DEVELOPMENT AND QUALITY EVALUATION OF OMEGA-3 FATTY ACID FORTIFIED BUTTER

THESIS SUBMITTED TO THE

ICAR-NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL

(DEEMED UNIVERSITY)

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE AWARD OF THE DEGREE OF

MASTER OF TECHNOLOGY

IN

DAIRY TECHNOLOGY

By

PANDULE VISHAL SHRIRANG

B. Tech. (Dairy Technology)

DAIRY TECHNOLOGY SECTION

ICAR-NATIONAL DAIRY RESEARCH INSTITUTE

SOUTHERN REGIONAL STATION

ADUGODI, BENGALURU- 560 030, INDIA

2019

Regn. No. 17-M-DT-11

DEVELOPMENT AND QUALITY EVALUATION OF OMEGA-3 FATTY ACID FORTIFIED BUTTER

By

Pandule Vishal Shrirang

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

------------------------------	------------------------------ 26/8/19
Dr. S. N. Rajakumar	**Dr. Monika Sharma**
Professor	**Scientist**
(External Examiner)	**(Major Advisor)**

Members of Advisory Committee

1. **Dr. B. C. Ghosh**
   (Principal Scientist, Dairy Technology)

2. **Mr. H. C. Devaraja**
   (Scientist, Dairy Technology)

3. **Dr. B. Surendra Nath**
   (Principal Scientist, Dairy Chemistry)

4. **Dr. Menon Rekha Ravindra**
   (Principal Scientist, Dairy Engineering)
   (Jt. Director's Nominee)

DAIRY TECHNOLOGY SECTION
ICAR-NATIONAL DAIRY RESEARCH INSTITUTE
(Deemed University)
SOUTHERN REGIONAL STATION
ADUGODI, BENGALURU-30

Dr. Monika Sharma
Scientist (Dairy Technology)

CERTIFICATE

This is to certify that the thesis entitled, "**DEVELOPMENT AND QUALITY EVALUATION OF OMEGA-3 FATTY ACID FORTIFIED BUTTER**" submitted by **PANDULE VISHAL SHRIRANG** towards the partial fulfillment for the award of the degree of **M. Tech** in **DAIRY TECHNOLOGY** of the **ICAR-NATIONAL DAIRY RESEARCH INSTITUTE (Deemed University)**, Karnal (Haryana), India, is a bonafide research work carried out by him under my guidance and no part of the thesis has been submitted for any other degree or diploma.

Dated:

(Dr. Monika Sharma)
Major Advisor & Chairman

DEDICATED TO

MY

BELOVED FAMILY

AND

RESPECTED GUIDE

Acknowledgement

At the onset, I bow in gratitude before the "Almighty" who gave me enough patience and strength to complete my study and learn something new every day during my project and to get rid of the few difficulties, which came across in my way during accomplishment of this endeavor.

I would like to extend my sincere appreciation and immense gratitude to ***Dr. Monika Sharma****. Her constant support and understanding as a Major Advisor (Guide) and Chairman Advisory Committee, was the strongest support during my period of my dissertation. I am now, and will remain, extremely grateful for the invaluable guidance she has provided and the wisdom she has imparted and recognize how fortunate I am to have been able to work under such an exceptional woman with such excellent leadership abilities. I admire her accuracy in planning the projects and immense ability of reasoning, and appreciate her cool demeanor, unperturbed composure and perseverance in tackling research problems. She understood me thoroughly and has been very kind and supportive to me all through the period we worked together. It has been an absolute privilege to work under madam and getting to learn so much more from her than just what is limited to my research problem.*

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. B. C. Ghosh, Dr. B. Surendra Nath, Dr,H.C. Devaraja*** *and* ***Dr. Menon Rekha Ravindra.***

I extend my sincere thanks to ***Dr. R.R.B.Singh,*** *Director, National Dairy Research Institute, Karnal,* ***Dr. K P Ramesha****, Head, SRS-NDRI, Bengaluru for providing all necessary facilities to carry out the present investigation. I whole-heartedly thank to* ***Dr. Mukund Khatakthalware,*** *Incharge Education for their kind support in academic matters and concern shown for me throughout the course work*

I also take this opportunity to thank all the scientists at NDRI who have always rendered their support to me in any situation and have always showered me with their affection which made NDRI-SRS feel like a home away from home to me.

I would also express my gratitude to ***Vimala*** *ma'am for all the support provided in doing analysis of my product.*

.

I gratefully acknowledge the valuable help and all possible cooperation extended by the staff members of dairy technology, dairy chemistry and dairy microbiology department. All the other staffs of NDRI deserve my special thanks for giving me so much during my stay here.

I extend my sincere gratitude to ***Mr. Muralidhar,*** *Indian Institute of Sciences, Benguluru;* ***Mr. Ramesha*** *and* ***Mr. Vijay Jadav****, ITC Ltd, AAK Kamani Oil Industry, Mumbai,* ***Dr. S. B. N. Rao sir****, NIANP for my analysis work.*

I am also thankful to all my batch mates ***Mr. Ashok, Mr. Geo Thomas, Mrs. Kavitha, Mr. Naveen, Mr. Abhinash, Ms. Seethu, Mr. Amol Adbhai, Ms Nandini*** *my seniors* ***Ms. Abila Krishna, Mr. Partha Pratim Debnath, Mr. Tambade Pramod Bhivasen, Mr. Jadhao Vishal Mangilal, Mr. Digvijay, Ms. Rashmi Kumari, Mr. Anup Aravindarao Suryawanshi, Mr. Pratik Singh Panwar, Ms. Soumyashree T.C. Mr. Karan Patial, Mr. Rajuayak, Mr. Devaraju, Ms. Priyanka Rangi, Mr. Rupesh Datir, Mr. Gajanan Deshmukh, Ms. Preeti Birwal, Mr. Saurabh Patel, Mr. Bhavesh, Mr. shiva, Mr. Rajshesker Mr. Shriramulu, Mr. Amit, Miss. Puja , Miss. Ammu Mr. Prashant shelke, Ms. Sonia, Mr. Dapaak sharma, Mr. Kaustubh, Mr. Prahant, Mr. Ankur Mr. Adarsh Kalla*** *and* ***Mr. M. Manjunatha*** *are acknowledged. I shall also thank my juniors* ***Ms Sreema, Mr. Shubham, Mr. Avinash, Mr. Omkar, Mr. Darshan, Ms. Devi, Mr. Rahul, Mr. Rahul Rathod*** *and* ***Mr. Aditya*** *for all the support and affection they showered on me. I will forever cherish the moments spent with them.*

Special thanks to ***Prof. Ananta Dhotre*** *and* ***Prof. V. Vijaya Geetha*** *for all the help and great company. I also thank to him for their moral support, affection and constant encouragement.*

Words are not sufficient to express my devotion and gratitude to my dearest parents ***Shrirang*** *and* ***Anitha*** *and loving family members for their motivation, possessiveness, nurture and blessings in counteracting every obstacle coming in the way of my evolution which ensured to have a sensible head on my shoulders. They are and will remain my whole and sole inspiration behind each and every achievement of my life. Without their invaluable sacrifices and moral support, it would have not been possible for me to reach this landmark.*

Date: **Pandule Vishal Shrirang**

Abstract

Butter is a good source of fat soluble vitamins. However, it lacks omega-3 fatty acids; a functional ingredient with several health benefits. Flaxseed oil is a rich source of omega-3 fatty acid i.e. alpha linolenic acid (ALA) and thus utilized for developing fortified butter. The process for omega-3 fatty acid fortified butter was optimized by studying the effect of flaxseed oil @ 2.9 to 5.1% and flaxseed oil emulsion @ 4.8 to 8.6% into cream at two different stages i.e. before ageing and after ageing on textural, sensory and colour characteristics. The sensory acceptability of fortified butter was comparable to control butter. Firmness decreased, while spreadability increased significantly ($p<0.05$) with increasing level of flaxseed oil. The L*, a*, b* and yellowness index decreased significantly ($p<0.05$) with the level of fortificant. Differential scanning calorimetry (DSC) confirmed the presence of flaxseed oil in fortified butter. The alpha-linolenic acid content was higher in the butter fortified with flaxseed oil (3.149%) as well as with emulsion (3.174%) than control (0.86%) as determined by gas chromatography-mass spectrometry. The optimized butter samples were examined for storage stability under refrigeration and frozen conditions for 90 days. Peroxide value, free fatty acids and TBA value increased significantly ($p<0.05$) during storage however, the values were within the acceptable range. The overall sensory acceptability decreased significantly ($p<0.05$) during storage but the scores were still above 7.5 on 9 point hedonic scale. No yeast and mould, coliform count was detected during 90 days storage. Fat and moisture in optimized butter prepared by adding flaxseed oil and emulsion were 85.22% and 14.06% and 87.371% and 12.16%, respectively. Developed butter provides nearly 25% recommended dietary allowance of ALA in one serving.

सारांश

मक्खन वसा में घुलनशील विटामिन का एक अच्छा स्रोत है। हालांकि, इसमें ओमेगा -3 वसीय अम्ल की कमी है; जो कई स्वास्थ्य लाभ के साथ एक कार्यात्मक घटक है। अलसी का तेल, ओमेगा -3 वसीय अम्ल यानी अल्फा लिनोलेनिक एसिड (अ. लि. ए.) का समृद्ध स्रोत है जिस कारण इसका उपयोग मक्खन का ओमेगा-3 वसीय अम्ल के साथ फोर्टीफिकेशन में किया गया। ओमेगा-3 वसीय अम्ल फोर्टीफाइड मक्खन की प्रक्रिया अलसी का तेल और अलसी के तेल का इमल्शन के स्तर के प्रभाव का अध्ययन करके अनुकूलित की गई थी और क्रीम में दो अलग-अलग चरणों में एजिंग से पहले और एजिंग के बाद मिलाया गया।अलसी का तेल @ 2.9% से 5.1% की दर से जबकि अलसी तेल-व्हेय प्रोटीन कंसन्ट्रेट इमल्शन @ 4.8 से 8.6% की दर से क्रीम के आधार पर मिलाया गया और इसके प्रभाव को मूल्यांकन बनावट, संवेदी और रंग विशेषताओं पर अध्ययन किया गया था। फोर्टीफाइड और नियंत्रण मक्खन का मूल्यांकन समीपस्थ रचना और (अ. लि. ए.) सामग्री के लिए किया गया था। फोर्टीफाइड मक्खन की संवेदी स्वीकार्यता नियंत्रित मक्खन के तुलनात्मक थी। अलसी के तेल के बढ़ते स्तर के साथ, दृढ़ता कम हो गई जबकि प्रसार में काफी वृद्धि (पी<0.05) हुई । फोर्टीफिकेन्ट के स्तर के साथ L*, a*, b* और पीलापन सूचकांक काफी कम (पी<0.05) हो गए । डिफरेंशियल स्कैनिंग केलोरीमेट्री (डीएससी) से फोर्टीफाइड मक्खन में अलसी के तेल की उपस्थिति की पुष्टि हुई । गैस क्रोमैटोग्राफी-द्रव्यमान माइक्रोस्कोपी द्वारा निर्धारित अल्फ़ा-लिनोलेनिक एसिड की मात्रा अलसी का तेल (3.149%) और इमल्सन (3.174%) को मिलाकर तैयार मक्खन में नियंत्रण नमूनों (0.86%) से अधिक थी। 90 दिनों के लिए प्रशीतित और उप शून्य तापमान पर भंडारण स्थिरता के लिए अनुकूलित मक्खन के नमूनों की जांच की गई। हालांकि, भंडारण के दौरान पेरोक्साइड मूल्य, मुक्त फैटी एसिड और टीबीए मूल्य में काफी वृद्धि (पी<0.05) हुई है, परंतु यह सभी मूल्य स्वीकार्य सीमा के भीतर थे। भंडारण के दौरान समग्र संवेदी स्वीकार्यता में थोड़ी कमी (पी<0.05) आई लेकिन स्कोर फिर भी 9 पॉइंट हेडोनिक पैमाने पर 7.5 से ऊपर था। 90 दिनों के भंडारण के दौरान नमूनों में कोई किण्व और मोल्ड नहीं पाये गए । अलसी के तेल और इमल्शन को मिलाकर तैयार मक्खन नमूनों में वसा और नमी क्रमशः 85.22% और 14.06% और 87.371% और 12.16% थी। विकसित मक्खन, एक सर्विंग में अल्फा लिनोलेनिक एसिड का लगभग 25% अनुशंसित आहार भत्ता प्रदान करता है।

CONTENTS

Chapter	Title	Page No.
1.0	INTRODUCTION	**1-3**
2.0	REVIEW OF LITERATURE	**4-26**
	2.1 Polyunsaturated fatty acids (PUFAs)	4-7
	2.1.1. Omega-3 fatty acids	4-5
	2.1.2. Omega-6 fatty acids	5
	2.1.3. Metabolic conversion of LA and ALA into EPA and DHA	6-7
	2.2 Health benefits of ω-3 fatty acids	7-11
	2.2.1 Anti diabetic properties of ω-3 fatty acids	7-8
	2.2.2 Reduces atherosclerosis	8-9
	2.2.3 Brain and neural development	9
	2.2.4 Antitumor and anti-cancer	9
	2.2.5 Prevention of kidney disease	9-10
	2.2.6 Reduction in CVDs	10
	2.2.7 Prevention of obesity	10
	2.2.8 Anti-inflammatory, antipyretic and analgesic Activities	11
	2.3 Sources of ω-3 fatty acids	11-12
	2.3.1. Animal sources	11
	2.3.2 Plant sources	11-12
	2.4 Flaxseed and Flaxseed oil	12
	2.5.Dietary recommendation of ω-3 fatty acids	12-13
	2.6 Food fortification	13-15
	2.6.1 Criteria for selection of vehicle for food Fortification	13-14
	2.6.2. Approaches for fortification with omega-3 fatty Acids	14-15

	2.6.2.1 Omega-3 fatty acids rich emulsions	15-116
	2.6.2.2 Oil	16
	2.6.2.3 Microcapsule	16
	2.7 Omega-3 fatty acids fortified food and dairy products	16-20
	2.7.1 Food products	16-18
	2.7. 1.1 Bakery products	16-17
	2.7.1.2. Infant formula	17
	2.7.1.3 Eggs	17-18
	2.7.1.4 Meat	18
	2.7.1.5 Cereal bars and chocolates	18
	2.7.2 Dairy products	18-20
	2.7.2.1 Milk	18-19
	2.7.2.2 Curd	19
	2.7.2.3 Cheese	19
	2.7.2.4 Yoghurt	19
	2.7.2.5 Ice cream	19
	2.7.2.6 Butter	20
	2.8 Quality characteristics of ω-3 fatty acids fortified butter	20-26
	2.8.1 Gas chromatography -mass spectrometry (GC-MS)	20-21
	2.8.2 Thermal properties of butter using Differential	21-22
	Scanning Calorimetry (DSC)	20-21
	2.8.3 Determination of solid fat content using Pulsed	
	Nuclear Magnetic Resonance (pNMR)	22
	2.8.4 Oxidative stability of butter	23-24
	2.8.4.1 Rancimat for studying Induction period	23
	2.8.4.2 Changes in butter quality attributes upon	23-24
	storage	
	2.8.5 Water activity of butter	24
	2.8.6 Colour properties of butter	25
	2.8.7 Textural properties of butter	25-26

3.0	SCOPE AND PLAN OF WORK	**27-29**
	3.1 Scope	27
	3.2 Plan of work	27-29
	3.2.1 Process optimization for preparation of flaxseed oil emulsion	27-28
	3.2.1.1 Selection of stabilizer/emulsifier	27
	3.2.1.2 Optimization of emulsion	27
	3.2.2 Storage study of the omega-3 fatty acid fortified Butter	29
4.0	MATERIALS AND METHODS	**30-48**
	4.1 Materials	30-31
	4.2 Equipment	31-33
	4.3 Apparatus and glassware	33
	4.4 Reagents	33-34
	4.4.1 Preparation of 0.01 N solution of sodium thiosulphate ($Na_2S_2O_3$)	33
	4.4.2 Saturated potassium iodide (KI) solution	34
	4.4.3 Starch indicator (1%)	34
	4.4.4 Phenolphthalein indicator solution	34
	4.4.5 Standard aqueous sodium hydroxide solution (NaOH 0.1N, 0.01N, 0.02N)	34
	4.4.6 Neutralized ethanol	34
	4.4.7 Trichloroacetic acid solution (7.5 and 1%)	34
	4.4.8 Thiobarbituric acid solution (1%)	34
	4.5 Microbiological media	34
	4.6. Methods	35-48
	4.6.1. Optimization of flaxseed oil –WPC emulsion	35
	4.6.1.1 Preparation of flaxseed oil-WPC Emulsion	35
	4.6.1.2 Physical stability	35

	4.6.2 Method for preparation of omega-3 fatty acid fortified butter	36
	4.6.3 Sensory evaluation of the butter samples	37
	4.6.4 Textural analysis of butter	37-38
	4.6.5 Colour analysis of butter	38-39
	4.6.6 Proximate composition of optimized butter	39-42
	4.6.6.1 Determination of fat content in butter	39-40
	4.6.6.2 Determination of moisture content of butter	40
	4.6.6.3 Determination of protein content of butter	40-41
	4.6.6.4 Determination of ash content of butter	41-42
	4.6.6.5 Determination of total carbohydrate content of butter	42
	4.6.7 Titratable acidity of butter	42
	4.6.8 pH of butter	42
	4.6.9 Storage study of the butter	43-44
	4.6.9.1 Peroxide Value (PV)	43
	4.6.9.2 Free Fatty Acids (FFA) Value	43
	4.6.9.3 Thiobarbutiric Acid (TBA) Value	44
	4.6.10 Microbiological analysis.	44-45
	4.6.10.1 Preparation of phosphate buffer	44
	4.6.10.2 Determination of coliform count	44
	4.6.10.3 Determination of yeast & mould count	45
	4.6.11 Determination of Omega-3 fatty acid (ALA) content	45-46
	4.6.11.1 Fat extraction	45
	4.6.11.2 Preparation of fatty acid methyl esters (FAME)	45
	4.6.11.3 Determination of fatty acid	45-46

	compositions by Gas Chromatography- Mass Spectrometry (GC-MS)	
	4.6.12 Thermal properties of butter using Differential scanning Calorimetry (DSC)	46-47
	4.6.13 Oxidative stability in terms of Induction period by Rancimat	47
	4.6.14 Determination of solid fat content by pNMR	47-48
	4.6.15 Water activity of the optimized sample	48
5.0	RESULTS AND DISCUSSION	**49-97**
	5.1. Prologue	49
	5.2 Process optimization for preparation of flaxseed oil emulsion	49-51
	5.2.1 Selection of stabilizer/emulsifier	49
	5.2.2 Selection of level of emulsifier	50
	5.2.3 Optimization of oil load preparation for flaxseed oil emulsion	50-51
	5.3 Optimization of ageing time of cream	51
	5.4 Process optimization for fortification of butter with omega-3 fatty acids	51-67
	5.4.1 Effect of fortificant addition on churning efficiency and yield of butter	51-55
	5.4.1.1 Effect of fortificant addition on fat loss in buttermilk	51-52
	5.4.1.2 Effect of fortificant addition on yield of butter	52-53
	5.4.1.3 Effect of fortificant addition on churning time of cream	53-54
	5.4.2 Sensory characteristics of the control and fortified butter samples	54-58
	5.4.3 Textural properties of the control and	59-63

	fortified butter samples	
	5.4.4 Colour properties of the control and fortified butter samples	63-66
	5.4.5 Inference	67
	5.5 Characterization of Control and Omega-3 fatty acids fortified butter	67-77
	5.5.1 Alpha linolenic acid content in the fortified butter	67-70
	5.5.2 Melting and crystallization characteristics of the control and fortified butter using Differential Scanning Calorimetry (DSC)	70-73
	5.5.3 Solid fat content (SFC) in control and fortified butter	73-74
	5.5.4 Induction period of control and fortified butter	74-76
	5.5.5 Water activity of the control and fortified butter	76
	5.5.6 Proximate composition of optimized and control butter	76-77
	5.6 Storage study of the control and fortified butter	77-97
	5.6.1 Physico-chemical changes in fortified butter during storage	77-79
	5.6.1.1 Acidity of control and fortified butter during storage	77-78
	5.6.1.2 pH of control and fortified butter during storage	78-79
	5.6.2 Oxidative stability of control and fortified butter during storage	79-84
	5.6.2.1 Peroxide value (PV) control and fortified butter during storage	80-81
	5.6.2.2 Free fatty acids (FFA) of control and fortified	81-83

	butter during storage	
	5.6.2.3 Thiobarbituric acid value (TBA value) of control and fortified butter during storage	83-84
	5.6.3 Textural properties of control and fortified butter during storage	85-90
	5.6.4 Sensory characteristics of control and fortified butter during storage	91-95
	5.6.5 Alpha-linolenic acid (ALA) content of control and fortified butter during storage	96
	5.6.6 Microbial quality of control and fortified butter during storage	96-97
6.0	SUMMARY AND CONCLUSION	98-101
	BIBLIOGRAPHY	i-xix
	APPENDIX	

LIST OF TABLES

Table No.	Title	Page No.
2.1	Omega-3 fatty acids content in marine sources	11
2.2	ALA content of selected vegetable oil, seeds and nuts	12
3.1	Independent and dependent variables studied for process optimization	28
3.2	Methodology used for proximate composition of the optimized product	28
3.3	Methodology used for quality evaluation of the omega-3 fatty acid fortified butter	28
3.4	Parameters evaluated during storage study of the optimized butter	29
4.1	Technical specifications of flaxseed oil	30
4.2	Technical specifications of whey protein concentrate	31
4.3	Oil load and WPC content for preparation of emulsion samples	35
4.4	Texture Analyzer settings for spreadability and creep test	38
5.1	Effect of WPC content and oil load on physical stability of emulsion	50
5.2	Sensory properties (9-point hedonic scale) of butter as affected by level of flaxseed oil and stage of addition	56
5.3	Sensory properties (9-point hedonic scale) of butter as affected by level of flaxseed oil-WPC emulsion and stage of addition	58

5.4	Textural properties of butter as affected by level of flaxseed oil and stage of addition	60
5.5	Textural properties of butter as affected by level of flaxseed oil-WPC emulsion and stage of addition	62
5.6	Colour characteristics of butter as affected by level of flaxseed oil and stage of addition	65
5.7	Colour characteristics of butter as affected by level of flaxseed oil-WPC emulsion and stage of addition	66
5.8	Alpha-linolenic acid content of control, fortified butter and ghee made thereof	67
5.9	Thermal data of peaks of the melting and crystallization curve	71
5.10	Proximate composition of control and fortified samples	76
5.11	Effect of storage time on firmness (N) and work of shear (N.sec) of butter fortified with flaxseed oil and flaxseed oil-WPC emulsion when stored at 5 °C	86
5.12	Effect of storage time on Stickiness (N) and work of adhesion (N.sec) of butter fortified with flaxseed oil and flaxseed oil-WPC emulsion when stored at 5 °C	86
5.13	Effect of storage time on firmness (N) and work of shear (N.sec) of butter fortified with flaxseed oil and flaxseed oil-WPC emulsion when stored at -18 °C	88
5.14	Effect of storage time on Stickiness (N) and work of adhesion (N.sec) of butter fortified with flaxseed oil and flaxseed oil-WPC emulsion when stored at -18 °C	88

