |
| 6 | 7.72± 0.58 | 8.54± 0.29 | 8.04± 0.09 | 8.08± 0.11 | 7.76± 0.36 | 8.22± 0.4 | 7.78± 0.31 | 8.12± 0.22 |
| 9 | 7.56± 0.92 | 8.54± 0.29 | 8.00± 0.00 | 8.04± 0.09 | 7.9± 0.84 | 7.46± 0.88 | 8.08± 0.79 | 7.38± 0.46 |
| 11 | 7.56± 0.92 | 8.54± 0.29 | 7.60± 0.42 | 7.86± 0.26 | 7.56± 1.2 | 6.32± 0.98 | 6.98± 0.48 | 4.16± 0.45 |
| 13 | 7.4± 0.89 | 8.24± 0.25 | 4.6± 0.55 | 4.6± 0.55 | 6.78± 0.47 | 4.2± 0.45 | 6.98± 0.48 | 4.16± 0.45 |

CA – Colour and appearance, BT –Body and texture, Fl-Flavour, OA-Overall acceptability;

Control:0%CCC+2%Cocoa Sample:1.5% CCC+2% Cocoa

Ap-22 (/Fig.4.7): Effect of storage on sensory scores of CCC coloured sapota shrikhand

| Day of storage | CA | | BT | | Fl | | OA | |
|----------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| | Control | Sample | Control | Sample | Control | Sample | Control | Sample |
| 1 | 7.90± 0.22 | 8.54± 0.28 | 8.30± 0.45 | 8.30± 0.45 | 8.36± 0.41 | 8.44± 0.38 | 7.96± 0.29 | 8.54±0.29 |
| 3 | 7.86± 0.22 | 8.34± 0.23 | 8.3± 0.45 | 8.3± 0.45 | 8.36± 0.41 | 8.42± 0.43 | 7.94± 0.26 | 8.58±0.29 |
| 5 | 7.86± 0.22 | 8.34± 0.23 | 8.2± 0.27 | 8.2± 0.27 | 8.38± 0.41 | 8.42± 0.43 | 7.98± 0.29 | 8.52±0.20 |
| 8 | 7.86± 0.22 | 8.34± 0.23 | 8.10± 0.22 | 8.10± 0.22 | 8.34± 0.38 | 8.38± 0.38 | 7.92± 0.41 | 8.40± 0.14 |
| 10 | 7.86± 0.22 | 8.34± 0.23 | 8.08± 0.17 | 8.08± 0.18 | 8.3± 0.41 | 8.34± 4.02 | 7.92± 0.41 | 8.26±0.43 |
| 13 | 7.8± 0.22 | 8.3± 0.21 | 7.94± 0.26 | 7.94± 0.26 | 8.02± 0.04 | 8.00± 0.14 | 7.84± 0.36 | 8.04±0.36 |
| 15 | 7.80± 0.22 | 8.20 ±0.25 | 7.80± 0.3 | 7.80± 0.33 | 7.94± 0.13 | 7.96± 0.17 | 7.76± 0.33 | 7.94±0.30 |
| 18 | 7.86± 0.22 | 8.26± 0.25 | 7.68± 0.32 | 7.68± 0.33 | 7.82± 0.16 | 7.88± 0.22 | 7.7± 0.3 | 7.84±0.27 |
| 22 | 7.72± 0.22 | 7.92± 0.37 | 7.34± 0.42 | 7.34± 0.38 | 7.56± 0.31 | 7.72± 0.33 | 7.58± 0.18 | 7.82±0.18 |
| 26 | 7.68± 0.16 | 7.92± 0.37 | 7.30± 0.34 | 7.34± 0.38 | 7.52± 0.23 | 7.64± 0.29 | 7.56± 0.19 | 7.78±0.18 |
| 31 | 7.60± 0.19 | 7.96± 0.36 | 7.34± 0.23 | 7.44± 0.33 | 7.40± 0.14 | 7.58± 0.16 | 7.48± 0.04 | 7.72±0.13 |