5.15	Effect of storage time on Yield stress (N) of butter fortified with flaxseed oil and flaxseed oil-WPC emulsion	90
5.16	Effect of storage time on Retardation time (seconds) of butter fortified with flaxseed oil and flaxseed oil-WPC emulsion	90
5.17	Effect of storage time on color & appearance and body & texture of butter fortified with flaxseed oil and flaxseed oil-WPC emulsion when stored at 5°C	93
5.18	Effect of storage time on spreadability and flavour of butter fortified with flaxseed oil and flaxseed oil-WPC emulsion when stored at 5°C	93
5.19	Effect of storage time on overall acceptability butter fortified with flaxseed oil and flaxseed oil-WPC emulsion when stored at 5°C	94
5.20	Effect of storage time on Color & appearance and Body & texture of butter fortified with flaxseed oil and flaxseed oil-WPC emulsion when stored at -18°C	94
5.21	Effect of storage time on spreadability and flavour of butter fortified with flaxseed oil and flaxseed oil-WPC emulsion when stored at -18°C	95
5.22	Effect of storage time on overall acceptability butter fortified with flaxseed oil and flaxseed oil-WPC emulsion when stored at -18°C	95
5.23	Effect storage time on microbial quality of control and fortified butter	97

LIST OF FIGURES

Table No.	Title	Page No
2.1	Chemical structure of ω-3 and ω-6 fatty acids	6
2.2	Lipid oxidation mechanism in foods	15
4.1	Process flow chart for preparation of flaxseed oil–WPC emulsion	35
4.2	Preparation of omega-3 fatty acid fortified butter using flaxseed oil and emulsion	36
4.3	Experimental set up for determining spreadability and creep behavior using Texture analyser.	37
4.4	Kjel plus digestion and distillation assembly	41
4.5	GS-MS equipment	46
4.6	Differential scanning colorimeter (DSC)	47
4.7	Rancimat	47
4.8	Pulsed nuclear magnetic resonance (pNMR) analyser	48
4.9	Water activity meter	48
5.1	Flaxseed oil-WPC emulsions with varying oil load and WPC level	50
5.2	Effect of flaxseed oil addition on fat loss in buttermilk	52
5.3	Effect of flaxseed oil–WPC emulsion addition on fat loss in buttermilk	52
5.4	Effect of stage and level of flaxseed oil addition on yield of butter	53
5.5	Effect of stage and level of flaxseed oil–WPC emulsion addition on yield of butter	53
5.6	Effect of stage and rate of flaxseed oil addition on churning time of cream	54
5.7	Effect of stage and rate of flaxseed oil-WPC emulsion addition on churning time of cream	54
5.8	GC-MS chromatograms of fatty acid methyl esters of Control butter	68
5.9	GC-MS chromatograms of fatty acid methyl esters of fortified butter using flaxseed oil	69

5.10	GC-MS chromatograms of fatty acid methyl esters of fortified butter using flaxseed oil emulsion	69
5.11	GC-MS chromatograms of fatty acid methyl esters flaxseed oil	70
5.12	Melting curves for control and fortified samples	72
5.13	Crystallization curves for control and fortified samples	73
5.14	Solid fat content (SFC) in the control and fortified samples	74
5.15	Induction period (using Rancimat) of control butter	75
5.16	Induction period (using Rancimat) of butter prepared by adding flaxseed oil	75
5.17	Induction period (using Rancimat) of butter prepared by adding flaxseed oil-WPC emulsion	75
5.18	Acidity of control and fortified butter during storage at 5°C	77
5.19	Acidity of control and fortified butter during storage at -18°C	78
5.20	pH of control and fortified butter during storage at 5°C	79
5.21	pH of control and fortified butter during storage at -18°C	79
5.22	Peroxide value of control and fortified butter during storage at 5°C	80
5.23	Peroxide value of control and fortified butter during storage at -18°C	81
5.24	Free fatty acid (FFA) content of control and fortified butter during storage at 5°C	82
5.25	Free fatty acid (FFA) content of control and fortified butter during storage at -18°C	82
5.26	TBA value of control and fortified butter during storage at 5°C	84
5.27	TBA value of control and fortified butter during storage at -18°C	84
5.28	Effect of storage on alpha-linolenic acid content in control and fortified butter	96

LIST OF ABBREVIATIONS

ALA	Alpha linolenic acid
ANOVA	Analysis of Variance
AMDR	Acceptable Macronutrient Distribution Range
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists Society
AR	Analytical reagent
BHT	Butylated hydroxy toulene
cfu	Colony forming unit
CHD	Coronary heart diseases
CKD	Chronic kidney disease
CLA	Conjugated linoleic acid
cm	Centimetre
Conc.	Concentrated
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
DRI	Dietary Reference Intakes
DSC	Differential Scanning Calorimetry
DW	Distilled water
EAAA	Emulsion addition after ageing
EABA	Emulsion addition before ageing
EPA	Eicosapentaenoic acid
FAME	Fatty acid methyl esters
FAO	Food and Agricultural Organization
FDA	Food and Drug administration
FFA	Free fatty acids
FO	Flaxseed oil
FSSAI	Food Safety and Standards Authority of India
GC-MS	Gas Chromatography Mass Spectroscopy
g	Gram
h	Hour
HDL	High density lipoprotein
HMF	High melting fraction
IBD	Inflammatory Bowel Disease
ICMR	Indian Council of Medical Research
IHD	Ischemic heart disease
ISSFAL	International Society for Study of Fatty Acid and Lipids
kg	Kilogram
LA	Linoleic acid/ Lactic acid
LAB	Lactic acid bacteria

LDL	Low density lipoprotein
LMF	Low melting fraction
M	Molar
MFO	Microencapsulated flaxseed oil
MFOP	Microencapsulated flaxseed oil powder
mg	Milligram
min	Minute
ml	Millilitre
mm	Millimetre
MMF	Medium melting fraction
MSNF	Milk solids not fat
MUFA	Monounsaturated fatty acid
N	Normality/ Newton
NCDC	National Collection of Dairy Cultures
NIST	National Institute of Standards and Technology
No.	Number
N.s	Newton. Seconds
OAAA	Oil addition after ageing
OABA	Oil addition before ageing
o/w	Oil-in-water
pNMR	Pulsed nuclear magnetic resonance
PUFA	Polyunsaturated fatty acid
PV	Peroxide value
RDA	Recommended dietary allowances
s	Second
SD	Standard deviation
SFC	Solid fat content
SNF	Solids not fat
TBA value	Thiobarbituric acid value
v/s	Versus
VLDL	Very low density lipoprotein
wt	Weight
WPC	Whey Protein Concentrate
WPI	Whey Protein Isolate
w/v	Weight/volume
WHO	World Health Organization
mEq	Milliequivalent

1.0 INTRODUCTION

Butter is one of the oldest way of preserving milk fat with its mention in some of the historical records. In Ayurveda, butter has been used for many medicinal as well as cosmetic purposes. From ancient days, it is used as a sacrificial worship in the yagna. Food application of the butter has been observed in the earlier days for preparation of the sweets and in several homemade recipes. Nowadays it has found applications in nearly all the food processing sectors including bakery foods, snacks, etc.

Butter is a fat rich dairy product with up to 80% fat. India is the largest producer with a very high growth rate. Butter production in India is increasing in past few years with 5.4 Million Tonnes and 5.6 Million Tonnes in 2017 and 2018 respectively (Annon, 2018). Also, per capita consumption of butter in India increased from 0.4 kg to 0.5 kg from the year 2015 to 2016. Butter is a very good source of fat and fat soluble vitamins viz. A, E, K. However, it contains high level of cholesterol and saturated fats which can be a cause of health concern (Drouin *et al*., 2018). On the contrary, butter lacks in certain essential functional components which include omega-3 fatty acids.

The omega-3 fatty acids play a significant role in prevention of CVDs, eye health, reduces the risk of hypertension, inflammatory bowel disease (IBD), coronary heart diseases, neurogenerative disorder, cancers and diabetes (Chen *et al.,* 2013), anti-inflammatory (Mori *et al.,* 2000) and hypocholesterolemic effect (Dawczynski *et al.,* 2010). Due to the changing eating habits, the ratio of omega 6: omega 3 fatty acid is found to be very high to the level of 38-50:1 (Singh *et al.,* 2011 a & b) among the Indian urban population compared to the level of 5:1 (FAO/WHO, 2010). Thus, butter could be a potential vehicle for fortification with omega-3 fatty acids.

The ideal ratio can be only achieved by fortification of food products with omega-3 fatty acids. Also, fat rich dairy products are compatible for the maximum fortification with omega-3 fatty acid with added flavourings (Kolanowski *et al.,* 2007). Fortification could be achieved through various sources of omega-3 fatty acids such as oily fishes which majorly contain eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) while, alpha-linolenic acid (ALA) is found in vegetarian source like flaxseeds, soybean oil, wheat germ oil, canola oil and nuts (Gruenwald *et al.,* 2009, Mantzioris *et al.,* 2000). There are certain ethical issues related to the consumption of fish oil among the vegetarian groups of people. This has paved the way for vegetarian alternatives. Flaxseed is considered as rich vegetarian source of alpha-linolenic acid and thus offers several health benefits. This ALA is converted into DHA and EPA in human body through elongation and desaturation process. Further it is vital to note that, supplementation of α-linolenic acid (ALA) with dairy lipids increases the level of DHA in the tissues than ALA alone (Drouin *et al.,* 2018).

Flaxseed is one of the source of polyunsaturated fatty acid (PUFA). Chemically flaxseed is composed of fat 35-45%, total dietary fibre 28%, protein 20-30%, ash 3.4% and moisture 7.7% (Martinchik *et al.*, 2012). Lipids content in the flaxseed comprise of 9% saturated fatty acid, 18% monosaturated fatty acid and 73% polyunsaturated fatty acids (Brenna *et al.*, 2009). Along with higher content of polyunsaturated fatty acid, flaxseed contains some antinutritional factors like cyanogenic glycosides about 250-550 mg/100 g (Oomah and Mazza, 1998). Flaxseed damage triggers activity of β-glucosidases results in production of hydrogen cyanides and hydrogen cyanide is responsible for food poisoning. This problem can be resolved with the adequate processing of the foodstuff (Kucukoner and Haque, 2003). The anti-nutritional factors are not present in the flaxseed oil. Thus, flaxseed oil with higher ALA content is preferred for food fortification with omega-3 fatty acids.

As flaxseed oil mostly contains nearly 75 per cent polyunsaturated fatty acids such as alpha-linolenic and linoleic fatty acids, it is more prone to the oxidation (Ramcharitar *et al.*, 2005) and may result in toxic peroxides upon processing such as heat treatment and during handling. Thus, direct fortification of food and dairy products with flaxseed oil may results in products susceptible to oxidation which finally results in off flavour development. Further, flaxseed oil can be added in the form of emulsion for fortification purpose. The organoleptic properties of emulsion are better than oily solutions and pills forms. Also, the bioavailability of ALA has reported to be higher through emulsion (McKenna and Kilcast 2003; Couedelo *et al.*, 2011).

Various stabilizers or emulsifiers have been used for preparation of omega-3 fatty acid emulsion such as sodium caseinate, phospholipid, whey protein concentrate (WPC), gum arabic, whey protein isolate (WPI) etc. in order to maximize the oil load in the emulsion. It has been reported that, WPI and WPC have good emulsifying property.

Since butter is the fourth most consumed dairy product after fluid milk, ghee, and curd and it also lacks omega-3 fatty acids; there is enough scope for its fortification with ALA. In addition to this, it is a fat rich product and thus would be quite compatible with flaxseed oil. Thus, in the present study the butter has been selected for fortification with alpha-linolenic acid. Alpha-linolenic acid (ALA) 1.6 g/day, while EPA plus DHA 250 mg/day was recommended by the Indian Council of Medical Research (ICMR, 2010). Consequently, this would be an attempt for fortifying butter to have atleast 25% RDA of alpha-linolenic acid in one serving. Addition of flaxseed oil either as such or in the form of emulsion is expected to influence the physico-chemical, textural and sensory properties of fortified butter. Also, the stage of addition of flaxseed oil and emulsion needs to be optimized. Further, limited work has been reported on fortification of butter with omega-3 fatty acids especially with plant-based sources. Thus, the present study has been planned to fortify butter using flaxseed oil with the following objectives:

1. To optimize the process for fortification of butter with omega-3 fatty acid (flaxseed oil).
2. To evaluate the quality characteristics and storage stability of fortified butter.

2.0 REVIEW OF LITERATURE

This chapter reviews the current status of polyunsaturated fatty acids (PUFAs) especially α-linolenic acid; one of the ω-3 fatty acid, health benefits, sources, major challenges for fortification in the dairy products. This chapter also includes review of various food products fortified with ω-3 fatty acid and effect of ALA fortification on various physico-chemical and functional properties of butter.

2.1 Polyunsaturated fatty acids (PUFAs)

Dietary fatty acids show a significant role in supplying energy to the body as they are main source of energy. Along with providing energy, these are the precursor of some bio molecules and involve in the formation of cell membrane (Hulbert *et al.,* 2005). Dietary fatty acids not only help in survival but also well being of humans (Hwang and Rhee, 1999). The occurrence or absence of double bonds within the alkyl chain of fatty acids, they are categorized into two different groups. Saturated fatty acids show absence of double bond in the alkyl chain; while one or more double bonds are present in unsaturated fatty acids. Fatty acids with at least two unsaturated bonds in their structure are called as polyunsaturated fatty acid (Christie, 1995); The fatty acids can be further classified as long chain, medium chain or short chain depending upon the length of alkyl groups.

Structural arrangement further classified PUFAs into different groups; conjugated fatty acids, methylene interrupted polyenes and other PUFAs. Methylene interrupted polyenes showed divinyl- methane pattern having at least two cis double bonds partitioned from each other with single methylene ($-CH_2-$) bridge (Gunstone, 2006). Another group contains more than two conjugated double bonds in their structure and thus called conjugated fatty acids while third group includes podocarpic acids, pinolenic acid. As work in the present study is related with omega-3 and omega-6 fatty acids, they are discussed in detail in the subsequent section.

2.1.1 Omega-3 fatty acids

Omega-3 fatty acids are unsaturated fatty acid with more than one double bond. Among the two ends of fatty acid, one end with carboxyl group (-COOH) and on the other methyl group ($-CH_3$) exists. The first double bond appears after the third carbon atom from the methyl end of alkyl chain. Methyl end is also known as omega- (ω) end thereby the nomenclature of ω-3 fatty acids being used for such polyunsaturated fatty acids. The ω-3 fatty acids vary in the alkyl chain length and number of double bonds. When three conventionally double bonds are present in an 18-carbon fatty acid it is said to be alpha linolenic acid (C18:3); while 5 and 6 double bonds containing 20 and 22 carbon fatty acids are said to be Eicosapentaenoic acid (C20:5) and Docosahexaenoic acid (C22:6) respectively (Markiewicz-Keszycha *et al*, 2013). The long chain ω-3 fatty acids can be either directly taken from diet or synthesized in human body form ALA by elongation and desaturation pathways. However; this conversion may be limited to levels even below 10% in some individuals (Brenna *et al.*, 2009)

2.1.2 Omega-6 fatty acids

Omega-6 fatty acids also termed as ω-6 or n-6 fatty acids contain first double bond in the alkyl chain, sixth position from the methyl terminus of the chain. Linoleic acid (LA) accounts 85-90% of omega-6 PUFA and is an essential fatty acid with 18 carbon and 2 double bonds in the alkyl chain structure (Harris *et al.,* 2009). Absence of omega-3 denaturase in the mammalian cells results in inability to convert ω-6 fatty acid to ω-3 fatty acid (Bibus *et al.,* 2015). Linoleic acid undergoes desaturation and elongation to produce γ-linolenic and dihomo-γ-linolenic acids which include another group of omega-6 PUFAs These could be further converted into arachidonic acid (C20:4),various types of eicosanoids such as thromboxane A_2, leukotriene B_4 and prostaglandin E_2. They exhibit proinflammatory, proaggregatory, and/or vasoconstrictive properties responsible for inflammation and hypertension (Harris *et al.,* 2009).

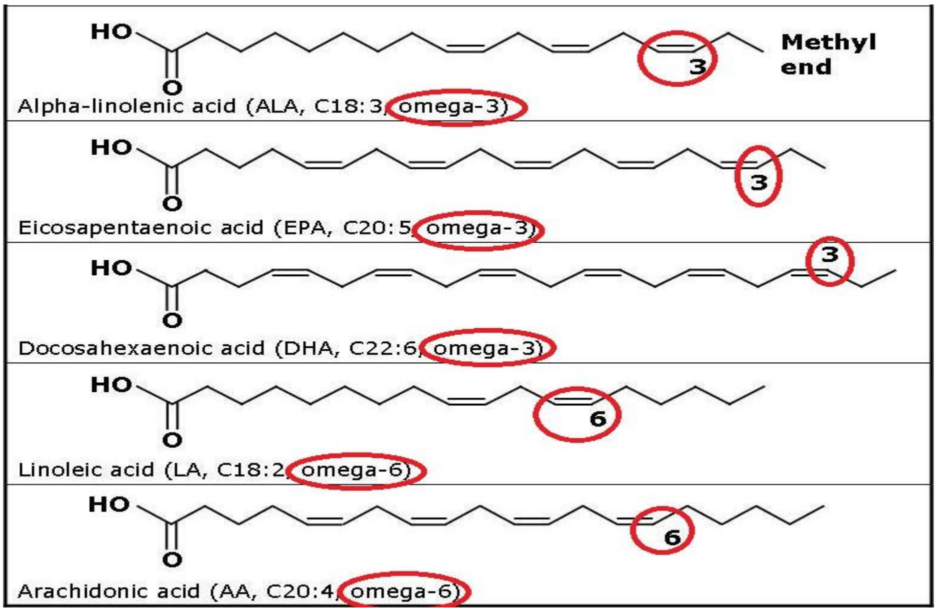


Figure 2.1 Chemical structure of ω-3 and ω-6 fatty acids

(Adopted from: biochemianzunited.wordpress.com**)**

2.1.3 Metabolic conversion of LA and ALA into EPA and DHA

The omega-3 and omega-6 fatty acids are said to be essential owing to their involvement in various structural and physiological functions. To begin with the structural components of cell membranes help in prostaglandin formation (Simopoulos, 2010). Linoleic acid (LA) upon conversion into 2- and 4- series prostacyclin and prostaglandin compounds i.e. eicosanoids like prostaglandin, thromboxanes and leukotrienes, encourage inflammation and platelet aggregation while opposite effect is observed for ω-3 fatty acids. (Gogus and Smith, 2010). Prostaglandin is the hormone like substance which involve in the relaxation and contraction of smooth muscle, blood vessels constriction and causes inflammation. Thromboxane is a lipid which causes constriction of the blood vessels. Leukotrienes is also a lipid and responsible for inflammation (Ghafoorunissa, 1998; Harris *et al.,* 2009). Among all ω-6 fatty acids arachidonic acid shows maximum inflammatory activity and stimulates the production of glutamate (neurotransmitter) causing neuron destruction by undue free radical production.

ω-3 PUFAs produced by prostaglandin1 has numerous beneficial effects such as calcium movement, controls cell growth and hormone regulation. It also produces prostaglandin 3 and prostacyclin 3 which has similar benefits like prostaglandin 1 i.e. anti-inflammatory activity and increases the level of trienoic prostaglandins, pentaenoic leukotrienes and thromboxane A3 (Trautwein, 2001). Incorporation of ω-3 in the phospholipids affect the membrane properties of cell such as flexibility, fluidity, permeability and most importantly the activity of membrane bound enzymes (Stillwell and Wassal, 2003). DHA elevates the extent of serotonin ("feel good" neurotransmitter) and acetylcholine (memory boosting chemical). It also carried out neutralization of the oxygen free radicals, decreases cellular and vascular inflammation in brain and keeps the cell membranes in the brain soft and pliable. Thus ω-3 PUFAs function significantly in treatment in various diseases mainly inflammatory bowel disease (IBD), psoriasis, rheumatoid arthritis and eczema (Cleland *et al.,* 2003).

The metabolic conversion of ω-3 fatty acid includes involvement of several enzymes. In case of ω-3 fatty acids, α-linolenic undergoes desaturation and elongation by the enzymes Δ6-desaturase and elongase respectively to form Eicosatetraenoic acid (20:4 ω-3). Again, it undergoes desaturation and elongation to form Eicosapentaenoic acid (20:5 ω-3). Finally, elongation, desaturation and β-oxidation EPA lead to the formation of Docosahexaenoic acid (DHA). Further EPA produces anti-inflammatory compounds (3-series prostaglandins, 3-series thromboxane, 5-series leukotriene and E-resolvin) and DHA produces D-resolvin and D-protectin (Venegas-Calerón *et al.,* 2010).

In ω-6 fatty acid, linoleic acid is converted into the γ-linoleic acid by Δ6-desaturase enzyme and further into the Dihomo-γ-linoleic acid by elongase. Dihomo-γ-linoleic acid is converted into the Arachidonic acid (20:4ω-6) by Δ5- desaturase. The arachidonic acid undergoes elongation, desaturation and β-oxidation to produce Docosapentaenoic acid (22:5ω-6). Arachidonic acid is responsible for the production of inflammatory compounds such as 2-series prostaglandins, 2-series thromboxane and 4-series leukotriene (Venegas-Calerón *et al.,* 2010).

2.2 Health benefits of ω-3 fatty acids

2.2.1 Antidiabetic properties of ω-3 fatty acids

In diabetic patients; blood glucose level increases because of the body's inability to synthesis completely or partially insulin. In such case, insulin secretary glands called pancreas becomes non-functional because of damage to the insulin secreting cells. Diabetes categories into two types i.e. Type 1 and Type 2 diabetes. Type 1 diabetes is caused by inability to produce insulin while Type 2 diabetes is cause by inability not only in insulin production but also in its utilization. Among the two, Type 2 is very common among the population.

There are several reports where positive health benefits are observed in diabetic patients when fed with omega-3 fatty acids. Han *et al.* (2016) observed the influence of ω-3 fatty acids supplementation in patients having diabetes and hypertriglyceridemia. In this examination, total 344 patients were examined having type-2 diabetes. These patients were divided into subgroups based on their daily doses (1, 2 and 4 g per day) of ω-3 fatty acids. In this study, albumin to creatinine ratio and glomerular filtrate was examined immediately after administration and after 8 h of fasting. It was found that out of 344 patients 172 did not face the problem of renal non-functioning and 125 patients found positive slope in glomerular filtrate. Also, patients given with higher dose found better renal maintenance that those given with lower doses.

Vuksan *et al.* (2007) studied effect of feeding Salba (*Salvia hispanica L.*) which rich source of ALA and fibre on improvement in the cardiovascular disease of patients having type 2 diabetes. This study was conducted with the 20 individuals (11 males and 9 females) and given Salba or wheat bran for 12 weeks. Control group provided with the wheat bran while the other with Salba (37 ± 4 g per day). Observations revealed that reduction in, high-sensitivity C-reactive protein, vonWillebrand factor and systolic blood pressure. Similarly, significant decrease in the fibrinogen and A_1C was observed in the salba supplemented group not in control. Thus, supplementation of Salba for longer period of time helpful in systolic blood pressure reduction, lipid control and maintaining proper glycemic index.

Similar kind of relationship has been found when flaxseed or its products are used as a source of ω-3 fatty acids. Kelley *et al.* (2009) observed 20% glycemia reduction by supplementation of 0.5% CLA and 0.5 % flaxseed in the rats susceptible to diabetic tumours. Kapoor *et al.* (2011) supplemented flaxseed powder @ 15 and 20 g of per day over a period of 2 months and observed the effect on blood sugar level. Decrease in the blood sugar level by 7.9% and 19.1% in the rats fed with 15 g and 20g respectively was observed. Similar kind of study has been reported by Nazni *et al.* (2006) on 25 diabetic patients. It was found that there was significant reduction in the blood glucose level of diabetic patients due to supplementation flaxseed powder form of bread for 90 days.