Sample: sapota shrikhand containing 1.5% CCC

Ap-23 (Fig.4.8): Effect of storage on sensory scores of CCC coloured yoghurt

| Day of storage | CA | | BT | | Fl | | OA | |
|----------------|---------------|---------------|-----------------|---------------|---------------|---------------|----------------|---------------|
| | Control | Sample | Control | Sample | Control | Sample | Control | Sample |
| 1 | 7.64± 0.47 | 8.2± 0.27 | 8.04± 0.09 | 8.04± 0.09 | 8.08± 0.11 | 8.26± 0.19 | 7.98± 0.29 | 8.34±0.2 3 |
| 3 | 7.6± 0.42 | 8.24 ±0.25 | 8.04± 0.09 | 8.04± 0.09 | 8.04± 0.9 | 8.22± 0.23 | 7.68± 0.260 | 8.61±0.2 7 |
| 5 | 7.64± 0.47 | 8.24±0.2 5 | 8.14± 0.22 | 8.14± 0.22 | 8.04± 0.17 | 8.22± 0.15 | 8.04±0. 36 | 8.04±0.2 2 |
| 7 | 7.64± 0.47 | 8.24±0.2 5 | 8.06± 0.30 | 8.06± 0.30 | 7.98± 0.28 | 8.16± 0.17 | 7.96±0. 39 | 8.3±0.2 1 |
| 10 | 7.60± 0.42 | 8.20± 0.27 | 8.00± 0.38 | 8.00± 0.38 | 7.94± 0.30 | 8.12± 0.23 | 7.84± 0.52 | 8.2± 0.31 |
| 12 | 7.6± 0.42 | 8.20± 0.27 | 8.00± 0.38 | 8.00± 0.38 | 8.00± 0.38 | 8.18± 0.29 | 7.84± 0.52 | 8.20± 0.31 |
| 15 | 7.60± 0.42 | 8.20± 0.27 | 7.98± 0.41 | 7.98± 0.41 | 7.92± 0.31 | 8.12± 0.23 | 7.78± 0.47 | 8.13±0.2 6 |
| 18 | 7.56± 0.49 | 8.16±0.3 2 | 7.98± 0.41 | 7.98± 0.41 | 7.70± 0.41 | 7.80± 0.47 | 7.72± 0.48 | 7.96±0.4 3 |
| 22 | 7.33± 0.46 | 7.90± 0.42 | 7.53± 0.0.40 | 7.82± 0.30 | 6.96± 0.29 | 7.06± 0.36 | 7.06± 0.26 | 7.30± 0.21 |
| 24 | - | 7.10±0.2 3 | - | 7.56± 0.51 | - | 7.10± 0.32 | - | 7.16±0.3 2 |

CA – Colour and appearance, BT –Body and texture, Fl-Flavour, OA-Overall acceptability;
Sample:2% CCC

Ap-24 (Fig. 4.9): Effect of storage on sensory scores of CCC coloured flavoured milk

| Day of storage | CA | | BT | | Fl | | OA | |
|----------------|---------------|---------------|--------------|---------------|---------------|---------------|---------------|---------------|
| | Control | Sample | Control | Sample | Control | Sample | Control | Sample |
| 1 | 7.9± 0.22 | 8.35± 0.34 | 7.8± 0.27 | 7.85± 0.22 | 7.76± 0.25 | 8.06± 0.26 | 7.76± 0.25 | 8.15± 0.33 |
| 4 | 7.9± 0.22 | 8.35± 0.34 | 7.8± 0.27 | 7.85± 0.22 | 7.76± 0.25 | 8.06± 0.26 | 7.7± 0.27 | 8.15± 0.33 |
| 6 | 7.84± 0.36 | 8.25± 0.35 | 7.8± 0.27 | 7.85± 0.22 | 7.76± 0.25 | 8.06± 0.26 | 7.7± 0.27 | 8.15± 0.33 |
| 8 | 7.84± 0.36 | 8.16± 0.48 | 7.7± 0.44 | 7.8± 0.27 | 7.7± 0.27 | 7.9± 0.41 | 7.7± 0.27 | 8.1± 0.42 |
| 10 | 7.5± 0.35 | 7.9± 0.58 | 7.5± 0.5 | 7.74± 0.37 | 7.6± 0.42 | 7.66± 0.42 | 7.4± 0.42 | 7.96± 0.36 |
| 12 | 7.36± 0.41 | 7.8± 0.57 | 7.4± 0.54 | 7.52± 0.47 | 7.3± 0.45 | 7.5± 0.35 | 7.3± 0.44 | 7.44± 0.26 |
| 14 | 6.68± 0.43 | 6.76± 0.42 | 6.7± 0.4 | 6.82± 0.46 | 4.6± 0.89 | 4.64± 0.79 | 4.1± 0.74 | 4.36± 0.61 |

CA – Colour and appearance, BT –Body and texture, Fl-Flavour, OA-Overall acceptability;
Control: plain sterilised FM, Sample:coloured Sterilised FM containing 0.8% CCC