2.2.2 Reduces atherosclerosis

Atherosclerosis is the disease in which blood flow get obstructed due to formation of plaque. Plaque is made of cholesterol, fat, other compounds present in the blood. Plaque gradually grows up and causes hardening of the arteries which supplies oxygenated blood to the heart and other part of the body. This ultimately leads to stroke, heart attack and also peripheral vascular disease. This is where comes the beneficial effects of ω-3 fatty acids in preventing atherosclerosis. Dyerberg *et al.* (2004) studied the same behaviour in case of healthy males. They conducted an intervention study for 8 weeks by supplementing three different diets (trans fat enriched diet using partially hydrogenated soybean oil, ω-3 fatty acids enriched diet and control diets) and observed the effect on HDL cholesterol, blood pressure and other parameters. HDL cholesterol level of the group supplemented with trans fatty acid was decreased and ω-3 fatty acid supplementation showed lower blood pressure; thereby preventing atherosclerosis.

2.2.3 Brain and neural development

The ω-3 fatty acids also play a very significant role in the brain, neural and cognitive development. Supplementation of new-born animals with α-linolenic acid deficient milk formulas resulted in decrease in DHA as well as increase in level of ω-6 fatty acids (adrenic acid, osbond acid) in the retina and brain both (Arbuckle and Innis, 1992; Ward *et al.,* 1998). The decreased level of DHA in the brain of the animals supplemented with deficient in alpha- linolenic acid leads to alteration in the neurotransmitter metabolism along with serotonin and dopamine and membrane linked enzymes (Innis and Jacobson, 2007). Supplementary studies indicate that deficiency of ω-3 fatty acid decreases the size of neurons in the various organs such as parietal cortex, hypothalamus and reduces the trouble of cortical dendritic arborization (Wainwright *et al.,* 1998; Ahmad *et al.,* 2002).

2.2.4 Antitumor and anti-cancer

Globally, out of 6 deaths 1 is due to the cancer contributing to about 9.6 million deaths in 2018. Omega-3 fatty acids have a role to play in this context with antitumor benefits. Being source omega-3 fatty acids, flaxseed oil can be used in treatment of cancer. Truan *et al.* (2010) established the effect of feeding flaxseed oil to rats for 8 weeks, with 33% reduction in tumour area, 38% reduce in proliferation of tumour cells and thereby causing elevation in the apoptosis (110%). Similarly, Chen *et al.* (2007) also observed 26 % and 38 % reduction on tumor cells growth by feeding 5 and 10% flaxseed, respectively.

2.2.5 Prevention of kidney disease

Chronic kidney disease is general condition in all age groups which increases the risk of kidney failure, CKD and other kidney related complications (National Kidney Foundation, 2002; Sarnak *et al.,* 2003; Levey *et al.,* 2005). Anti-inflammatory properties of ω-3 fatty acids exhibit protective action against these diseases. Gopinath *et al.* (2011)

studied the effect of ω-3 PUFA on fibrosis and renal inflammation. Observations revealed that increased intake reduces occurrence of CKD. Cicero *et al.* (2010) observed the declined in systolic and diastolic blood pressure with long-term feeding of ω-3 PUFA and thus helpful in prevention of CKD by decreasing hypertension, one of the risk factors for kidney disease. An *et al.* (2009) reported that long term supplementation of ω-3 fatty acids can reduce the upregulation of prooxidant, profibrotic and proinflammatory pathways and helpful in attenuation of tubulointerstitial fibrosis in the remnant kidney.

2.2.6 Reduction in CVDs

Cardiovascular diseases are related to blood vessels and heart (Pekka *et al.,* 2011). It includes a group of disease namely congenital heart disease, heart failure, stroke, peripheral artery disease, venous thrombosis etc. (Pekka *et al.,* 2011; GBD, 2013). There is direct relationship between lipid profile of the blood serum and CVDs. Hypocholesterolmic activity of flaxseed has been studied on various animal including mice, rats and rabbits (Cardozo *et al.*, 2010; Barakat and Mehmoud, 2011; Leyvaa *et al.,* 2011; Mani *et al.*, 2011; Khalesi *et al.*, 2011; Kristensen *et al.*, 2012; Hassan *et al.,* 2012). Dawczynski *et al*. (2010) had given about 40 g fat, partially replaced by fish oil, rapseed oil containing a daily dose of 3 g omega-3 fatty acids daily to patients and observed that the consumption of dairy products containing n-3 PUFA decreases cardiovascular risk. Similarly, Vijaimohan *et al.* (2006) fed rats with high fat diet containing flaxseed oil (1 mL/kg b.w.) for 60 days and observed the hypocholestrolemic activity through dramatically lowered plasma cholesterol, liver weight, LDL, VLDL and HDL. Jennifer *et al.* (2010) reported improvement in the vascular relaxation responses by inhibiting ventricular fibrillation incidences due to the supplementation of dietary flaxseed and thus protect from Ischemic heart disease (IHD).

2.2.7 Prevention of obesity

Obesity is the metabolic disorder of the human being and its prevalence is increasing day by day. According to WHO report in 2018, 18% children and adolescents, 39% men and 39% women were obese. Flaxseed oil has laxative effect and therefore helpful in the problem related with obesity by improving metabolic functions. Owing to this advantage traditionally also flaxseed was used in treatment of obesity as well as diabetics (Santos *et al.*, 2010; Singh *et al.*, 2011b).

2.2.8 Anti-inflammatory, antipyretic and analgesic activities

Flaxseed oil provides several health benefits i.e. anti-inflammatory, antipyretic and analgesic in addition to the above discussed beneficial effects. Wistar and Swiss albino rats when fed with 3 mg/kg body wt., exhibited analgesic activity comparable to aspirin (Kaithwas *et al.*, 2011)

2.3 Sources of ω-3 fatty acids

Omega-3 fatty acids are essential fatty acids and have to be taken from the food to fulfil the daily requirement of the body. Food sources of the ω-3 fatty acids comprise both plant origin and animal origin sources.

2.3.1 Animal sources

Marine sources seaweed and algae are rich in EPA and DHA (Adarme-Vega *et al.*, 2014) which includes oily fishes (Cod, Salmon, Mackerel, Seabass, Sardines, Trout) (Calder, 2015), shrimps and oysters. ω-3 fatty acids content in some fish oil is shown in the Table 2.1

Table 2.1 Omega-3 fatty acids content in marine sources

Source	**EPA+DHA content,(g/g oil)**	**Amount required to provide ~1g of EPA+DHA (g oil)**
Cod (Pacific)	0.13	23
Salmon (chum)	0.68	4.5
Mackerel	0.34-1.57	2-8.5
Sardines	0.98-1.70	2-3
Trout (Farmed)	0.98	3
Herring (Pacific)	1.81	1.5
Oyster (Pacific)	1.17	2.5
Shrimp mixed species	0.27	11

(Adapted from USDA Nutrient Data Laboratory, 2002)

2.3.2. Plant sources

Plant sources contain omega-3 and omega-6 fatty acids in the form of ALA and LA. ALA is obtained from plant origin sources. Some of the plant sources of ALA includes certain oils such as flaxseed oil, wheat germ oil, soybean oil, canola oil etc., seeds (flaxseed, hemp, pumpkin) and nuts (walnuts) (Covington, 2004; Gruenwald *et al.,* 2009) content of the selected vegetable oil, seeds and nuts is given in Table 2.2.

Table 2.2 ALA content of selected vegetable oil, seeds and nuts

Source	α-Linolenic Acid content (%)
Olive oil	0.7
Soybean oil	6.38
Canola oil	9.21
Flaxseed oil	60.28
Walnut oil	9.92
Flaxseeds	15.62
Walnuts	4.96

(Adapted from USDA Nutrient Data Laboratory, 2002)

2.4 Flaxseed and Flaxseed oil

Flaxseed is an oilseed crop with maximum production by Canada, Ethiopia, China, United States and India. Canada ranks 1st in flaxseed production and contributing to about 80% of total production (Oomah and Mazza, 1998). India produces 10.2% of total production and ranks 3rd (Singh *et al.,* 2011a). Flaxseed belongs to the family linaceae and its latin name is *Linum usitatissimum* which means "very useful". Depending upon the application, there two varieties of flaxseed i.e. seed flax and fiber flax. Oil flax is grown for oil production which is used for direct consumption as well as utilised for production of soap, paints, oil cloths, varnishes and linoleum etc.

Flaxseed is one of the rich plant source of polyunsaturated fatty acid (PUFA). Chemically flaxseed composed of fat 35-45%, total dietary fibre 28%, protein 20-30%, ash 3.4% and moisture 7.7% (Martinchik *et al.,* 2012). Flaxseed oil is extracted from the flaxseeds by cold pressing of dried seeds. Flaxseed oil is also called as linseed oil and mostly used for human consumption because of its several health benefits. Flaxseed oil mostly contains unsaturated fatty acids such as ω-3 and ω-6 fatty acids. It contains nearly 16% linoleic acid, whereas approximately 57-60% α-linolenic acid of flaxseed oil (Ramcharitar *et al.,* 2005).

2.5. Dietary recommendation of ω-3 fatty acids

A deficiency of essential ω-3 fatty acids causes various health problems such as dermatitis, rough skin and scaly skin. Decrease in the DHA concentration tissue and plasma is also cause by deficiency of ω-3 fatty acids. Omega-3 fatty acids prevent cardiovascular diseases and improves the functioning and development of retina, brain and testis (Simopoulos *et al.,* 1999; Connor, 2000; Banning, 2005). Omega-6 to omega-3 ratio in Indian urban and Western diets is 38-50:1 and 20:1, respectively (Singh *et al.,* 2011a and b) which is much than FAO/WHO 2010 recommendation of omega-6 to omega-3 ratio i.e. 5:1. In light of all these points various agencies have given different recommendation level.

Indian Council of Medical Research has given Recommended dietary allowances (RDA) for ALA, 1.6 g/day and for EPA & DHA, >250 mg/day (ICMR, 2010). Similarly, International Society for the Study of Fatty Acids and Lipids suggested RDA level for ALA, 2.2 g/day and for EPA/DHA, 500 mg/day (ISSFAL, 2004). The minimum intake levels given by ICMR (2010), is out of total energy, 2.5% from linoleic acid 0.5 % from α-linolenic acid for prevention of deficiency symptoms. Some of the countries like Canada, United Kingdom, Sweden, Japan, Australia and World Health Organization (WHO) have decided dietary recommendations for ω-3 fatty acids. This includes ALA about 0.8 to 1.1 g/day and EPA+DHA about 0.3 to 0.5 g/day. Dietary Reference Intakes (DRI) for energy and macronutrients was given by Institute of Medicine and Food and Nutrition Board, in association with Health Canada. The Acceptable Macronutrient Distribution Range (AMDR) is given on the basis of energy utilized from ALA i.e. 0.6% to 1.2% of energy, based on daily need i.e. 1.3 to 2.7 g/day and based on calorie i.e. 2000-calorie of total diet (Kris-Etherton *et al.,* 2002). This is much more than the current consumption of EPA+DHA.

2.6 Food fortification

Food fortification process includes addition of one or more essential nutrients to improve the functional properties of food. Food fortification plays a major role in dealing with problems related to deficiency diseases. The Codex General Principles for the Addition of Essential Nutrients to Foods (61) defines "fortification", or synonymously "enrichment", as "the addition of one or more essential nutrients to a food whether or not it is normally present in the food, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups" (FAO/WHO, 1996).

2.6.1 Criteria for selection of vehicle for food fortification

Selection of an ideal vehicle for fortification of food products is the most important step. Depending upon the nature of fortificant and its compatibility with the food system, the carrier has to be selected. Criteria for selection of vehicle for food fortification are given below**.**

- Commonly consumed by the target population
- Constant consumption pattern among the target population with a minimum risk associated with excess consumption
- Good storage stability
- Relatively low cost
- Centrally processed with minimal stratification of the fortificant
- No interactions between the fortificant and the carrier food
- Contained in most meals, with the availability unrelated to socio-economic status
- Linked to energy intake (FAO/WHO, 1996)

2.6.2 Approaches for fortification with ω-3 fatty acids:

Dietary lipids are prone to the oxidation and forms unsaturated carbonyls and other compounds during food processing (Eunok and David, 2006). Fatty acids present in the lipids converted into lipid alkyl radical with removal of hydrogen atom from the structure. The factors responsible for conversion are metal catalysts present in the lipids, heat and UV light exposure (Ganesan *et al.,* 2014). Atmospheric triplet oxygen reacts with lipid alkyl radical and produces allylic hydroperoxide. These allylic hydroperoxide takes hydrogen atom from another molecules and forms more reactive lipid peroxyl radicals (Ganesan *et al.,* 2014). Presence of transition metals such as iron, copper helps in elevation rate of autooxidation by decreasing the activation energy required for initiation. Additionally, photosensitizer (type II) and light converted triplet oxygen into singlet oxygen which further react with lipids and form peroxyl radicals. The autoxidation process ends with the formation of stable end products from reaction of hydroperoxides and / or free radicals. Another reasons for elevation of lipid oxidation is free fatty acids. Antioxidants prolongs the induction period of autooxidation and another mechanism of action is free radicals and/ or hydroperoxide scavenging effect to delay the process of autooxidation (Choe and Min, 2007). Preparation of emulsion makes the lipids unavailable for oxidant (Let *et al.,* 2007).

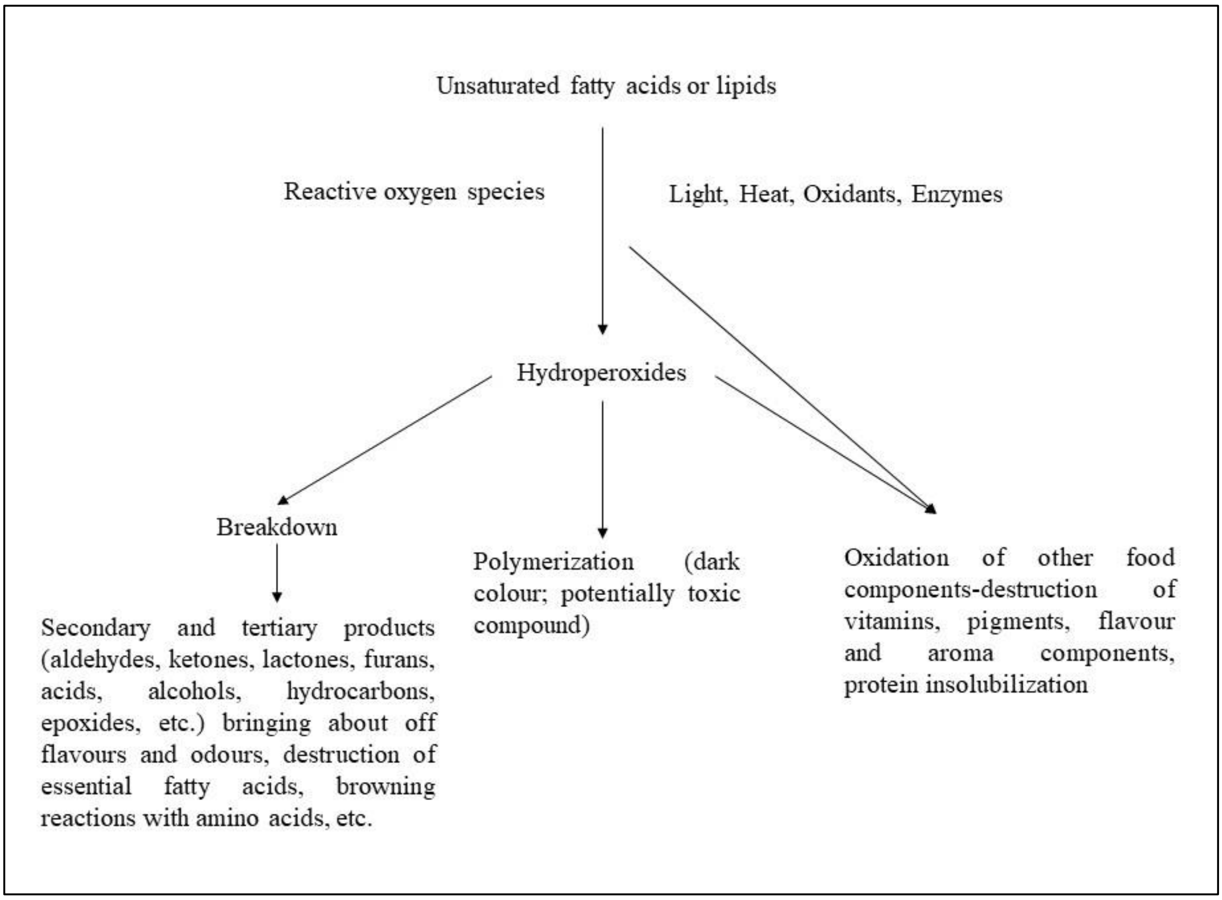


Figure 2.2 Lipid oxidation mechanism in foods (Adopted from Kocchar, 1995)

Higher unsaturated fatty acids content in the flaxseed oil makes it more prone to the oxidation. Different approaches were found for fortification of food products i.e. emulsion, oil and microcapsules.

2.6.2.1 Omega-3 fatty acids rich emulsions

The oils either plant or marine origin which are rich source of ω-3 fatty acids i.e. α-linolenic acid and other unsaturated fatty acids, have higher susceptibility to oxidation. To overcome this problem, various efforts have been made for preparation of emulsion so that the oil is protected from external oxygen supply thereby reducing the extent of oxidation. Kartal *et al.* (2016) prepared multilayer oil in water emulsion with 10% oil load (flaxseed oil) using pectin and Na-caseinate by micro fluidization process and observed that multilayer membrane improves emulsion stability at pH values between (3 - 5) and also oxidative stability compared to a monolayer membrane. Lee and Choo (2015) prepared flaxseed oil emulsion with 40% oil load using WPI, gum arabic, xanthan gum, potassium sorbate, citric acid and tween 80 and observed that surfactants has more emulsifying capacity compared to proteins in flaxseed oil. Lin *et al.* (2014) fortified dairy products with soy lecithin-stabilized emulsions containing 50 wt.% algal oil, 6 wt.% soy lecithin, and 44 wt.% Milli-Q water. This resulted in increased stability of emulsion at gastric pH and improved bio accessibility of omega-3 fatty acids. Horn *et al*. (2011) compared the oxidative stability of phospholipids and protein-based fish oil emulsion (70% oil load) prepared using micro fluidization. They observed that phospholipids function better at acidic pH while protein behaves better at neutral pH.

2.6.2.2 Oil

Divya *et al.* (2013) prepared fortified milk using DHA and flaxseed oil and observed the rancid flavour after 3 days of refrigerated storage. Lim *et al.* (2010) developed ice cream added with flaxseed oil and reported decrease in the flavour score with increasing level of addition. Thus, fortification of ω-3 fatty acids by direct oil makes the product prone to the oxidation as well as affect the sensory quality of the product.

2.6.2.3 Microcapsules

Goyal *et al.* (2015) prepared ω-3 fatty acids fortified milk using flaxseed oil microcapsules and observed the similar sensory quality with the control upto 5 days of storage. Tambade (2018) prepared pasteurized and sterilized flavour milk fortified with ω-3 fatty acids using flaxseed oil microcapsules. During storage the pasteurized and sterilized milk were stable upto 6 and 28 days respectively. Estrada *et al.* (2011) prepared strawberry flavoured yoghurt by adding salmon oil microcapsules and observed the increase in the peroxide as well TBA value without change in the colour characteristics of the product. Thus emulsion and microcapsules provides better oxidative stability over the direct oil without much affecting the sensory quality of product.

2.7 Omega-3 fatty acids fortified food and dairy products

2.7.1 Food products

2.7. 1.1 Bakery products

Wheat is one of the staple food in Western diet and comes on 3rd position after rice and corn in terms of production (Ganesan *et al.*, 2014). Bread making process includes additions of various oils during dough making. Replacement of these oils with ω-3 fatty acids enriched oils is difficult as it affects the sensory quality as well as durability of the product during storage (Serna-Saldivaretal *et al.*, 2006). Various bakery products such as biscuits, cookies, breads have been prepared by replacing oils with the ω-3 fatty acids enriched oils but only bread could grasped the commercial production. Gokmen *et al.* (2011) prepared bread with enhanced ω-3 fatty acids content by adding flaxseed oil in the form of microparticle powder (made of flaxseed oil and corn starch rich in amylose nanosized complexes). Fortification with the nano encapsulated powder of flaxseed oil improved bread quality and storage stability. Yep *et al.* (2002) supplemented ω-3 fatty acids in bread using microencapsulated tuna oil and feed the same for a period of 3 weeks. They observed an increase in ω-3 fatty acid level in the blood plasma of all the subjects. Umesha *et al.* (2015) carried out a comparative study for fortification of biscuits (1 g ALA/ 100 g biscuits). Microencapsulated garden cress seed oil was used for fortification. Among the two microencapsulated oil showed better sensory, physical and oxidative quality during storage. Rajiv *et al.* (2012) made cookies by replacing some percent of wheat flour with the dried and ground flaxseed powder. The fatty acid profile especially linolenic acid analysed by gas chromatography was higher for cookies made with flaxseed powder (4.75-5.31%) than control values. Ramcharitar *et al.* (2005) prepared muffins containing 11.6% milled flaxseed but partially acceptable. Moraes *et al.* (2010) prepared cakes by partially replacing wheat flour with the flaxseed flour (upto 45%) and found that up to 30% flaxseed four replacement gives acceptable sensory quality with functional benefits of linolenic acids and fibre.

2.7.1.2. Infant formula

Before conception lipids from mother are transferred to the placenta either in the form of triacylglycerol or free PUFAs with the help of membrane fatty acid binding protein (Herrera *et al.,* 2006). After conception infants are capable for synthesis and absorption of PUFAs from mothers milk (Herrera, 2002). ω-3PUFAs plays an important role in visual and brain development in infants (Innis *et al.,* 1999; Fewtrell, 2006; Agostoni, 2008). Human milk has similar fat percent that of cow milk but contains 7 times more DHA and deficient in EPA (Gunstone, 1996). So in case of formula fed infants devoid of others milk; ω-3 fatty acids need to be supplemented in the infant formula. PUFA content in the commercial formulae varies from 13 to 26% with fortification using ALA (Jensen *et al.,* 1992), EPA as well as DHA (Douaud, 2006; Starling, 2008).

2.7.1.3 Eggs

Eggs contains high amount of protein (12.6%) and fat (10.6%). Chicken eggs are deficient in PUFA i.e. it contains about 10–15 mg/g fat and out of that > 95% of PUFA is linoleic acid (Cantor *et al.,* 2000). ALA content in the eggs is negligible and thus efforts have been made to fortify eggs with ω-3 PUFAs. Farrell (1998) developed ω-3 PUFAs enriched eggs by feeding hens with fish oil or a combination of fish oil with vegetable oil. The ω-3 fatty acids level increased in the blood samples of people fed with ω-3 PUFAs enriched eggs. The malonaldehyde of eggs enriched in ω-3 PUFA increased significantly upto 28 days of storage but antioxidant makes it relatively stable during storage. Mridula *et al.* (2012) also fed flaxseed rich diet to poultry and observed increased level of ω-3 fatty acids in the eggs.

2.7.1.4 Meat

Dietary lipids can be obtained from meat products as it contributes majorly in the adult diet of non-vegetarian people. However, ω-3 fatty acids cannot be synthesized in terrestrial animal and thus they are deficient in ω-3 PUFAs but contains higher amount linoleic and arachidonic acid in muscle and adipose tissue. Therefore, level of ω-3 PUFAs in the meat and its products has been increased with feeding ω-3 PUFAs enriched diets to the animals.

2.7.1.5 Cereal bars and chocolates

Breakfast products including cereals and bars have also been fortified with ω-3 PUFAs. Mridula *et al.* (2013) optimized a process for preparation of energy bar with varying level of flaxseed (0-20%) and sweeteners (45, 50 and 55%). The product made from 15% flaxseed, 45% sweeteners and other components has acceptable sensory quality. Other products such as sauces and chocolates are commercially available in the market.

2.7.2 Dairy products

2.7.2.1 Milk

Milk is an ideal carrier for fortification of essential nutrients and almost consumed by all age groups peoples. Thus, various efforts have been made for fortification of milk with ω-3 fatty acids. Milk fortification is done either by direct addition of oil or feeding of animals. Divya *et al.* (2013) observed occurrence of rancid flavour after 3 days of refrigerated storage when fortified milk with DHA and flaxseed oil. Algal and fish oil are also good source of omega-3 fatty acids and thus used for fortification. Baer *et al.* (2001) made an attempt to fortify milk with fish oil and found perceptible difference in the flavour of control and fortified sample during storage. Considering oxidation stability of algal oil and impact on sensory quality, Gallaher *et al.* (2005) studied effect of fortification with algal oil emulsion on storage stability of milk and no significant difference was observed between the fortified and control sample. Goyal *et al.* (2015) fortified market milk with ω-3 fatty acids by using flaxseed oil micro encapsulated (made with milk protein) powder at 1% level. The fortified milk showed similar sensory qualities like control sample up to 5 days in storage. Similar kind of study has been conducted by Tambade (2018) for fortification of milk with microencapsulated flaxseed oil powder prepared by soy protein isolate and modified starch. Pasteurized and sterilized flavoured milk were stable for 6 and 28 days respectively. Attempts have also been made to increase the natural level of ω-3 fatty acids in milk by feeding diet rich in these fatty acids to the cattle. Nelson and Martini (2009) supplied diet containing fish oil and its calcium salts for 9 weeks and observed the effect on fatty acid content and flavour of the milk. Results showed that elevation in omega-3 fatty acids without affecting the flavour of milk during the experimental period.

2.7.2.2 Curd

Goyal *et al.* (2016) optimized the process for fortification of dahi with ω-3 fatty acids using flaxseed oil in the form of microcapsule. Addition of 2% microcapsule showed comparable sensory attributes to the control curd. Similar study was reported by Veena *et al.* (2017) for fortification of milk and curd using flaxseed oil and observed slightly lesser flavour in case of fortified products when compared to control.

2.7.2.3 Cheese

Bermudez-Aguirre and Barbosa-Canovas (2011) prepared cheddar (C), Queso fresco (QF) & mozzarella (M) cheeses fortified with ω-3 fatty acids by fish oil & ME fish oil and studied effect of pasteurization, curdling and salting on ω-3 addition and concluded that the highest ω-3 fatty acid retention (8.69 mg/g MFO; 5.08 mg/g FO) was observed in cheddar cheese when added during salting.

2.7.2.4 Yoghurt

Chee *et al.* (2005) manufactured strawberry flavoured yoghurt containing algal oil emulsion and observed the perception of fishy flavour in fortified sample after 15 days of storage. In another study, strawberry yoghurt was prepared by Estrada *et al.* (2011) using salmon oil microcapsules (2%). Increase in peroxide and TBA value was noticed without change in the colour characteristics during storage.

2.7.2.5 Ice cream

Lim *et al.* (2010) manufactured ice-cream containing flaxseed oil and reported the decrease in the flavour score with increasing level of fortification. Furthermore, Ullah *et al.* (2017) supplemented chia oil (olein fraction) into the ice-cream and found improvement in the antioxidant properties with increasing level of ω-3 fatty acids. Similarly, Gowda *et al.* (2018) prepared omega-3 fatty acids especially alpha-linolenic acid fortified ice cream using microencapsulated flaxseed oil powder. Fortification 4% MFOP provides approximately 45% of RDA level of alpha-linolenic acid in 100 g of fortified ice cream.

2.7.2.6. Butter

Kolanowski *et al. (*2007) evaluated the sensory characteristics of butter fortified with 0.1 to 8% fish oil and found that butter fortified with 3% fish oil shows better sensory characteristics. Ivanov *et al. (*2011) added flaxseed additive (@ 0.8, 1.2 and 1.6%) in butter, and reported reduction of structure destruction with improvement in convalescing ability of butter structure. Devaraja *et al.* (2013) prepared CLA and phytosterol enriched butter using sunflower oil and observed that functional butter with 4% CLA and 12% phytosterol was organoleptically acceptable. Bobe *et al. (*2007) fed Holstein cows with flaxseed oil (0.9%) and roasted soybeans (5%) for 3 weeks and found that unsaturated fats were higher in the butter obtained from milk of the cows with soft texture. Silva-Kazama *et al.* (2010) studied the changes in fatty acid profile of butter obtained from cows fed with ground or intact flaxseed (0.02% on a dry matter basis) for 21 days storage period and observed that percentages of saturated as well as medium-chain fatty acids increased with progression of storage period, in the butter obtained from fed cows. Landis (2006) developed peanut butter with addition of flaxseed oil (0, 3.8, 7.6 and 11g), among all the levels of addition peanut butter with flaxseed oil 3.8 level had highest palatability. In another study, Kolanowski *et al.* (2001) prepared spreadable fats enriched with omega-3 fatty acids using fish oil at the rate of 3, 4, and 5% and observed that there was no significant influence on sensory acceptability of low calorie spreadable fats enriched upto 1% EPA and DHA.

2.8 Quality characteristics of ω-3 fatty acids fortified butter

Butter is the fat rich dairy product and rich source of fat soluble vitamins. Butter contains higher amount of saturated fats and cholesterol which are having many adverse health effects (Cox *et al.*,1995, Drouin *et al*., 2018). Butter lacks in the essential fatty acids such as alpha-linolenic acid. The total omega-3 fatty acids and ALA content in cow milk is 0.56 and 0.5 g per 100 g of total fatty acids respectively. **(**Woods and Fearon, 2009; Markiewicz-Kęszycka *et al*., 2013), which cannot meet the Recommended Daily Allowance (RDA) of ALA (as per ICMR 1.6 g/ day). Thus, in the present study, an attempt was made to fortify butter with omega-3 fatty acid using flaxseed oil and the recovery of the flaxseed oil in the butter fat was confirmed by various techniques. The review of the techniques is discussed in the subsequent sections.

2.8.1 Gas chromatography-mass spectrometry (GC-MS)

In the present study, flaxseed oil was incorporated into the butterfat which can be detected by determining the fatty acid composition of fortified butter using GC-MS.

Butterfat is expensive than several vegetable oils thus adulteration of butter with cheaper oils has been a serious problem. Butter adulteration has been detected by GC-MS because of its distinct fatty acid profile. Changes in the fatty acid profile indicates butter adulteration with foreign fat. Popa *et al.* (2012) analysed the fatty acid profile of the flaxseed oil by GC-MS and reported presence of 17.25% linoleic acid and 53.21% linolenic acid in the oil. Goyal *et al.* (2016) prepared omega-3 fatty acid fortified dahi using microencapsulated flaxseed oil powder (MFOP) and the ALA content in the product was determined by GC-MS. They found that approximately 21% reduction in the ALA content was occurred after 15 days of storage. Further, Mondello *et al.* (2004) analysed various samples such as butter, tallow, lard, corn, peanut, soya, olive, sunflower and menhaden oils with two different methods i.e. gas chromatography-mass spectrometry and fast gas chromatography in order to cut the analysis time. They found that fast GC showed decrease in the efficiency which cannot be avoided but quite good analytical results and also analysis time was reduced over 95%. Flaxseed oil is the costlier oil than butterfat; owing to health benefits associated with significant amount of ALA, which is essential fatty acids. Thus, in the present study, the determination of ALA content in fortified samples would be carried out using GC-MS as distinct retention time, structural and mass difference will form the basis for ALA identification.

2.8.2 Thermal properties of butter using Differential Scanning Calorimetry (DSC)

Fortification of the butter using flaxseed oil changes the proportion of low melting, medium melting and high melting fraction of the butterfat and thus affect thermal properties of fortified butter which can be determined using DSC. Until recently, DSC analysis of butter was majorly focused on detection of different adulterants in a quick time. But the same principle could be useful in the studies where butter is intended to be fortified with certain essential oils such as omega-3 or ALA rich oils. However, in the absence of such studies, in the present review the available studies pertaining to adulteration. Tomaszewska-Gras (2016) described a method for rapid determining palm oil adulteration in butter using DSC. Addition of palm oil to the butterfat altered the melting curve of butterfat. Increase in palm oil content from 0-35% resulted an increase in temperature corresponding to first peak i.e. low melting fraction peak by 2°C, area of low melting fraction from 34 to 55% and height of the peak from 145 to 234 mW g^{-1}. In case of medium melting fraction, the peak temperature decreased by 2°C, area decreased from 31 to 10% and the peak height decreased from 345 to 200 mW g^{-1}. Thus, based on the shift in peaks, that DSC was used for quantitative analysis of butter adulteration with palm oil. Similarly, butter adulteration with water was also determined using DSC. Tomaszewska-Gras (2012) detected the water content variation of 0.2 to 2.6%, when measured for melting curve and for crystallization curve it varied from 1 to 5.6%. Thus, it can be deduced that DSC could be used for determination of even minor constituents' addition with considerable accuracy. Aktaş and Kaya (2001) investigated adulteration of butterfat with margarine and beef body fat using DSC. Rachana *et al.* (2013) analysed the melting and crystallization behavior of the commercial ghee and compared with the laboratory ghee and their blends. The commercial ghee samples with poor granularity resembled with blend of laboratory ghee and low melting fraction (LMF), while the commercial ghee with bigger grains resembled with blend of laboratory ghee and high melting fraction (HMF).

2.8.3 Determination of solid fat content using Pulsed Nuclear Magnetic Resonance (pNMR)

Pulsed nuclear magnetic resonance is a technique used for measuring solid fat content (SFC) at different temperature for fat and oil samples. Fortification of butter using flaxseed oil increases the liquid fat content in the fortified butter which can be detected by pNMR by determining SFC. Soft fat contains lower solid fat content even at lower temperature and helpful in determining the softness of butter, margarine etc. Therefore, it can be used for determination of butter adulteration with vegetable oil or any other fat. Avramis *et al.* (2003) analysed processing and physical parameters of various products such as milk, cheddar cheese and butter obtained from cows fed with fish meal. Supplementation of fish meal changed the fatty acid composition of butter and analysed by pNMR. Butter obtained from cows milk supplemented with fish meal showed lower SFC than control sample from 5 to 20°C. Rousseau *et al.* (2003) investigated relationship between SFC and stability of various products such as butter, low fat margarine and margarine. They observed critical SFC for butter, reduced fat margarine and margarine as 9, 6.5 and 5% respectively. Thus, it was concluded that there is relationship between SFC and stability of products. Bornaz *et al.* (1994) elevated solid fat content in the butter by adding trimyristin or tripalmitin and enzymatic interesterification or combination of thereof. Kalo and co-workers (Kalo *et al.,* 1986a, b, 1990) studied effect of interesterification methods (enzymatic and chemical) on crystallization and melting characteristics of butter. The crystallization and melting behaviour of interesterified butter was examined by pNMR and /or DSC. The melting properties and chemical composition of butter made by both the process was similar.

2.8.4 Oxidative stability of butter

Butter is high fat dairy product and contains 80% butterfat and thus, oxidation is most common problem during storage. Oxidation of fat causes rancid and off flavour in the butter and makes it unacceptable for human consumption. Normally two types of rancidity occurred in butter samples i.e. hydrolytic and oxidative rancidity.

2.8.4.1 Rancimat for studying Induction period

Fortification of butter using flaxseed oil increases the unsaturated fatty acid content and thereby reduces the induction period. Lower induction period than the control butter confirms the recovery of the flaxseed oil into the butterfat. Butter is one of the most popular high fat dairy product. It is generally used for cooking and sweet preparation. Replacement of butterfat with the vegetable oil adversely affect the shelf life of butter (Garcia *et al.*, 2003) and makes it more prone to oxidation. Higher temperature of storage promotes rancid flavour development (Potter and Hotchkiss, 1998) and affect the palatability of product. Nadeem *et al.* (2013) studied the antioxidant characteristics of leaf extract obtained from *Moringa oleifera* in butter samples. The leaf extract was added into the sample in three different concentration (400 ppm, 600 ppm and 800 ppm) and stored at refrigeration temperature for 90 days. Butter added with leaf extract showed lesser FFA, peroxide value, p-anisidine value and the induction period was increased (8.91 h) than control sample (6.35 h). Similarly, Gramza-Michalowska *et al.* (2007) studied the effect of season and leaf extract on oxidative stability of butter. The antioxidant potential of rosemary and green tea extract was analysed in comparison with antioxidants (tocopherol and BHT) by adding into the lipid samples at rate of 0.02% and stability was measured by rancimat and oxidograph test. Among them the green tea extract showed highest antioxidant potential. In summer season the induction period was 71.22 h and 81.23 h for rancimat and oxidograph test respectively, while in winter season it is 66.5 h and 64.0 h. Shiota *et al.* (1999) studied oxidative stability of fish oil when blended with butter. The oxidation stability of fish oil and blend of fish oil with butter was analysed by peroxide value and rancimat. The induction period was less than 3 h for fish oil, 4.05 h for unsalted butter blended with 10% oil and 4.19 h for salted butter blended with 10% fish oil. Thus, it has been concluded that combination of fish oil and butter had higher the oxidative stability of than fish oil alone, due to increase in total saturated fat content; which are relatively stable towards oxidation.

2.8.4.2 Changes in butter quality attributes upon storage

Oxidative rancidity could be determined by thiobarbituric acid (TBA) and peroxide value while, the hydrolytic rancidity is determined by Free fatty acids (FFA). Ozcan and

Ayar (2003) studied antioxidant properties of propolis extract when butter was stored at 5°C and 25°C. The propolis extract was added in two different concentrations (0.02 and 0.05%) and compared with butylated hydroxy toluene (BHT) added at rate of 0.02%. At 25°C, propolis extract had significant impact on reducing peroxide value, TBA value and FFA content compared to control sample. At 5°C propolis extract (0.05%) had similar effect as BHA (0.02%). Similarly, Najgebauer-Lejko *et al.* (2009) prepared butter from sour cream and studied effect of rosemary and dried sage addition on butter quality and stability during storage. Butter added with rosemary extract showed better stability than dried sage. But during storage control sample was more stable in lipolysis retardation than prepared with rosemary and dried sage. Kolanowski *et al.* (2004) prepared low fat spread with the addition of fish oil (30 g/kg product) and studied the effect on oxidative stability for 3 months of storage. There was no substantial change in oxidation indicators during storage. Devdhara *et al.* (1991) studied the changes in the FFA content of butter during storage at 4°C and 10°C. The FFA content of the stored samples was increased slightly (0.11 to 0.53) but steadily over a period of storage on both the temperatures. Increase in FFA content was higher butter without preservatives (0.85 to 1%) than with preservatives (0.11 to 0.53%). Similarly, Devdhara *et al.* (1991) studied the changes in the peroxide value of butter during storage at 4 and 10°C. The peroxide value of the butter stored at both temperatures increased steadily throughout the storage period. The butter added with preservative showed increase in PV from 0.2 to 1.1 meq O_2/ kg fat when stored at 4°C and from 0.2 to 1.2 meq O_2/ kg fat when stored at 10°C. The peroxide value of the butter without preservative was increased from 0.2 to 1.5 meq O_2/ kg fat when stored at 4°C and from 0.2 to 1.8 meq O_2/ kg fat when stored at 10°C. Thus, storage temperature affects significant for elevation in the peroxide value.

2.8.5 Water activity of butter

Water activity is the ratio of partial vapour pressure of water in the product to the partial vapour pressure of pure water. Many chemical reactions, enzymatic activities and bacterial growth occurs at a specific water activity. Thus, water activity is very relevant property than water content of the samples deforming the spoilage of food products. The water activity of unsalted butter at 24°C is 0.961 (Schmidt and Fontana Jr, 2008). The rheological and physicochemical properties of supercritical fractionated butter samples. The samples with high meting fat showed lower activity than the control samples. High melting butter can be made by reducing saturated fatty acids and cholesterol content which attracts more attention of the consumers Shukla *et al.* (1994).

2.8.6 Colour properties of butter

From consumer point of view, colour and appearance is one of the most important attributes. Addition of foreign fat/oil or any other material to the butter affect the colour characteristics. Thus, colour analysis plays an important role in customer satisfaction and determination of foreign material in the butter. Shukla *et al.* (1994) analysed the colour of both control and high melting triglyceride butter using a Macbeth colour eye spectrophotometer with hunter scale. Butter with high melting triglycerides showed low 'L value' and higher 'a' as well as 'b value'. Similarly, Gonzalez *et al.* (2003) observed slight change in the colour characteristics of control butter and butter containing higher unsaturated fatty acids obtained by feeding animals with unsaturated fatty acid reached meals.

2.8.7 Textural properties of butter

Spreadability and firmness are two important parameters in textural quality of butter perceived by the consumers (Prentice, 1972). Both the parameters are affected by the composition of butter. Firmness of the butter increases proportionately with solid fat content and spreadability has inverse relationship with the SFC (Fearon, 2001). Spreadability can be increased by altering the composition of butter by various ways such as feeding of animals with oilseeds (DePeters *et al.*, 1985; Mohamed *et al.,* 1988)., blending with vegetable oil (Amer and Myhr, 1973), fractionation (McGillivary, 1972; Deffense, 1987; Makhlouf *et al.*, 1987; Kaylegian and Lindsay, 1992) etc. Addition of vegetable fats decreases SFC of the samples and thus improves the spreadability. Glibowski *et al.* (2008) analysed the textural and rheological characteristics of the table fats such as the margarine, blends and butter. Spreadability was determined by using texture analyser and margarine showed higher spreadability than the butter samples. Kulkarni (1983) prepared butter spread by blending butter with vegetable oil (20, 30 and 40%) and SMP, 30% addition of safflower or sunflower oil showed good spreadability based on sensory acceptability. Similarly, Amer and Myhr (1973) analysed effect of 30% replacement of milk fat with sunflower oil and found improvement in the butter spreadability. Blending of vegetable oils with butter resulted in decrease in melting point and elevation in unsaturation level (Amer and Myhr, 1973; Ahmed *et al.,* 1979; Wilbey, 1994).

Further, the textural properties undergo changes during storage. Schaap *et al.* (1981) observed the satisfactory temperature for winter butter was 16°C and for summer butter was 14°C. The firmness of butter was increased upto 21 days of storage when stored at 14°C for 40 days. Nabar *et al.* (1969) prepared butter from the buffalo milk cream and reported the hardness of butter increases with storage. Kulkarni and Rama Murthy (1985) stored butter at different temperature (0, 5, 15 and -20°C) and studied the effect on yield stress, penetration value, viscosity and extrusion thrust. They observed that, maximum changes take place during first week of storage and resulted in increased firmness of butter. Desai *et al.* (1994) studied the effect on consistency of butter when stored at 5 and 15°C. Temperature and storage period affect significantly ($P<0.01$) on penetration value of butter. During first week, the penetration values of butter decreased significantly and thereafter slightly. Thus, we can conclude that firmness of the butter samples increases during storage.

Based on the above review, it is concluded that fortification of the butter with omega-3 fatty acids may not only provide health benefits but also likely to improve the textural properties of the butter (spreadability). The recovery of the flaxseed oil could be confirmed by the several techniques discussed in the review. Further, there are only limited reports available on fortification of butter using flaxseed oil. Thus, the present study focuses on process optimization and quality characteristics of the fortified butter.

3.0 SCOPE AND PLAN OF WORK

3.1 Scope

Functional foods are the foods which provide physiological benefits along with basic nutrition. Among the methods of preparation of functional foods, fortification with bioactive components is the most common one. Mostly essential nutrients i.e. not present in a particular food, are generally used to fortify food products to fulfill the daily requirement and exhibit physiological benefit. Omega-3 fatty acid is one such bioactive which is getting more attention because of its health benefits. Lack of omega-3 fatty acid in Indian diet and its availability in some vegetarian sources such as flaxseed oil creates opportunities for fortification. The dairy products also contain limited amount of omega-3 fatty acids. There have not been enough evidences of omega-3 fatty acid fortification in butter. Keeping all the points into consideration, present study has been planned to fortify butter with omega-3 fatty acid with flaxseed oil and emulsion. The flaxseed oil-WPC emulsion was prepared and its effect was compared with direct flaxseed oil addition on the physico-chemical, textural and sensory attributes of the fortified butter. The samples were also evaluated for storage stability.

3.2 Plan of work

3.2.1 Process optimization for preparation of flaxseed oil emulsion

3.2.1.1 Selection of stabilizer/emulsifier

i) Preliminary trials for selection of stabilizer/emulsifier: Gum arabic, Guar gum, Sodium alginate, Modified starches, Sodium caseinate, WPC, WPI, combination of WPC and Sodium caseinate.

ii) Oil load: 45, 60 and 75%

iii) Emulsion prepared using hand blender (20000 rpm) for 5 min

3.2.1.2 Optimization of emulsion

i) Level of whey protein concentrate: 5, 7.5 and 10%

ii) Oil load: 45, 60 and 75%

iii) Parameters evaluated: Physical stability

3.2.2 Process optimization for fortification of butter with omega-3 fatty acid

Process for fortification of butter with omega-3 fatty acid will be optimized based on the sensory acceptability of the samples and fat loss in the buttermilk. Three different independent variables will be taken for optimization i.e. fortificant type (flaxseed oil and flaxseed oil emulsion), stage of addition of fortificant (Before ageing and after ageing) and level of addition i.e. flaxseed oil at rate of 2.9, 4.1 and 5.1% while, flaxseed oil emulsion at the rate of 4.6, 6.8 and 8.6% in order to meet 25, 35 and 45% of RDA level of α-linolenic acid respectively given by ICMR (2010).

Table 3.1 Independent and dependent variables studied for process optimization

Independent variables (Level/stage of addition)		Dependent variables
Flaxseed oil	2.9, 3.1 and 4.1% on cream basis	• Textural analysis (Spreadability and creep test) • Sensory evaluation • Colour • Churning efficiency
Flaxseed oil emulsion	4.8, 6.8 and 8.6% on cream basis	
Stage of addition	Before ageing and after ageing	

Table 3.2 Methodology used for proximate composition of the optimized product

S. No.	Constituent	Analytical method
1	Fat	Rose-gottlieb method given in 6.2 of IS: 3507-1966.
2	Moisture	Gravimetric method given in 4.2 of IS: 3507-1966.
3	Protein	Kjeldahl method (AOAC, 2005)
4	Carbohydrate	Difference method
5	Ash	Gravimetric method (AOAC, 2005).

Table 3.3 Methodology used for quality evaluation of the omega-3 fatty acid fortified butter

S. No.	Characteristics	Analytical method/Instrument
1	Omega-3 fatty acid content	Gas chromatography-mass spectrometry (GC-MS)
2	Thermal characterization by Differential Scanning Calorimetry (DSC)	Method described by Tomaszewska-Gras, (2016)
3	Oxidative stability by rancimat	Method given in Ω Metrohm application bulletin 204/2e
4	Water activity	Water activity meter (AQUA Lab, Decagon Devices, WA, USA)
5	pNMR	AOCS official method Cd 16-81 (1993)
6	Colour	Adobe photoshop
7	Texture	Spreadability and Creep test using TA-XT Texture analyser
8	Sensory evaluation of fortified butter	Nine-point hedonic scale

3.2.2 Storage study of the omega-3 fatty acid fortified butter:

i) Storage temperature: 5 and -18 °C
ii) Storage container: PP cups
iii) Duration: 90 days
iv) Interval of analysis: 15 days

Table 3.4 Parameters evaluated during storage study of the optimized butter

S. No.	Characteristics	Analytical method/ Instrument
1	Sensory evaluation of fortified butter	Nine-point hedonic scale
2	Texture	Spreadability and Creep test using TA-XT Texture analyser
3	pH	Section 9.2 of IS: 3507-1966.
4	Titrable acidity	Section 8.0 of IS: 3507-1966.
5	Coliform count	Section 13 of IS: 3507-1966.
6	Yeast and mould count	Section 14 of IS: 3507-1966.
7	Free fatty acid content	Method described in Kuruppu *et al.* (1983) and Amr (1991).
8	TBA value	Method described by Kuruppu *et al.* (1983)
9	Peroxide value	Method Cd 8-53 of the AOCS (1993).

4.0 MATERIALS AND METHODS

This chapter deals with the details of the materials and instruments used in the study. The experimental methodologies, procedure followed are also discussed in detail.

4.1 Materials

Cow milk (3.5 % fat and 8.5 % SNF), skim milk and cream were collected from Experimental dairy of NDRI-SRS, Bangalore. Butter culture (NCDC-193, *Lactococcus lactis spp. Lactis*) was obtained from National Collection of Dairy Cultures (NCDC), NDRI, Karnal. Glass bottles and poly propylene cups (6 cm diameter and 1mm thickness) were purchased from local market of Bangalore, for storage of emulsion and storage study of butter respectively.

Cold pressed flaxseed oil was obtained free of cost from AAK Kamani Pvt. Ltd. Andheri, Mumbai (Maharashtra) The physicochemical parameters for the oil are presented in Table 4.1

Table 4.1 Technical specifications of flaxseed oil

Sr. No.	**Parameters**	**Value**
01	Colour (1"Cell) Lovibond	3.0Y+0.4R=5.0
02	Moisture content In %	0.015%
03	Refractive Index at 40 C	1.4725
04	Free fatty acids (% oleic acid)	0.06%
05	Acid value	0.12
06	Iodine value	173.25
07	Peroxide value	0.44
08	Saponification value	191.58
09	Unsaponifiable value in %	0.29%

WPC was procured from Shridurga Sales Corporation (Imported from Friesland Campina DMV B.V. Netherland). The physico-chemical and microbial characteristics are mentioned in Table 4.2.

Table 4.2 Technical specifications of whey protein concentrate

Physico-chemical characteristics		
S. No.	**Parameters**	**Results**
01	Protein	78.8%
02	Protein / dry matter ratio	82.2%
03	Moisture	4.10%
04	Ash	4.90%
05	Scorched particles(ADPI)	A
06	Fat	4.7%
07	Bulk Density	289 g/l
08	Colour	Pass
09	Smell	Good
10	Taste	Good
11	Dispersibility (sec.)	9
Microbiological characteristics		
01	Mesophilic aerobic plate count	140 cfu / g
02	Yeast and Mould	<5 cfu /g
03	Coagulase positive Staphylococci (1 g)	Negative
04	Listeria spp. (25 g)	Negative
05	Salmonella (750 g)	Negative

4.2. Equipment

- ✓ ***Autoclave:*** Sterilization of skim milk and microbiological media was carried in Autoclave (Excel Scientific, Bangalore, India)
- ✓ ***Bacteriological incubator:*** Used for ripening of cream (Metrex Scientific Instruments Ltd., New Delhi)
- ✓ ***Cream separator:*** Used to obtain cream and skim milk from raw milk. (Kamdhenu, New Delhi, India)
- ✓ ***Deep freezer:*** Used to store butter samples during storage at -18 °C (Blue star, India)
- ✓ ***Differential Scanning colorimetry:*** Used to study the phase transition such as melting, glass transition or exothermic decomposition of the samples available at IISC, Bangalore (Mettler-Toledo, Switzerland)

- ✓ ***Flatbed scanner:*** Used to acquire scanned images of butter for color analysis (Canon CanoScan 9000F MarkII, Japan)
- ✓ ***Gas chromatography-mass spectrometry (GC-MS):*** Used for quantification of ALA content in the control and fortified samples (Agilent Tchnologies, United states)
- ✓ ***Gerber centrifuge:*** Used for estimation of fat of skim milk, cream and buttermilk (Sanraj Industries, New Delhi)
- ✓ ***Hand blender:*** Used for preparing emulsion of flaxseed oil and whey protein concentrate (Maharaja Whiteline Turbomix 350-Watt, Speed: 20000 rpm, India)
- ✓ ***Hot air oven:*** Used for estimation of moisture and curd content in butter (Falcon Scientific Instruments, India)
- ✓ ***Hot plate:*** Used for charring of samples for estimation of ash content (Rivotek Instruments Ltd, India)
- ✓ ***Induction heater:*** Ceramic Induction heater was used for pasteurization of cream (Phillips, Netherland)
- ✓ ***Insta Madhani :*** Used for churning cream, speed 1500 rpm (Insta madhani, India)
- ✓ ***Kjel plus digestion and distillation assembly***: Kjeldahl digestion unit and Kjel plus distillation unit is used to estimate nitrogen content in butter samples (Gerhardt Instruments, Chennai)
- ✓ ***Laboratory centrifuge:*** Used for centrifuging the samples (R8C, Remi motors, Ltd, Mumbai)
- ✓ ***Laminar air flow:*** Used for microbiological analysis (Excel Scientific, Bangalore)
- ✓ ***Magnetic stirrer:*** Used for determination of peroxide value of butter (Remi, Mumbai)
- ✓ ***Muffle furnace:*** Used for estimation of ash content of butter samples (Murphy Scientific, Bangalore)
- ✓ ***pH meter:*** Used for determination of pH of cream (Eutech Instruments, Singapore)
- ✓ ***pNMR*: S**olid fat content in the optimized and control sample was determined (Bruker Minispec pNMR Analyser, Model no. 120, Rheinstetten, Germany)

- ✓ ***Rancimat:*** Oxidative stability of the butter samples was analyzed (Metrohm rancimat 679 , Ω Metrohm, Switzerland).
- ✓ ***Refrigerator:*** Double door refrigerator was used to store butter samples at 4-7°C during storage (LG, India)
- ✓ ***Spectrophotometer:*** Used for colorimetric estimation of TBA value of the butter samples during storage study (Antheli Junior, France)
- ✓ ***TA-XT Plus Texture Analyzer***: Used for measuring textural properties of butter (Stable Micro System, Godalming, Surrey, England)
- ✓ ***Vortex mixer:*** Used for determination of TBA value of butter (Spinix, India)
- ✓ ***Water activity meter:*** Water activity of the control and optimized samples of butter was measured by Water activity meter (AQUA Lab, Decagon Devices, WA, USA)
- ✓ ***Water bath:*** Used for fat estimation of butter (Rivotek Instruments, Singapore)
- ✓ ***Weighing balance:*** Used for weighing (Shimadzu corporation, Japan)

4.3 Apparatus and glassware

All volumetric flasks, burettes, conical flasks and pipettes were cleaned and dried before use. Burette (50 mL), funnels (small and large), measuring cylinder (10, 50, 100, 250, 500 and 1000 ml), separating funnels (250 and 500 ml), beakers (100, 150 and 250 ml), conical flasks (100, 150 and 250 ml), volumetric flasks (100, 250 and 500 ml) and Glass pipettes (1, 2, 5, 10, 10.75 and 25 ml), glass test tubes, flat bottom flasks (150 and 250 ml) and round bottom flask (500 ml) were procured from Borosil India Ltd., Mumbai, India; parafilm from Sigma Aldrich, St. Louis, MO, USA; and filter papers (HIMEDIA filter paper no. 1, 4 and 41) from Hi Media Laboratories Pvt. Ltd., Mumbai.

4.4 Reagents

4.4.1 Preparation of 0.01 N solution of sodium thiosulphate ($Na_2S_2O_3$)

For preparation of 0.01 N sodium thiosulphate solution, 24.8181 g of $Na_2S_2O_3.5H_2O$ was added in 500 mL of distilled water (or freshly boiled and cooled deionised water) and mixed. Then, 2 to 3 drops of trichloromethane ($CHCl_3$) (or also 0.4 g of NaOH) was added and the volume was made up to 1000 mL in a volumetric flask.

4.4.2 Saturated potassium iodide (KI) solution

Saturated solution of potassium iodide was prepared by adding sufficient quantity of potassium iodide in previously boiled and cooled distilled water (~10 g KI in 6 mL water).

4.4.3 Starch indicator (1%)

One gram of soluble starch was taken and made into a paste by adding it into 30 mL water. Further the paste was transferred to 100 ml boiled water and heated till clear solution was obtained. The solution was stored in air tight and stoppered reagent bottle.

4.4.4 Phenolphthalein indicator solution

Firstly, dissolved one g of phenolphthalein in 50 mL of 95% ethanol in a 100 mL volumetric by shaking vigorously for few minutes. A clear solution was obtained by adding 20 mL more ethanol and finally volume was made to 100 mL with 95% ethanol.

4.4.5 Standard aqueous sodium hydroxide solution (0.1N, 0.01N and 0.02N)

0.1 N aqueous NaOH solution was prepared by dissolving 4 gm of NaOH pellets in 1000 ml distilled water and stored in an amber colored glass bottle. 0.01 N aqueous NaOH solution was prepared by dissolving 0.4 gm of NaOH pellets in 1000 mL distilled water and stored in an amber colored glass bottle. 0.02 N aqueous NaOH solution was prepared by dissolving 0.8 gm of NaOH pellets in 1000 ml distilled water and stored in an amber colored glass bottle.

4.4.6 Neutralized ethanol

Neutralized ethanol was prepared immediately before use by adding 0.1 N NaOH using phenolphthalein indicator till light pink colour appears.

4.4.7 Trichloroacetic acid solution (7.5 and 1%)

7.5% Trichloroacetic acid (TCA) solution was prepared by dissolving 7.5 g of TCA in sufficient quantity of distilled water and volume was made up to 100 mL with distilled water.

4.4.8 Thiobarbituric acid solution (1%)

To prepare 1% thiobarbituric acid (TBA) solution, dissolved 1 g TBA in 100 mL 90% glacial acetic acid and 90% glacial acetic acid prepared by adding 10 ml distilled water in 90 mL glacial acetic acid.

4.5 Microbiological media

Potato dextrose agar and violet red bile agar required for microbiological analysis were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India).

4.6 Methods

4.6.1 Optimization of flaxseed oil –WPC emulsion

For preparation of emulsion, three different levels, 5, 7.5 and 10% of WPC were taken. The oil load in the emulsion varied with 45, 60 and 75% and the prepared emulsion were evaluated for physical stability as given in the Table 4.3

Table 4.3 Oil load and WPC content for preparation of emulsion samples

S. No.	WPC (%)	Oil load (%)
1	5	45
		60
		75
2	7.5	45
		60
		75
3	10	45
		60
		75

4.6.1.1 Preparation of flaxseed oil –WPC emulsion

Flaxseed oil emulsion was prepared by dissolving WPC in distilled water and blending using hand blender (Maharaja Whiteline, India) for 1 min, and then adding flaxseed oil followed by mixing using hand blender for 5 min. The emulsion was prepared at temperature about 28°C. The flow chart for preparation of emulsion is given as Figure 4.1.

Mixing of WPC and distilled water

↓

Premixing by Hand blender for 1 min.

↓

Addition of oil

↓

Mixing by hand blender for 5 min.

↓

Storage at 5-7°C

Figure 4.1 Process flow chart for preparation of flaxseed oil –WPC emulsion

4.6.1.2. Physical stability

Physical stability of the emulsion was visually observed for oil separation during storage of the emulsions in glass bottles of capacity 100 mL (Internal diameter = 4.5 cm, height = 7.5 cm, volume of emulsion=60 mL) at 5-7 °C.

4.6.2 Method for preparation of omega-3 fatty acid fortified butter

The process for fortification of butter with omega-3 fatty acid was optimized by studying the effect of flaxseed oil and flaxseed oil emulsion addition during two different stages into the cream i.e. before ageing and before churning of cream. Firstly, cream was standardized to 40% fat by using skim milk. Further, standardized cream was heated to 85-90°C for 10 min and cooled to inoculation temperature of the butter culture i.e. 30°C. Cream was ripened by adding 2% culture NCDC 193 and incubated at 30°C for 8 h so as to obtained good diacetyl flavor in the butter. The oil was added with different rates such as 2.9, 4.1 and 5.1% on the basis of cream in first stage before inoculation with culture while, in second stage it was added after ageing. Similarly, emulsion was added at the rate of 4.8, 6.8 and 8.6%. Cream was aged (4-7°C) for 20 h after ripening. Then cream was churned in butter churner (Insta Madhani, India) till pea size butter granules were formed. Washing of the butter granule was done with potable chilled water (7-8°C), followed by working of the butter. Finally, the butter was packed in the polypropylene (PP) cups and stored two temperatures 5°C as well as at -18°C. Flow chart for the preparation of omega-3 fatty acid fortified butter is given in Figure 4.2

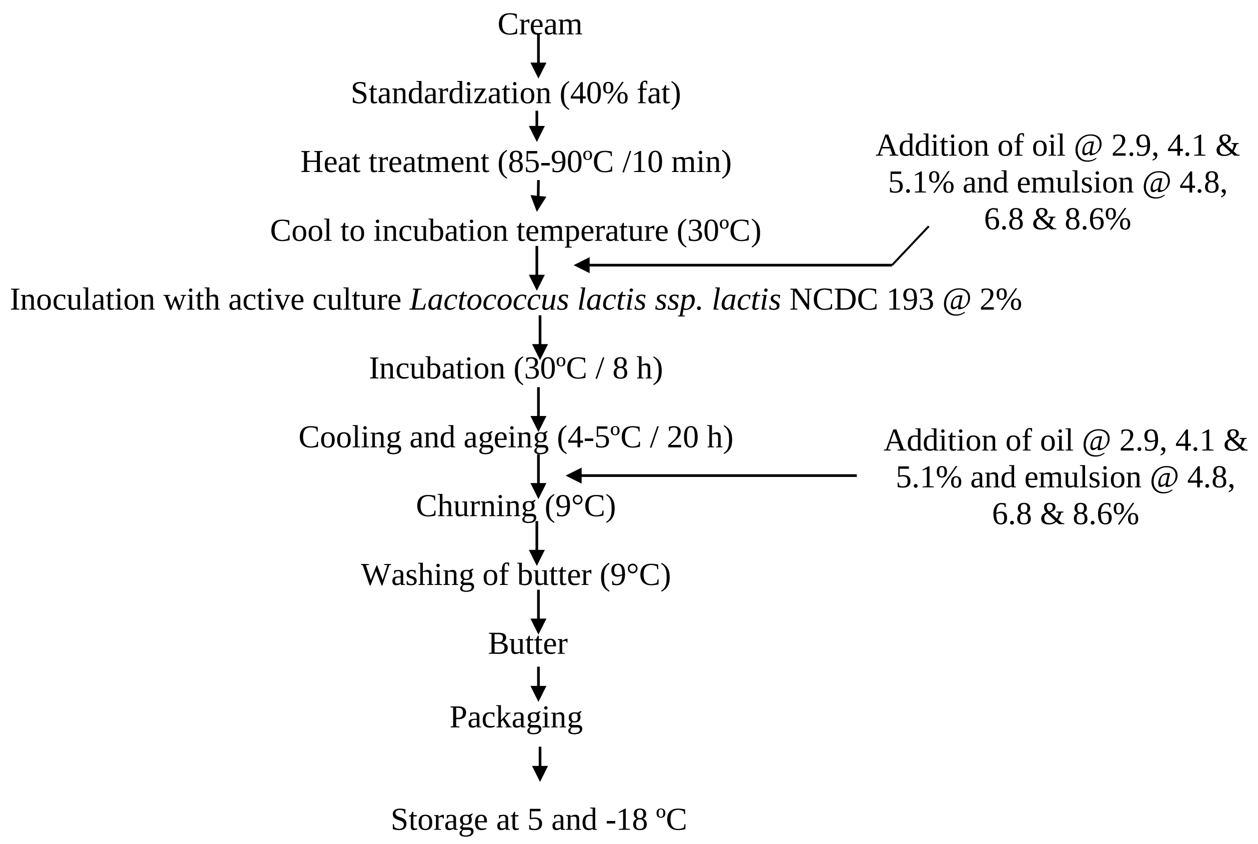


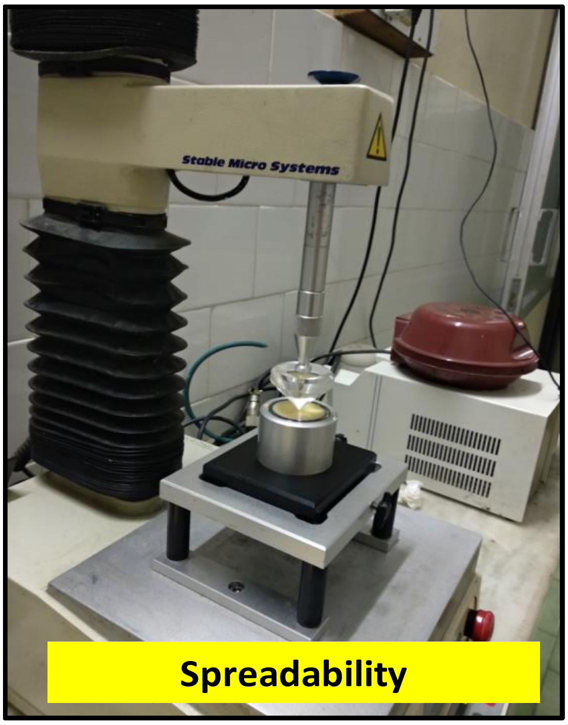
Figure 4.2 Preparation of omega-3 fatty acid fortified butter using flaxseed oil and emulsion

4.6.3 Sensory evaluation of butter samples

Freshly prepared samples were kept for overnight in the refrigerator. They were tempered for 15 min at room temperature before sensory evaluation by the panelist. The semi trained panelist consists of staff and students of dairy processing group of SRS of ICAR, NDRI, Bangalore. Butter samples were organoleptically analyzed by the panelist using 9-points hedonic scale in order to check overall acceptability for further process optimization. (9-Like Extremely, 8-Like Very Much, 7-Like Moderately, 6-Like Slightly, 5-Neither Like nor Dislike, 4-Dislike Slightly, 3-Dislike Moderately, 2-Dislike Very Much, 1-Dislike Extremely)

4.6.4 Textural analysis of butter

The samples were tempered to 24°C by keeping it at room temperature for about 4 h before analyzing textural properties. Spreadability of the butter was determined by spreadability rig using TA-XT. plus Texture Analyser (Figure 4.3). In case of creep test, 20 mm X 20 mm X 20 mm cubes were cut and tempered to 24°C. Creep test was carried out by using P/75 Platen probe. The texture analyzer programme settings used for spreadability and creep test is given in the Table 4.4



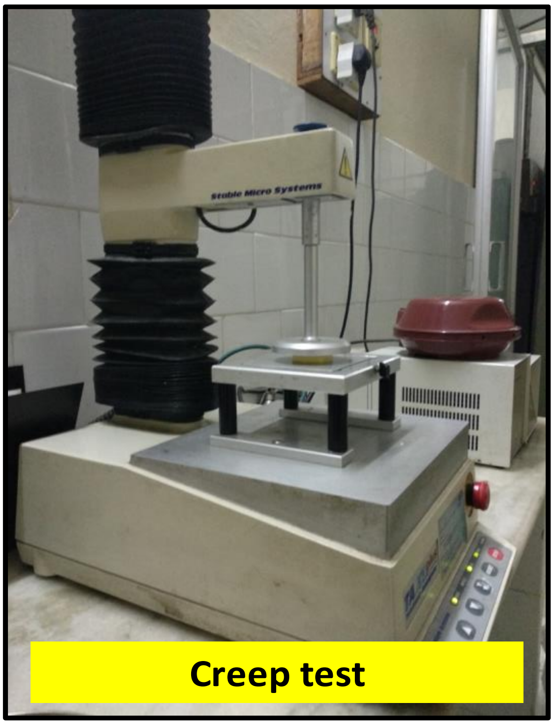


Figure 4.3 – Experimental set up for determining spreadability and creep behavior using Texture analyser

Table 4.4 Texture Analyzer settings for spreadability and creep test

S. No.	Parameters	Spreadability	Creep test
1	Test mode	Compression	Compression
2	Pre-test speed	1.0 mm/sec	1.0 mm/sec
3	Test speed	3.0 mm/sec	2.0 mm/sec
4	Post-test speed	10.0 mm/sec	10.0 mm/sec
5	Target mode	Distance	Force
6	Force	-	100 g
7	Trigger type	Button	Auto (force)
8	Trigger force	-	5.0 g
9	Distance	18 mm	-
10	Break mode	Off	-
11	Stop plot at	start operation	-
12	Tare mode	Auto	-
13	Advanced option	On	Off
14	Control option	Disabled	-

4.6.5 Colour analysis of butter

The images required for the study were acquired using the flatbed scanner (Canon CanoScan 9000F Mark II, Japan) by the Wasnik *et al.* (2017) method. The surface colour of images acquired by scanner was measured using Adobe Photoshop in Lab mode. The data obtained was converted in terms of CIELAB, L* lightness ranging from zero (black) to 100 (white), a* ranging from +60 (red) to -60 (green) and b* ranging from +60 (yellow) to -60 (blue) using Equations 1 to 4. From the extracted values, colour descriptors like yellowness index, hue, chroma and ΔE were derived by computation (Pathare *et al.*, 2013), as indicated in Equation 4 to 7, respectively.

$$L^* = \frac{100 \times L}{255} \quad \ldots\ldots\ldots\ldots (Eq. 1)$$

$$a^* = \frac{240 \times a}{255} - 120 \quad \ldots\ldots\ldots.. (Eq. 2)$$

$$b^* = \frac{240 \times b}{255} - 120 \quad \ldots\ldots\ldots.. (Eq. 3)$$

$$Yellowness\ Index(YI) = \frac{142.86 \times b^*}{L^*} \ \ldots\ldots\ldots.. (Eq. 4)$$

$$Hue\ value = ATAN\left(\frac{b^*}{a^*}\right) * \frac{180}{\pi} \ldots\ldots\ldots\ldots\ldots (Eq. 5)$$

$$Chroma = \sqrt{{a^*}^2 + {b^*}^2} \ldots\ldots\ldots\ldots\ldots.. (Eq. 6)$$

$$\Delta E = SQRT\ \{({L^*}_{Sample} - {L^*}_{Control})^2 + ({a^*}_{Sample} - {a^*}_{Control})^2 + ({b^*}_{Sample} - {b^*}_{Control})^2\} \ldots\ldots (Eq. 7),$$

Where; L* represents lightness

a* represents

b* represents

ΔE represents total colour change

4.6.6 Proximate composition of optimized butter

4.6.6.1 Determination of fat content in butter

The fat in butter was determined using one gram of the sample by Rose-gottlieb method given in section 6.2 of IS: 3507-1966. About 1 g of the butter was weighed into a 50-mL beaker and 9 mL of sodium chloride solution was added into the beaker. 10 mL ethyl alcohol was used to transfer the content to the Mojonnier fat-extraction apparatus and mixed well. Then 25 mL diethyl ether was then added through the beaker. Tube was stoppered with cork (or stopper) which is wetted with water before insertion and shaken vigorously for one minute. Again 25 mL of light petroleum ether boiling point 60°C was added and shake vigorously for one minute. The contents were left undisturbed for 30 min. Supernatant and separated fat was decanted as much as possible. Decantation was done carefully by gradually bringing the cylindrical bulb of the tube into a horizontal position. The cork or stopper was washed with mixed solvent and the rinsing were collected in the flask. Both inside and outside of the tube neck was washed with 4 to 5 mL of mixed solvent, and decanted. Extraction of milk fat residue and the subsequent operations was repeated but using 15 mL of diethyl ether and 15 mL of petroleum. Finally, repeat the extraction and subsequent operations once more with 15 mL diethyl ether and 15 mL petroleum. The collected solution was distilled for complete removal of the solvent. The flask was wiped and dried residual fat in the oven at 98 to 100°C for one hour. The flask was cooled to room temperature in a desiccator. The process of heating in oven, cooling and weighing was repeated until successive weighing do not show a loss in weight by more than one milligram. The weight difference before and after extraction showed the weight of milk fat extracted from butter.

4.6.6.2 Determination of moisture content of butter

Previously cleaned dish and glass rod was dried in the oven maintained at l00 ± 1°C for at least one hour. The dish and glass rod were allowed to cool to the room temperature in a desiccator and weight of the dish was taken. Accurately 3 g of the prepared butter sample was weighed in dish. The dish was placed on a steam-bath supported on a clay pipe triangle for 20 min. The bottom of the dish was wiped and transferred it to the oven maintained at 100 ± 1°C and kept it for 90 min. Further, the dish was allowed to cool in the desiccator as before and weighed. The dish was heated again in the oven for 30 min. The process of heating, cooling and weighing was repeated until the difference between two consecutive weights does not exceed 0·1 mg. Finally, the lowest weight was recorded. Difference in the weights of dish before and after heating represented amount of moisture present in the samples.

4.6.6.3 Determination of protein content of butter

Total protein content in the butter samples was determined by AOAC (2005) method given in the by using 6.38 as a conversion factor. In this method, 1 g of butter sample was taken in the digestion flask. After this, 10 g digestion mixer and 200 mL conc. sulphuric acid was added and flask was kept for digestion for 3 h in the Kjeldahl digestion unit. Distillation was carried out by Kjel plus distillation unit followed by neutralization with 0.2 N HCl. Volume of 0.2 N HCl required for blank and the samples was noted and total protein content was calculated by using equation 8.

$$Protein\ (\%) = \frac{(A-B) \times N \times 1.4007 \times 6.38}{W} \ldots\ldots\ldots..(Eq. 8)$$

Where, A- mL of 0.2 N HCl required for sample

B- mL of 0.2 N HCl required for blank

N- Normality of HCl

W-Weight of the sample taken in g

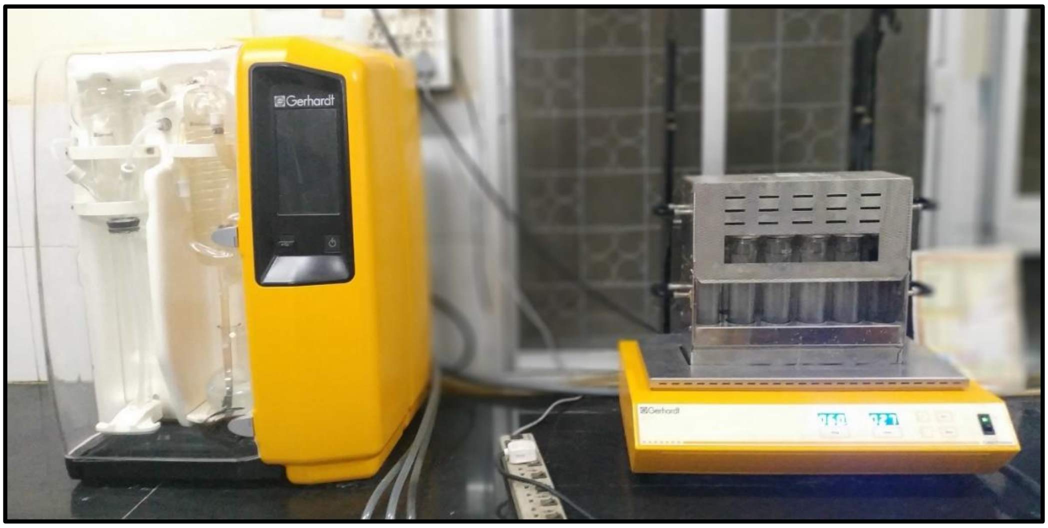


Figure 4.4 Kjel plus digestion and distillation assembly

4.6.6.4 Determination of ash content of butter

Ash content of the butter was determined by AOAC (2005) method. Properly cleaned and dried crucibles were kept overnight in the muffle furnace at 550°C and cooled down in the desiccator for 30 min. Empty crucible was weighed and 5 gm of butter was added into the crucible and charred till fumes stop coming from the samples. Then, charred samples were kept in muffle furnace maintained at 550°C for 6 h and cooled in the desiccator for 30 min. Finally, weight of the crucibles was taken and ash content was determined by the formula given below in equation 9.

$$Ash\ (\%) = \frac{Weight\ of\ ash}{Wight\ of\ sample} \times 100 \ldots \ldots \ldots \ldots (Eq. 9)$$

4.6.6.5 Determination of total carbohydrate content of butter

Total carbohydrate content of butter was determined by difference method. In this method total sum of fat, protein, moisture, ash content (%) was subtracted from 100.

4.6.7 Titratable acidity of butter

Titratable acidity of butter was determined by method given in section 8.0 of IS: 3507-1966. About 20 g of the butter sample was taken in a dry 250 mL conical flask. To this 90 mL previously boiled, hot distilled water was added and mixed. The contents of the flask were titrated with 0.02 N NaOH in hot condition by using 1 mL of the phenolphthalein indicator. The completion of titration was indicated by appearance of light pink colour and titre value was noted. The titratable acidity of the butter was calculated by using formula given in equation 10.

$$Titratable\ acidity\ (\%LA) = \frac{9 \times A \times N}{W} \ldots \ldots \ldots \ldots (Eq. 10)$$

Where,

A – amount (in mL) of 0.02 N NaOH required for titration
N – normality of NaOH
W – weight of butter taken for titration (g)

4.6.8 pH of butter

Method for determination of pH of butter was given in section 9.2 of IS: 3507-1966. About 100 g of butter was taken in a beaker and melted at a temperature not exceeding 50°C. The content in the beaker mixed properly and transferred to a separating funnel. The content in the funnel was allowed to stand undisturbed in order to separate by gravity, maintaining the fat in the liquid condition. After fat separation, serum was collected in small beakers without disturbing the fat phase and pH was measured by previously calibrated pH meter. (Eutech Instuments, Cyberscan, Singapore)

4.6.9 Storage study of the butter

4.6.9.1 Peroxide Value (PV)

Peroxide value was determined according to Method Cd 8-53 of the Association of Official Analytical Chemists (2005). In this method 5 g of butter was dissolved in 30 mL acetic acid-chloroform mixture (3/2. vol/vol) and a saturated solution of KI (1 mL) was then added. Then the mixture was kept for liberation of iodine. The liberated iodine was titrated with 0.01 N sodium thiosulphate solution till yellow colour turns to white. The mixture was then titrated against sodium thiosulphate using 1 mL starch indicator (1%) till blue colour of the mixture turns white and the peroxide value was calculated by using following Equation 11.

$$Peroxide\ value\ (meqO_2/kg\ of\ fat) = \frac{100 \times V \times N}{W} \ldots\ldots\ldots\ldots (Eq. 11)$$

Where,

V – Volume (mL) of 0.01 N Sodium thiosulphate required for titration

N – normality of Sodium thiosulphate

W – weight of butter taken for titration (g)

4.6.9.2 Free Fatty Acids (FFA) Value

The FFA content was evaluated according to the method described in Kuruppu *et al.* (1983) and Amr (1991). In this method, 5 g butter sample was weighed into an Erlenmeyer flask and 30 mL neutralized ethanol was then added. After adding few drops of phenolphthalein indicator, the mixture was warmed to facilitate dissolution. The solution was titrated with 0.01 N NaOH solution until a pink colour was stable for at least 20 s. The free fatty acids were calculated by using formula given below in Equation 12.

$$FFA\ (\%\ oleic\ acid) = \frac{2.82 \times V \times N}{Weight\ of\ sample} \ldots\ldots\ldots\ldots (Eq. 12)$$

Where,

V – Volume (mL) of 0.01 N NaOH required for titration

N – Normality of NaOH

4.6.9.3 Thiobarbutiric Acid (TBA) Value

TBA value was estimated according to method described by Kuruppu *et al.* (1983) with slight modification. About 1g butter sample was weighed in a screw-capped test tube and to this 10 mL of a 7.5% trichloroacetic acid solution was added. The mixture was shaken vigorously for 1 min using of a Vortex mixer (Spinix, India). After that, 3 mL of a 1% solution of thiobarbutiric acid in 90% glacial acetic acid was added and the tube was placed in a boiling water bath for 40 min. After cooling, the fat was removed from the reaction mixture by shaking with 3 mL of chloroform followed by centrifugation at 2500 G. The optical density of the pink aqueous phase containing the reaction product was measured at 530 nm.

4.6.10 Microbiological analysis

4.6.10.1 Preparation of phosphate buffer

Stock solution was prepared by dissolving 32 g of solution (Potassium Dihydrogen Phosphate = 26.22 g and sodium carbonate = 7.78 g) in one Litre of distilled water, autoclaved and stored at refrigeration temperature. Finally, pH of the buffer was adjusted to 7.2 ± 0.2 at 25°C.

Working solution was prepared by adding 1.25 mL of stock solution and 5 mL of Magnesium Chloride solution (81.1 g of $MgCl_2$. $6H_2O$ in one litre of distilled water) by making final volume to 1 litre using distilled water. Blanks were prepared by filling 99 ml of working solution into dilution bottles as well as 9 mL in test tubes and autoclaved. Final pH of the blank solution was adjusted to 7.2 ± 0.2 upon cooling to 25°C.

4.6.10.2 Determination of coliform count

Coliform count of the butter samples was determined by method section 13 of IS: 3507-1966. Before taking samples, butter was melted in a water bath maintained at 43-45°C. Blanks (99 mL) were warmed to 40°C and to this 11 mL of melted butter was added. Contents were mixed properly to obtain a dilution of 1:10. Further one millilitre of 1:10 dilution was added to 9 mL dilution blank to get 1:100 dilution. One millilitre of dilution was transferred to previously marked petri plates and every dilution was taken in duplicates. Then 10-15 mL of Violet Red Bile Agar was added to each plate, mixed well and allowed to solidify. After solidification second layer, 5-6 mL of agar was added and again allowed to solidify. Finally, plates were incubated at 37 ± 0.05°C for 24 h. Dark red colonies were counted and coliform count was calculated.

4.6.10.3 Determination of yeast and mould count

Yeast and mould count of the butter samples was determined by method given in section 14 of IS: 3507-1966. The procedure is nearly same as that followed for (sec. 4.6.10.2) for coliform count. Difference in the yeast and mould determination is potato dextrose agar instead of VRBA and the plates were incubated for 5 days at 21 or 25°C. There was no second layer of media in yeast and mould determination.

4.6.11 Determination of Omega-3 fatty acid (ALA) content

4.6.11.1 Fat extraction

Extraction of milk fat from butter was done by heating in water bath at temperature of 40°C for 15 min. In order to attain fat separation, samples were centrifuged for 15 min at 40°C, 1400 rpm and the milk fat was collected from the supernatant carefully for further analysis.

4.6.11.2 Preparation of fatty acid methyl esters (FAME)

The internal standard tridecanoic acid (200 μL, 25 mg/mL) was added to the extracted fat (100 mg) and was dissolved in 5 mL of dichloromethane, then vortexed vigorously for 1 min to achieve sample uniformity. From this 1 mL was taken in the centrifuge tube and added 5 mL of methanol. Transesterification of the samples was conducted at 55°C for 45 min by adding 3 mL 2 N KOH solution in methanol (with periodic shaking). To neutralize excess of potassium hydroxide, 3.5 mL of 12 N sulphuric acid was added and shaken. The samples were cooled and centrifuged at 7000 rpm for 15 min. The adequate hexane layer was transferred into a glass ampoule.

4.6.11.3 Determination of fatty acid compositions by Gas Chromatography-Mass Spectrometry

DB5 MS (30 m × 0.25 mm × 0.25 μm) capillary column was used to separate FAME with helium as a carrier gas at a flow rate of 1 mL/min. The injector temperature was 230°C, and the column temperature was programmed as follows: 50°C in the beginning for 1 min, subsequent increase to 220°C at the rate of 3°C /min and maintained for 1 min. In between; 2°C /min rate was also followed from 40 min time till the end. The interface temperature for GC-MS (Agilent Technologies, United states) was 220°C. The temperature of ion source was 200°C ionisation energy was 70 V. The total ion monitoring (TIC) was used to detect fatty acids (m/z ranged 50–500). Tridecanoic acid was used as internal standard to quantify FAME. Identification of fatty acids was made on the basis of mass spectral libraries (NIST 47, NIST 147 and Wiley

175), as well as data from literature and by comparison of their retention times with Supalco-37 standards GLC-85 and FAME Mix GLC-90. Results were expressed as w/w (%) total fatty acid.

Figure 4.5 GS-MS equipment

4.6.12 Thermal properties of butter using Differential scanning calorimetry (DSC)

Fat from the butter was separated according to the method given in Lorinczy *et al.* (2007). Butter was melted at 60°C, then centrifugated at the same temperature at 6000 rpm and the separated milk fat was dehydrated at 105°C for an hour.

Differential scanning calorimeter (Mettler-Toledo, Switzerland) equipped with an Intracooler II and running under Pyris software was used to examine the thermal properties of butterfat. Nitrogen (99.999% purity) was used as the purge gas. The DSC was calibrated using indium (M.P. 156.6 °C, ΔHf=28.45 J g^{-1}) and n-dodecane (M.P. -9.65°C, 151 ΔHf=216.73 J g^{-1}). 6 mg of fat was weighed into aluminium pans of 20 µL (Perkin Elmer, No. 0219-0062) and hermetically sealed. The reference was an empty, hermetically sealed aluminium pan. The calibration of the calorimeter was controlled with capric acid melting (M.P. 31.6 °C). The sample pan was placed in the calorimeter at 25 °C and then subjected to the following time–temperature program: (1) heating and isotherm for 5 min at 60°C to melt all crystals and nuclei; (2) cooling at 5°C min^{-1} to −40°C; (3) isotherm for 20 min at −40 °C; and (4) heating at 5°C·min^{-1} to 60 °C. Three replicates were analysed for each sample. The following parameters were analyzed from the melting and crystallizing curve; T_{onset} ,T_{peak} and T_{enset} of all the melting and crystallization peaks was determined . Also, area under each peak was calculated.

Figure 4.6 Differential scanning colorimeter (DSC)

4.6.13 Oxidative stability in terms of Induction period using Rancimat

Induction period was measured automatically by taking 2.5 ± 0.05 g samples in Rancimat (Metrohm, Switzerland) reaction vessels where they were oxidised at 120 ± 0.2°C by passing oxygen from the bottom of the samples according to the instructions given in the instruction manual of Metrohm Rancimat 679 (Metrohm 1993)

Figure 4.7 Rancimat

4.6.14 Determination of solid fat content by pNMR

The solid fat content in the butter samples was determined from 5 to 40°C by using a pulsed nuclear magnetic resonance analyzer (Bruker Minispec pNMR Analyser

Model no. 120, Rheinstetten, Germany). Before placing in NMR tubes, fat samples were melted at 60°C for 30 min to erase the crystal history. Then NMR tubes were filled with samples, kept at 60°C for 30 min, next chilled to 0 °C and left for 60 min. Prior to measurements the NMR tubes were placed in water bath at 5, 10, 15, 25, 30, 35 and 40 °C for 60 min. NMR data was automatically given by instrument software to provide SFC.

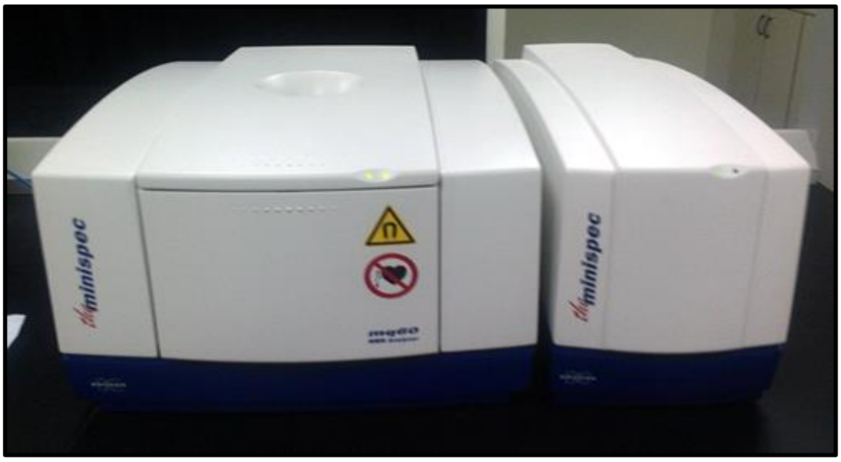


Figure 4.8 Pulsed nuclear magnetic resonance (pNMR) analyser

4.6.15 Water activity of the optimized sample

Water activity of the product was determined by using water activity meter (Aqua lab Model Series 3 TE, Decagon Devices, WA, USA)). Before placing the sample, instrument was stabilized for 10 min. after attaining the stable condition of temperature and water activity. Then sample was small quantity of sample was taken in a dish and reading was noted after equilibration time of the instrument.

Figure 4.9 Water activity meter

5.0 RESULTS AND DISCUSSION

5.1 Prologue

The present study mainly focused on the development of omega-3 fatty acid fortified butter as it is the fourth most consumed dairy product in India. This attempt would prove helpful in increasing the omega-3 fatty acids in the fortified butter through fortification using flaxseed oil; one of the richest plant sources of alpha linolenic acid. Fortification of butter with omega-3 fatty acids improves spreadability along with imparting health benefits. This chapter includes results obtained during the present investigation on 'Development and quality evaluation of omega-3 fatty acid fortified butter'.

The results are discussed in three different sections according to the objectives of study. The first section deals with the preliminary trials for ageing time and ripening time, optimization of emulsion process and process optimization for fortification of butter using direct flaxseed oil as well as flaxseed oil emulsion. The second section comprise of characterization of optimized samples for various thermal and physico-chemical characteristics using Differential Scanning Calorimetry (DSC), Pulsed nuclear magnetic resonance (pNMR), Rancimat and Gas chromatography mass spectrometry (GC-MS). Finally, the third section explains the changes occurring during storage in terms of physico-chemical, oxidative, textural, microbiological and sensory characteristics. The results obtained are presented and discussed in this chapter under relevant headings and subheadings.

5.2 Process optimization for preparation of flaxseed oil emulsion

5.2.1 Selection of stabilizer/emulsifier

Preliminary trials were taken for selecting suitable stabilizer and/or emulsifier for the preparation of flaxseed oil emulsion. Gum arabic, Guar gum, Sodium alginate, Modified starches, Sodium caseinate, WPC, WPI, blend of WPC and Sodium caseinate have been evaluated for preparation of flaxseed oil emulsion. Emulsions were prepared with 45, 60, and 75% oil load and among these, emulsion prepared by using WPC and WPI had maximum physical stability with no oil separation. However, considering the same emulsifying effect for the studied oil load, WPC being a cheaper source was selected for the preparation of emulsion.

5.2.2 Selection of level of emulsifier

Level of the emulsifier has been optimized on the basis of physical stability of the formulated emulsion. For preparation of flaxseed oil emulsion, three levels of WPC (5, 7.5 and 10%) have been studied. Emulsion with 7.5 and 10% WPC had same stability but emulsion with 10% WPC had higher viscosity than 7.5% as shown in the Table 5.1. The higher viscosity with 10% WPC level might be due to higher surface load and thus negatively influencing the emulsion properties (McClements, 2004). Thus, 7.5% WPC was selected for making emulsion.

Table 5.1 Effect of WPC content and oil load on physical stability of emulsion

WPC	Oil load (%)	Physical stability
5%	45	+ +
	60	+ +
	75	-
7.5 %	45	+ + +
	60	+ + +
	75	-

where, ++: stable; + + +: very stable; -: not stable

5.2.3 Optimization of oil load preparation for flaxseed oil emulsion

Flaxseed oil emulsions were prepared by using 7.5 and 10% WPC dissolved in aqueous phase and added with three different oil loads i.e. 45, 60 and 75%. Emulsions with 45 and 60% oil load (OL) had better stability (stable for more than 5 days) under refrigeration condition while, the emulsion with 75% oil load lost its stability within 5-6 h (Figure 5.1).

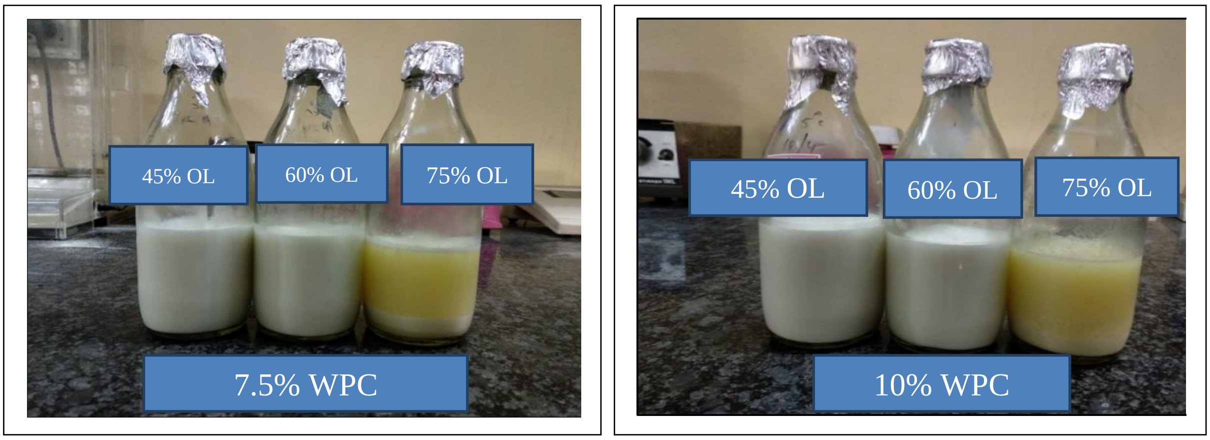


Figure 5.1 Flaxseed oil-WPC emulsions with varying oil load and WPC level

This may be due to the limited oil absorption capacity of WPC at higher oil load conditions. Further, emulsifier is required for entrapping the oil droplets, which in turn provides stability to the resultant emulsion. However, with increasing oil load, the emulsifier amount might be limited for sharing the active material between the oil droplets, which may result in separation of oil, thereby leading to less stable emulsions (Dickinson, 2001). Thus, based upon the higher physical stability, emulsion containing 7.5% WPC and 60% oil load was selected for incorporation in the cream for butter making.

5.3 Optimization of ageing time of cream

As ageing is an important processing step in making butter, attempt was made to study the effect of ageing time on fat loss in buttermilk. Two different ageing times (16 and 20 h) were selected for the optimization and ageing was carried out at refrigerated temperature i.e. 5-7 °C. Fat loss in the buttermilk was lesser (0.5%) when cream aged for 20 h than 16 h ageing (0.9%). This is due to the fact that a higher proportion of fat was partially converted into solid fat, thereby improving churning efficiency and in turn, lesser fat loss in the buttermilk. Thus, 20 h ageing time was selected during the present study.

5.4 Process optimization for fortification of butter with omega-3 fatty acids

The effect of type of fortificant (oil or emulsion), stage of addition of fortificant (before ageing and after ageing) and level of fortificant (25, 35 & 45% of RDA level of ALA) on various physico-chemical, sensory, textural and sensory characteristics was evaluated.

5.4.1 Effect of fortificant addition on churning efficiency and yield of butter

5.4.1.1 Effect of fortificant addition on fat loss in buttermilk

In the present study, it was observed that fat content in buttermilk obtained after churning of cream increased from 0.53 to 1.03% and 0.6 to 1.33% upon increasing the amount of oil in the cream before ageing and after ageing, respectively. Addition of oil before ageing showed lesser fat loss than when added after ageing. This might be due to entrapment of the oil droplets within the cream during ageing. Further, this could be due the formation of hydrophobic bonds resulting between flaxseed oil and cream constituents. Even though, the fat loss increased significantly ($P<0.05$) with the level of oil addition is shown in Figure 5.2, but it was still below 1.5% for all the levels of oil studied. The higher fat loss resulting increasing addition of flaxseed oil, could be due to increase in liquid/soft fat content in cream (De, 1980).

In addition to direct oil, flaxseed oil-WPC emulsion was also studied for its effect on fat loss and other properties. It was observed that the fat loss in buttermilk was 0.36-1.20% and 0.36-1.43% (Figure 5.3) when emulsion was added before ageing and after ageing respectively. It was also observed that fat loss was more in case of emulsion than oil. This might be due to the higher affinity of oil towards WPC. Further, WPC being water soluble, might be leached out in the buttermilk upon churning. Addition of emulsion to the cream before ageing resulted in lesser fat loss than when added after ageing and increased with the level addition. Similar results were obtained when oil was directly added into cream before its ageing. So, probably the same explaination holds well here also, that a considerable amount of flaxseed oil-WPC emulsion might have formed certain hydrophobic interactions with the cream constituents and thereby escaped the leaching in buttermilk after churning.

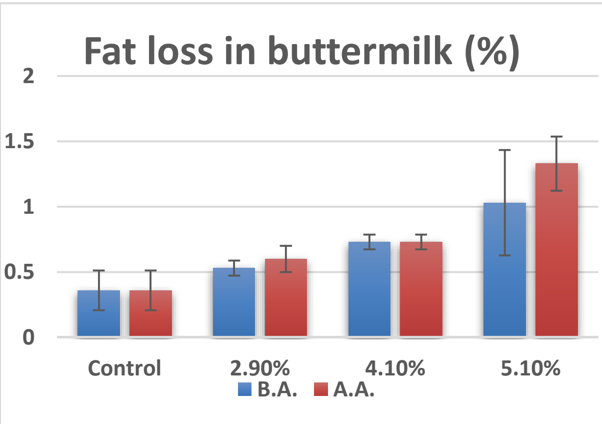


Figure 5.2 Effect of flaxseed oil addition fat loss in buttermilk

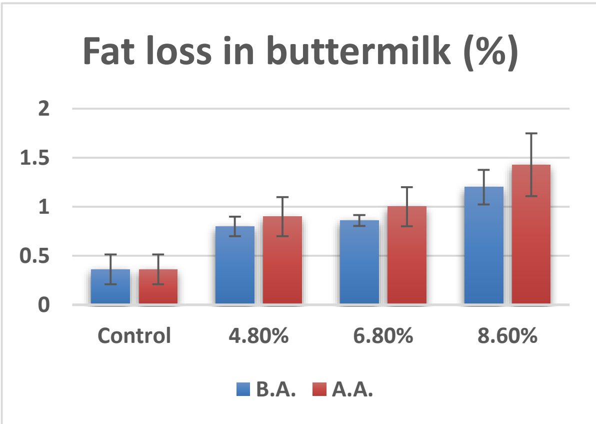


Figure 5.3 Effect of flaxseed oil-WPC emulsion addition on fat loss in buttermilk

5.4.1.2 Effect of fortificant addition on yield of butter

Yield of the butter was calculated as quantity of butter obtained per kg of cream and it is expressed as per cent yield. During the study, it was observed that the yield of butter varied from 49.13-50.41% when oil was added to the cream before ageing and 46.41-49.65 % when added after ageing (Figure 5.4). However, statistically there was no significant ($P<0.05$) difference in the yield of the butter with increasing level of oil. Similarly, in case of flaxseed oil-WPC emulsion, yield of the butter varied from 49.03-

47.46% when emulsion was added before ageing and 49.03-49.95% (Figure 5.5) when added after ageing. Further, there was no significant ($P<0.05$) difference in the yield of butter with increasing level of oil and emulsion.

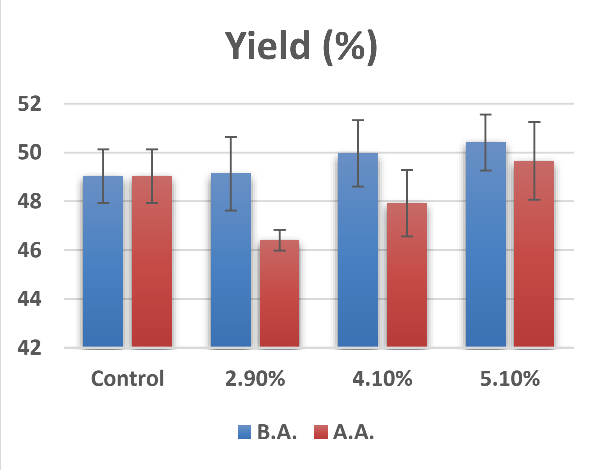


Figure 5.4 Effect of stage and level of flaxseed oil addition on yield of butter

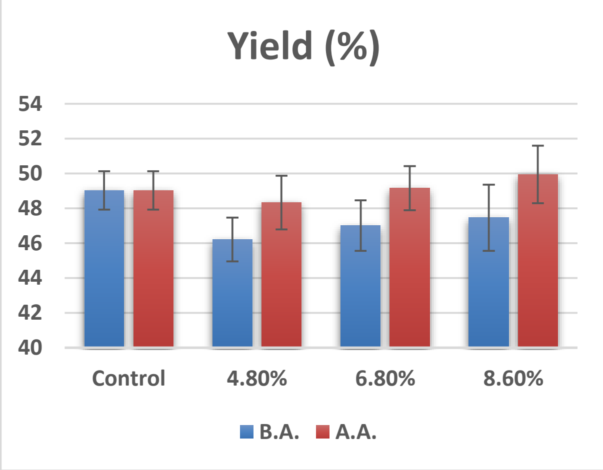


Figure 5.5 Effect of stage and rate of flaxseed oil-WPC emulsion addition on yield of butter

5.4.1.3 Effect of fortificant addition on churning time of cream

Churning is the process of agitating cream at a suitable temperature in order to facilitate the adhering of fat globules to each other to form larger fat masses until complete separation of fat and serum is obtained. It was observed that time required for churning of cream decreased significantly ($P<0.05$) from 49 to 25.66 min and 49 to 20.33 min with increasing the amount of flaxseed oil added before and after ageing, respectively (Figure 5.6). Similar trend was observed in case of butter samples prepared with addition of flaxseed oil-WPC emulsion in cream. It was observed that time required for churning of cream added with emulsion before ageing decreased significantly (from 49.33 to 26 min and from 49.33 to 29.33 min, when added after ageing (Figure 5.7). Generally, optimum time required for churning of normal cream is 30-60 min in order to obtain butter with good body and texture. However, for the butter samples prepared from cream containing 5.10% flaxseed oil and 8.60 % emulsion had churning time less than 30 minutes resulting weaker body. The less churning time is due to the increase in proportion of soft fat (De, 1980).

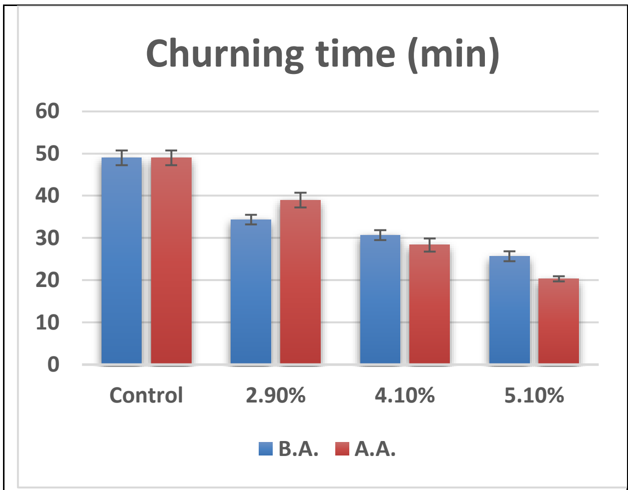
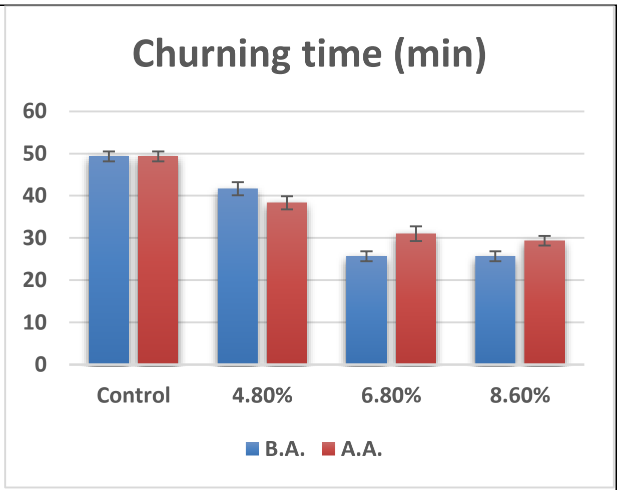
	

Figure 5.6 Effect of stage and rate of flaxseed oil addition on churning time of cream	**Figure 5.7** Effect of stage and rate of flaxseed oil-WPC emulsion addition on churning time of cream

5.4.2 Sensory characteristics of the control and fortified butter samples

Sensory evaluation of the butter samples was carried out by semi-trained panellist using 9-points hedonic scale in order to check overall acceptability for further process optimization. The colour & appearance decreased significantly ($P<0.05$) with increase in the level of addition, particularly the scores are lesser for the butter samples prepared with oil addition after ageing of cream (Table 5.2). This might be due to improper mixing or entrapment of flaxseed oil with milk fat globules and therefore perceivable difference was there in the butter appearance.

The spreadability of the samples increased significantly ($P<0.05$) with increase in the level of oil addition. The sample containing 5.1% oil on cream basis added before ageing showed highest spreadability score (8.40) and the lowest spreadability score (7.51) was reported in control sample. The increase in spreadability is due to the presence of more amount of liquid or soft fat in fortified samples.

However, the score for body and texture decreased significantly ($P<0.05$) with the increase in flaxseed oil content. This might be due to the weaker body resulting from less churning duration in the butter samples fortified with flaxseed oil. The stage of addition didn't have a greater effect on body and texture as evident from very similar sensory scores.

There was a considerable significant ($P<0.05$) decrease in the flavour scores of fortified butter samples as compared to control due to the characteristic flavour of flaxseed oil being perceived by the sensory panelist especially for the highest level of fortificant.

All the fortified butter samples were sensorially acceptable with sensory scores ranging from 7 (like moderately) to 9 (like extremely). However, the overall acceptability of the samples decreased significantly ($P<0.05$) with the increase in the level of flaxseed oil for both the stages of addition. The control sample showed highest sensory score (8.06) and sample prepared with maximum level of flaxseed oil added after ageing of cream (providing 45% RDA level of ALA) had least (7.65) overall acceptability score due to the perceived flaxseed oil flavour.

Further, the samples prepared by adding flaxseed oil before ageing had comparatively better sensory scores than the samples prepared from addition of oil after ageing of cream. This could be due to the effective entrapment of flaxseed oil within the milk fat emulsion matrix and thereby reduced flavour perception in the fortified butter.

Similar results were obtained by Kolanowski *et al.* (2007) in the butter fortified with omega-3 LC PUFA by blending fish oil in the butter. Blending of fish oil with butter showed acceptable sensory quality up to 30 g/kg and above this significantly affect the flavour of the product. Thus, it can be inferred that human olfactory system can easily detect the addition of flaxseed or fish oil above 30 g/kg level which in turn significantly affects the sensory quality of product. Therefore, the level of about 3% might be added without significantly affecting the sensory acceptability.

In case of flaxseed oil emulsion addition, similar kind of trend was found and the results are mentioned in Table 5.3. All the sensory properties were significantly ($P<0.05$) affected by the variation in amount of flaxseed oil-WPC emulsion. The decreasing trend was observed for colour and appearance, body and texture, flavour, while, increasing trend for spreadability was found with the increasing level of fortificant. There was significant ($P<0.05$) difference in the overall acceptability of the samples.

Table 5.2 Sensory properties (9-point hedonic scale) of butter as affected by level of flaxseed oil and stage of addition

Samples	Colour & Appearance	Body & Texture	Spreadability	Flavour	Overall acceptability
Control	8.17±0.36^{a}	8.17±0.43^{a}	7.51±0.31^{d}	8.42±0.45^{a}	8.06±0.19^{a}
2.9% OABA	7.88±0.28^{b}	7.98±0.77^{ab}	8.00±0.24^{b}	7.88±0.31^{b}	7.94±0.29^{b}
4.1% OABA	7.83±0.45^{b}	7.77±0.50^{bc}	8.08±0.23^{b}	7.80±0.36^{b}	7.87±0.24^{b}
5.1% OABA	7.74±0.42^{c}	7.73±0.46^{bc}	8.40±0.42^{a}	7.41±0.41^{cd}	7.82±0.20^{b}
2.9% OAAA	7.76±0.50^{bc}	7.72±0.43^{bc}	7.54±0.30^{cd}	7.79±0.23^{b}	7.70±0.18^{c}
4.1% OAAA	7.75±0.24^{bc}	7.64±0.35^{bc}	7.70±0.30^{c}	7.56±0.26^{c}	7.66±0.10^{c}
5.1% OAAA	7.59±0.36^{bc}	7.70±0.39^{c}	7.95±0.23^{b}	7.35±0.30^{d}	7.65±0.19^{c}

n=27, results are expressed as Mean± SD, different small letters superscript (a, b, c) within column differ significantly ($P<0.05$) among samples; EABA: Emulsion addition before ageing, EAAA: Emulsion addition after ageing

For the same amount of emulsion, the stage of addition also affected the overall sensory acceptability. When emulsion was added after ageing of cream, the sensory scores were reported to be lower as compared to the sensory acceptability scores for the butter samples prepared from cream in which emulsion was added before ageing. The spreadability of the samples increased owing to the presence of more soft fat in fortified samples due to higher proportion of unsaturated fatty acids in the flaxseed oil.

Control sample had highest (8.06) and sample (8.6% EABA) prepared with flaxseed oil emulsion added after ageing had lowest (7.56) overall acceptability score. The sample 4.1% OABA and 6.8% EABA had comparable overall acceptability scores with the control sample without affecting much to the flavour. Increase in the concentration above 4.1% flaxseed oil and 6.8% flaxseed oil emulsion affected drastically to the flavour of the product. Thus, sample 4.1% OABA and 6.8% EABA has been optimized for the further studies.

Table 5.3 Sensory properties (9-point hedonic scale) of butter as affected by level of flaxseed oil-WPC emulsion and stage of addition

Samples	Colour & Appearance	Body & Texture	Spreadability	Flavour	Overall acceptability
Control	8.17±0.36^{a}	8.17±0.43^{a}	7.51±0.31^{d}	8.42±0.45^{a}	8.06±0.19^{a}
4.8% EABA	8.21±0.38^{a}	7.96±0.38^{b}	7.96±0.34^{abc}	7.90±0.37^{b}	8.01±0.28^{ab}
6.8% EABA	8.10±0.28^{a}	7.94±0.27^{b}	8.11±0.39^{ab}	7.74±0.32^{bc}	7.97±0.19^{ab}
8.6% EABA	7.82±0.43^{b}	7.88±0.31^{b}	8.10±0.66^{a}	7.34±0.41^{d}	7.78±0.26^{cd}
4.8% EAAA	7.91±0.41^{b}	7.90±0.30^{b}	7.75±0.40^{c}	7.91±0.41^{b}	7.90±0.20^{bc}
6.8% EAAA	7.89±0.25^{b}	7.69±0.36^{c}	7.86±0.28^{bc}	7.63±0.50^{c}	7.77±0.19^{d}
8.6% EAAA	7.70±0.35^{b}	7.50±0.38^{c}	7.90±0.29^{abc}	7.31±0.29^{d}	7.56±0.23^{e}

n=27, results are expressed as Mean± SD, different small letters superscript (a, b, c) within column differ significantly ($P<0.05$) among samples; EABA: Emulsion addition before ageing, EAAA: Emulsion addition after ageing

5.4.3 Textural properties of the control and fortified butter samples

Texture is one of the most important parameters for predicting the quality of the product. Texture can be analysed by sensory as well as instrumental methods. For butter, spreadability is one of the important textural properties. In the present study, the texture of the butter was analysed using the spreadability rig and creep test.

Addition of flaxseed oil had influence on firmness of the butter and thus indirectly on spreadability. Firmness is related to the structural strength of any food matrix, the higher the value, the firmer is the product with increased resistance to applied shear forces. Firmness of the fortified butter samples decreased significantly ($P<0.05$) from 7.38±0.649 to 5.09±0.55 N with increase in the level of flaxseed oil. This could be due to the decreasing amount of solid milk fat and increasing amount of unsaturated liquid fat through addition of flaxseed oil which is having more than 75% unsaturated fatty acids.

The samples prepared from oil addition before ageing had significantly ($P<0.05$) higher firmness than the samples prepared using cream in which oil was added after ageing (Table 5.4).

Work of shear is an indicator of spreadability being inversely proportional to spreadability. The work of shear decreased significantly ($P<0.05$) from 7.88±0.665 to 5.03±0.360 N.s upon increasing the level of flaxseed oil. It shows that with the increase in level of addition, spreadability of the fortified butter samples increased. Similarly, work of adhesion and stickiness decreased significantly ($P<0.05$) with the level of oil addition.

Table 5.4 Textural properties of butter as affected by level of flaxseed oil and stage of addition

Sample	Firmness(N)	Stickiness(N)	Work of shear (N.sec)	Work of adhesion (N.sec)	Yield stress (N)	Retardation time (sec)
Control	7.38±0.649[a]	-6.66±0.607[a]	7.88±0.665[a]	-1.95±0.25[a]	0.741±0.011[a]	2.08±0.12[a]
2.9% OABA	6.37±0.632[b]	-5.72±0.636[bc]	6.76±0.750[b]	-1.74±0.266[b]	0.722±0.015[b]	1.81±0.11[b]
4.1% OABA	5.95±0.671[bc]	-5.25±0.672[cd]	6.32±0.684[b]	-1.64±0.171[bcd]	0.719±0.008[bc]	1.75±0.133[bc]
5.1% OABA	5.36±0.671[cd]	-4.91±0.743[d]	5.47±0.670[bc]	-1.47±0.198[de]	0.706±0.007[cd]	1.63±0.094[cd]
2.9% OAAA	6.49±0.763[b]	-5.93±0.804[b]	6.48±0.720[b]	-1.66±0.232[bc]	0.705±0.017[d]	1.71±0.145[bc]
4.1% OAAA	5.71±0.555[c]	-5.31±0.566[bcd]	5.93±0.472[bc]	-1.5±0.135[cde]	0.698±0.022[de]	1.67±0.177[bc]
5.1% OAAA	5.09±0.553[d]	-4.85±0.705[d]	5.03±0.360[c]	-1.35±0.095[e]	0.685±0.012[e]	1.54±0.146[d]

n=9, results are expressed as Mean± SD, different small letters superscript (a, b, c) within column differ significantly ($P<0.05$) among samples; OABA: Oil addition before ageing, OAAA: Oil addition after ageing

In creep test, retardation time decreased significantly ($P<0.05$) from 2.08±0.12 to 1.54±0.146 sec and yield stress from 0.741±0.011 to 0.685±0.012 N with the increase in level of flaxseed oil addition. Yield stress is a rheological property defined as the applied stress at which irreversible plastic deformation is first observed across the sample. It can also be termed as the minimum stress required for starting the flow of the material. Higher yield stress is required for firmer material to deform. The yield stress and retardation time results are also in agreement with the firmness values of these butter samples.

The textural properties were also affected by the stage of addition. Addition of oil before ageing had higher yield stress and retardation time than after ageing as shown in the Table 5.4. In before ageing stage, all the parameters analysed in spreadability and creep test showed comparable values with the control sample than after ageing samples.

In case of butter prepared by adding emulsion, firmness decreased significantly ($P<0.05$) from 7.25±0.722 to 4.36±0.429 N, work of shear from 7.66±0.743 to 4.18±0.426 N.sec. At the same time stickiness and work of adhesion was decreased significantly ($P<0.05$) with the increase in level of emulsion.

The retardation time also significantly ($P<0.05$) decreased from 1.81±0.113 to 1.47±0.086 sec and yield stress from 0.72±0.015 to 0.644±0.016 N (Table 5.5) with increasing amount of emulsion. Decrease in yield stress indicates that lesser force is required to start deformation of the butter samples fortified with flaxseed oil-WPC emulsion and thus the firmness of the samples decreased with increase in emulsion level. Contrary to what was noticed for oil added samples, fortified butter prepared from emulsion added after ageing showed higher values of textural parameters than before ageing. To be clearer, the butter samples containing emulsion added before ageing of cream showed higher spreadability than after ageing, while the reverse was observed for oil containing samples.

Table 5.5 Textural properties of butter as affected by level of flaxseed oil-WPC emulsion and stage of addition

Sample	Firmness(N)	Stickiness(N)	Work of shear (N.sec)	Work of adhesion (N.sec)	Yield stress (N)	Retardation time (sec)
Control	7.38±0.649[a]	-6.66±0.607[a]	7.88±0.665[a]	-1.95±0.25[a]	0.741±0.011[a]	2.08±0.12[a]
4.8% EABA	5.08±0.477[cd]	-4.9±0.673[c]	4.84±0.272[cd]	-1.34±0.091[cd]	0.685±0.009[cd]	1.7±0.148[ab]
6.8% EABA	4.85±0.548[d]	-4.75±0.648[c]	4.62±0.447[d]	-1.28±0.117[d]	0.676±0.02[d]	1.55±0.203[cd]
8.6% EABA	4.36±0.429[e]	-4.36±0.467[c]	4.18±0.426[e]	-1.16±0.082[e]	0.644±0.016[e]	1.47±0.086[d]
4.8% EAAA	6.46±0.646[b]	-5.79±0.706[b]	6.73±0.606[a]	-1.69±0.128[b]	0.715±0.013[b]	1.76±0.12[ab]
6.8% EAAA	5.47±0.285[c]	-4.94±0.321[c]	5.65±0.330[b]	-1.41±0.09[c]	0.704±0.015[ab]	1.73±0.137[ab]
8.6% EAAA	5.15±0.566[cd]	-4.7±0.686[c]	5.27±0.481[bc]	-1.39±0.145[cd]	0.697±0.008[bc]	1.65±0.132[bc]

n=9, results are expressed as Mean± SD, different small letters superscript (a, b, c) within column differ significantly (P<0.05) among samples; OABA: Oil addition before ageing, OAAA: Oil addition after ageing

The firmness of the sample having 4.1% oil added before ageing of cream and 6.8% emulsion added before ageing of cream was 5.95±0.671 N and 4.85±0.548 N respectively. It is significantly ($P<0.05$) lower than the control sample (7.38±0.649 N). Similarly, stickiness, work of shear, work of adhesion, yield stress and retardation time was significantly ($P<0.05$) lower than the control. Thus, it can be inferred that fortification of butter with omega-3 fatty acid using flaxseed oil or emulsion not only has health benefit but also improve the techno-functionality of butter by improving its spreadability. Similar findings were reported by Kulkarni (1983); where increasing the level of vegetable oils such as sunflower oil or safflower oil in the dairy spread resulted in increase in the spreadability as analysed by FIRA NIRD Extruder. Thus, it can be concluded that spreadability of the butter increases with increase in the level of flaxseed oil.

5.4.4 Colour properties of the control and fortified butter samples

Addition of foreign fat into the butter affects its colour and appearance. Colour is perceived as the reflectance of light by any sample. So, changing the constituents of the food matrix will definitely alter the extent of light reflectance, thereby resulting in different colour values.

In colour analysis, there was significant ($P<0.05$) increase in lightness value from 82.77 to 84.38, yellowness index from 60.89 to 62.38, chroma from 35.32 to 37.05 and total colour change from 0.49 to 3.16 with the increase in the level of flaxseed oil in cream which was further used for making fortified butter. But the Hue value was decreased significantly ($P<0.05$) from 87.22 to 84.01. In comparison with control, addition of flaxseed oil after ageing showed significantly ($P<0.05$) lesser change in the colour characteristics than before ageing (Table 5.6). This variation in colour characteristics could be attributed to variation in the triglycerides composition.

Similarly, there was significant ($P<0.05$) increase in lightness value from 82.77 to 83.96, yellowness index from 60.89 to 62.00, chroma from 35.32 to 36.55 and delta E from 0.49 to 2.06 with the increase in the level of flaxseed oil emulsion in the cream used for preparing butter (Table 5.7). But the Hue value decreased significantly ($P<0.05$) from 87.22 to 85.59. Addition of emulsion before ageing of cream significantly ($P<0.05$) changed the colour characteristics to higher extent than its addition after ageing of cream. Therefore, both the stage and level of addition of flaxseed oil emulsion affected the colour characteristics of fortified butter.

The optimized samples i.e. 4.1% OABE and 6.8% EABA had significant change in the colour characteristics as compared to the control sample. Similar studies also reported that increase in the concentration of unsaturated fatty acids or changes in the fatty acid composition of the butter affected the colour characteristics of the butter. The results obtained by Shukla *et al.* (1994) found agreement with the colour characteristics particularly for L values observed in the present study. However, the results for higher 'a' as well as 'b value' are in contradiction to those obtained by them. The increase in L*, a* and b* value could be attributed to the colour characteristics of flaxseed oil. Gonzalez *et al.* (2003) also observed slight change in the colour characteristics of control butter and butter containing higher unsaturated fatty acids made by feeding animals with unsaturated fatty acid reached meals. Thus, it can be concluded that addition of flaxseed oil or emulsion increases the lightness, a* and b* values of the fortified butter and thereby, increases the yellowness index.

Table 5.6 Colour characteristics of butter as affected by level of flaxseed oil and stage of addition

Samples	L*	a*	b*	Hue	Chroma	YI	delta E
Control	82.77±0.15^{d}	1.72±0.15^{c}	35.28±0.46^{d}	87.22±0.23^{a}	35.32±0.47^{d}	60.89±0.77^{b}	0.49±0.14^{d}
2.9% OABA	83.25±0.19^{c}	2.02±0.33^{c}	35.73±0.28^{c}	86.76±0.52^{a}	35.79±0.29^{c}	61.31±0.54^{b}	0.86±0.15^{cd}
4.1% OABA	83.98±0.19^{b}	2.51±0.25^{b}	36.17±0.36^{b}	86.02±0.40^{b}	36.26±0.35^{b}	61.54±0.66^{b}	1.75±0.25^{b}
5.1% OABA	84.38±0.27^{a}	3.87±0.38^{a}	36.84±0.43^{a}	84.01±0.62^{c}	37.05±0.41^{a}	62.38±0.78^{a}	3.16±0.26^{a}
2.9% OAAA	82.80±0.15^{d}	1.74±0.49^{c}	35.31±0.72^{d}	87.17±0.80^{a}	35.36±0.44^{d}	60.92±1.27^{b}	0.65±0.22^{de}
4.1% OAAA	83.41±0.22^{c}	2.04±0.55^{c}	35.41±0.29^{cd}	86.72±0.89a	35.68±0.27^{cd}	61.01±0.61^{b}	1.02±0.16^{c}
5.1% OAAA	83.90±0.29^{b}	2.75±0.50^{b}	36.65±0.37^{bc}	85.61±0.80^{b}	35.97±0.38^{bc}	61.06±0.79^{b}	1.76±0.28^{b}

n=9, results are expressed as Mean± SD, different small letters superscript (a, b, c) within column differ significantly (P<0.05) among samples;

OABA: Oil addition before ageing, OAAA: Oil addition after ageing

Table 5.7 Colour characteristics of butter as affected by level of flaxseed oil-WPC emulsion and stage of addition

Samples	L*	a*	b*	Hue	Chroma	YI	delta E
Control	82.77±0.15^{d}	1.72±0.15^{b}	35.28±0.46^{c}	87.22±0.23^{a}	35.32±0.47^{c}	60.89±0.77^{b}	0.49±0.14^{c}
4.8% EABA	82.80±0.24^{d}	1.78±0.41^{b}	35.52±0.54^{bc}	87.14±0.64^{a}	35.57±0.55^{bc}	61.29±1.03^{ab}	0.72±0.25^{c}
6.8% EABA	83.37±0.16^{c}	1.98±0.41^{b}	35.92±0.50^{b}	86.85±0.64^{a}	35.98±0.51^{b}	61.55±0.88^{ab}	1.07±0.38^{b}
8.6% EABA	83.96±0.30^{a}	2.81±0.39^{a}	36.44±0.40^{a}	85.59±0.62^{b}	36.55±0.39^{a}	62.00±0.76^{a}	2.06±0.38^{a}
4.8% EAAA	82.79±0.14^{d}	1.77±0.46^{b}	35.33±0.36^{c}	87.14±0.74^{a}	35.38±0.36^{c}	60.97±0.65^{b}	0.56±0.22^{c}
6.8% EAAA	82.97±0.24^{d}	1.90±0.44^{b}	35.52±0.36^{bc}	86.94±0.71^{a}	35.58±0.36^{bc}	61.16±0.66^{ab}	0.69±0.19^{c}
8.6% EAAA	83.63±0.28^{b}	2.19±0.63^{b}	35.91±0.27^{b}	86.51±1.00^{a}	35.98±0.28^{b}	61.34±0.54^{ab}	1.35±0.30^{b}

n=9, results are expressed as Mean± SD, different small letters superscript (a, b, c) within column differ significantly ($P<0.05$) among samples; EABA: Emulsion addition before ageing, EAAA: Emulsion addition after ageing

5.4.5 Inference

Fortification of butter with omega-3 fatty acid using flaxseed oil or emulsion significantly affected the sensory, textural and colour characteristics of the butter. The stage and level of addition has significant effect on churning time of butter. The churning time of butter was significantly lower (below 30 min) at 5.1 and 8.6% addition of flaxseed oil and emulsion, respectively. This had in turn resulted in weaker body of the oil fortified butter as well as higher fat loss in the buttermilk. Recovery of the flaxseed oil and emulsion is primary objective of the study without much affecting the sensory quality of the product. In the sensory evaluation of the fortified butter, addition of flaxseed oil or emulsion at rate of 4.1 and 6.8% respectively showed the comparable sensory acceptability scores with the control butter. Further, increment in the level of addition increases the fat loss in the buttermilk as well as drastically affects flavour of the product. Addition of flaxseed oil and emulsion upto 4.1 and 6.8% respectively, showed slight change in the colour characteristics. Thus, the level 4.1 and 6.8% of oil and emulsion, respectively have been selected for further characterization and storage study of the butter.

5.5 Characterization of Control and Omega-3 fatty acids fortified butter

5.5.1 Alpha linolenic acid content in the fortified butter

Gas chromatography-mass spectrometry (GC-MS) provides data regarding the fatty acid composition of the butter and thus useful in detecting foreign fatty acid and its content. This technique was used to determine omega-3 fatty acid content especially alpha-linolenic acid content in the butter. As flaxseed oil is the rich source of alpha-linolenic acid, its addition is expected to enhance the ALA content in the butterfat. The flaxseed oil (4.1%) or flaxseed oil emulsion (6.8%) was added into the cream with the aim of obtaining about 35% RDA level of alpha-linolenic acid (ALA) content in the butter. The ALA content in the control butter, 4.1% OABA, 6.8% EABA and flaxseed oil was found to be 0.872, 3.149, 3.174 and 61.17% respectively using the GC-MS spectra (Table 5.8).

Table 5.8 Alpha-linolenic acid content of control, fortified butter and ghee made thereof

Samples	Alpha-linolenic acid content (%)	
	Fresh sample	Ghee
Control	0.872	0.972
4.1% OABA	3.149	3.71
6.8% EABA	3.174	2.64
Flaxseed oil	61.17	-

The GC-MS chromatograms for control, 4.1% OABA, 6.8% EABA and flaxseed oil shown in Figure 5.8 to Figure 5.11. The GC-MS chromatograms of control butter showed lowest peak of abundance corresponding to ALA in comparison with the flaxseed oil and fortified samples, indicating low ALA content in the control sample. The GC-MS chromatogram of flaxseed oil had highest value for abundance value among all the samples evaluated. This is because, the flaxseed oil is the richest source of omega-3 fatty acid especially ALA i.e. about >55% of flaxseed oil. Therefore, it can be concluded that fortification of butter using flaxseed oil has resulted in significant increase in ALA content in the fortified butter.

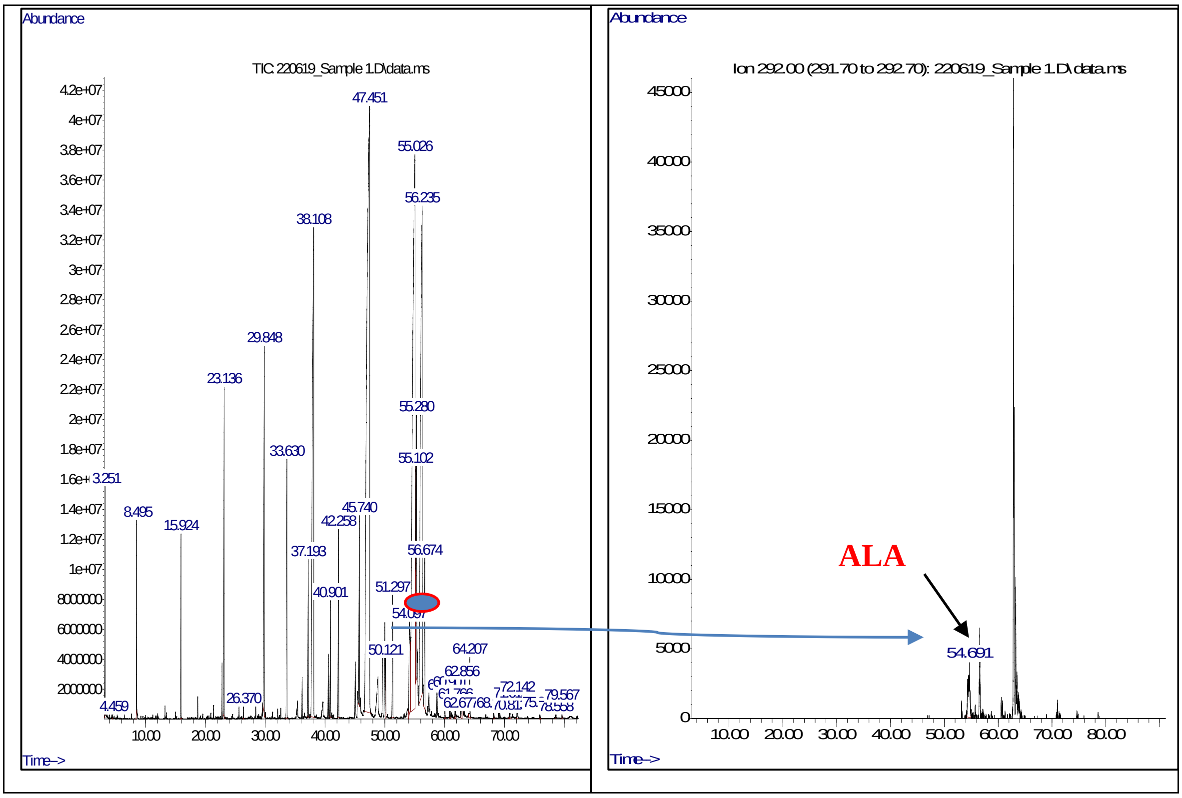


Figure 5.8 GC-MS chromatograms of fatty acid methyl esters of Control butter

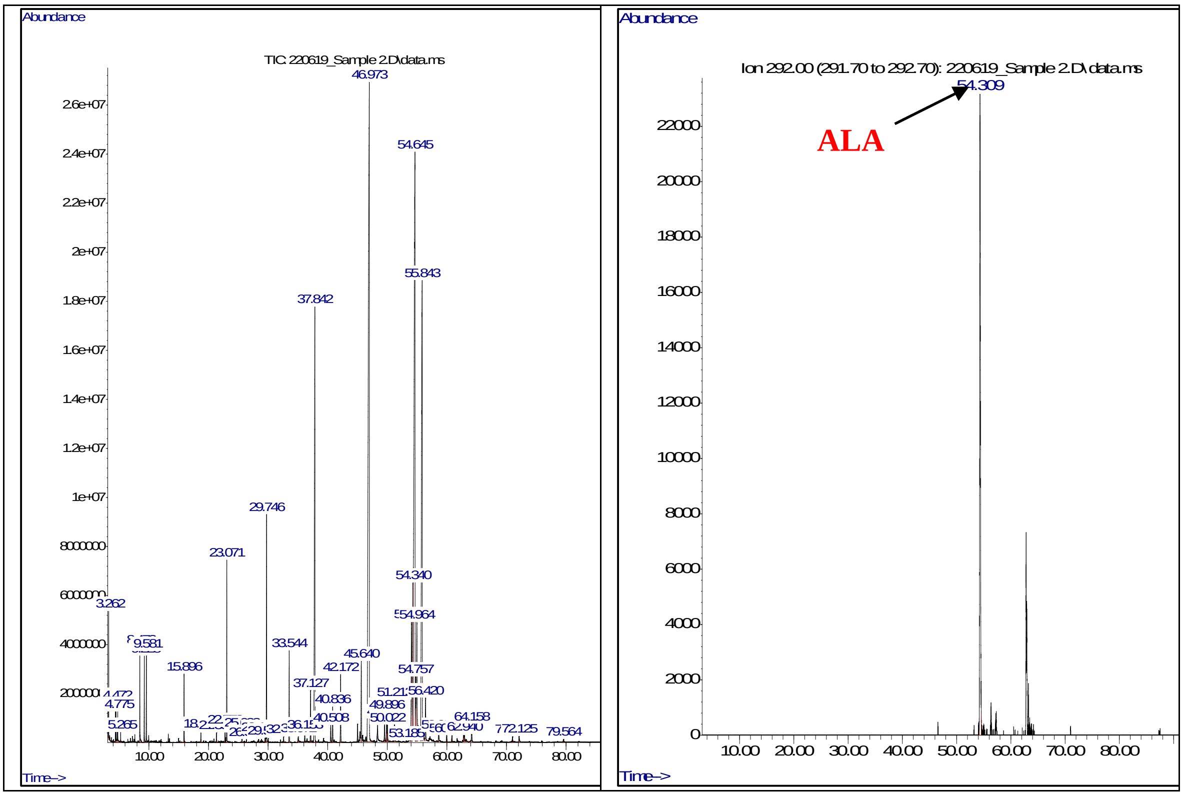


Figure 5.9 GC-MS chromatograms of fatty acid methyl esters of fortified butter using flaxseed oil

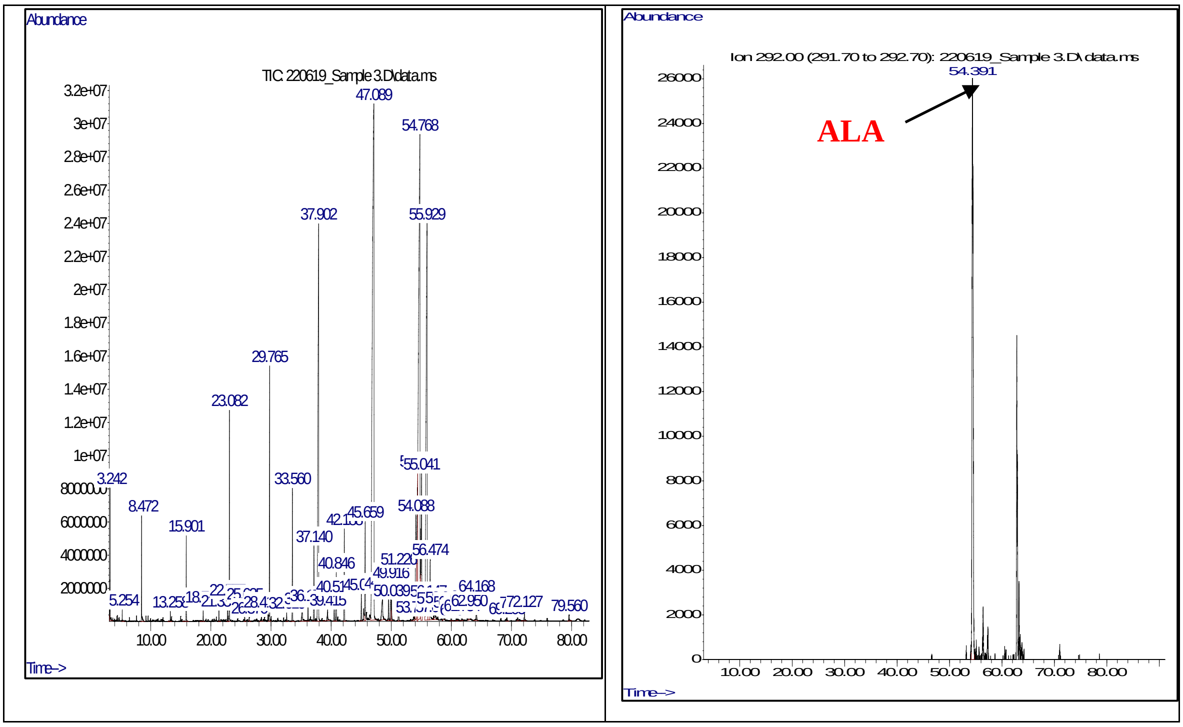


Figure 5.10 GC-MS chromatograms of fatty acid methyl esters of fortified butter using flaxseed oil emulsion

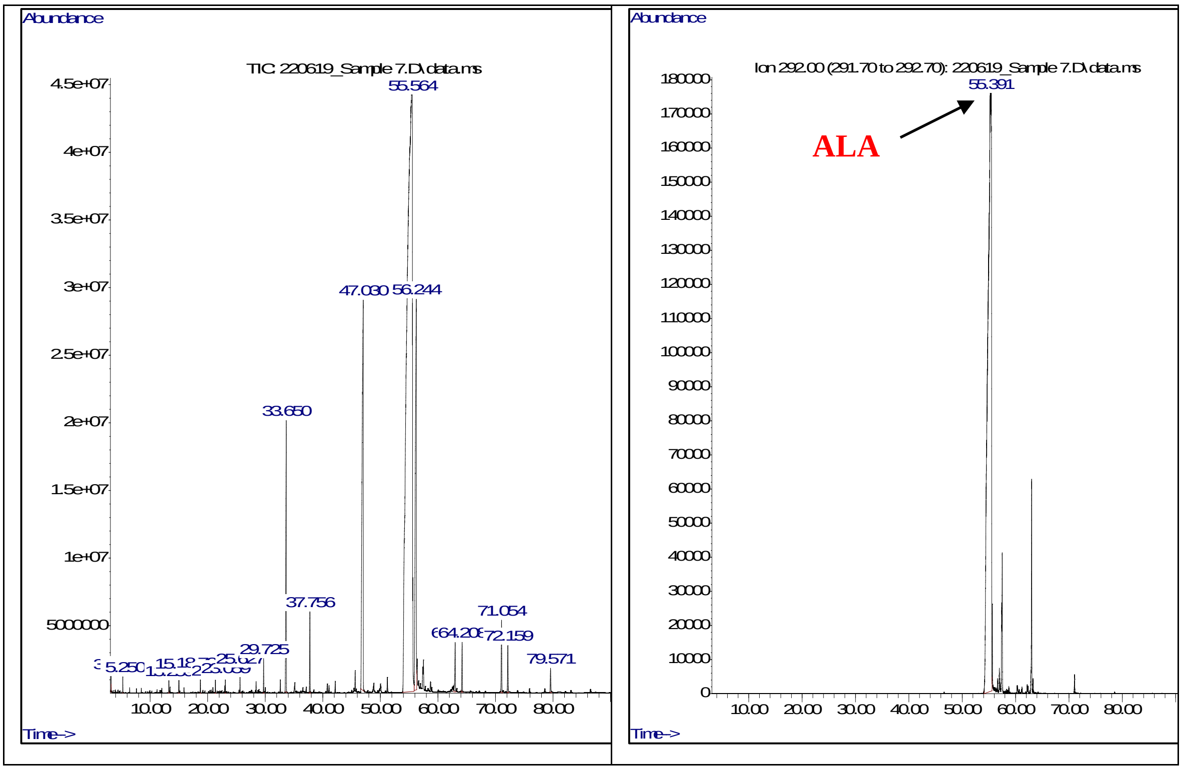


Figure 5.11 GC-MS chromatograms of fatty acid methyl esters flaxseed oil

These results are also in agreement with the findings of Gowda *et al.* (2018) for increase in ALA content in case of omega-3 fatty acid fortified ice-cream using flaxseed oil analysed by GC-MS. Similarly, Veena *et al.* (2017) found increase in ALA content in the *dahi* prepared from omega-3 fatty acid fortified milk analysed by GC-FID. Thus, results of the GC-MS concluded that fortification of butter with omega-3 fatty acids can be possible using flaxseed oil.

5.5.2 Melting and crystallization characteristics of the control and fortified butter using Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) is the technique which measures the difference in the amount of heat required for increasing the temperature of reference and sample is measured as a function of time. Fortification of butter with omega-3 fatty acid changes the fatty acid composition of the butter and thereby expected to change the melting and crystallization behaviour. Hence, Differential scanning calorimetry can be used to confirm the presence of flaxseed oil or emulsion into the fortified butterfat. This method has been used in the past for detection of vegetable oils as a source of adulteration in butter due to the shift in melting and crystallization curves. DSC is preferred over other methods as it is quite faster.

In the present study, fortified samples showed a shift in the onset temperature for melting curve towards lower temperature as compared to the control sample. The data of the melting and crystallization curves of control and fortified samples are shown in the Table 5.9. The melting (endothermic) curve showed three different peaks (P_1, P_2 and P_3) and similarly three peaks were also observed in the crystallization curve (P_4, P_5 and P_6) as shown in the Figure 5.12 and 5.13.

Table 5.9 Thermal data of peaks of the melting and crystallization curves

Temperature	Melting curve								
	Control			Oil			Emulsion		
	P_1	P_2	P_3	P_1	P_2	P_3	P_1	P_2	P_3
Onset (°C)*	9.30	14.06	24.85	6.86	11.98	24.03	7.63	11.23	24.72
Peak (°C)*	11.05	17.68	34.37	8.89	16.36	33.88	9.64	16.77	34.46
Endset (°C)*	13.02	20.23	37.12	10.51	19.68	3681	11.33	20.27	36.94
Temperature	Crystallization curve								
	Control			Oil			Emulsion		
	P_4	P_5	P_6	P_4	P_5	P_6	P_4	P_5	P_6
Onset (°C)*	19.55	15.13	-9.78	18.65	14.49	-10.93	18.95	14.85	-10.18
Peak (°C)*	18.13	11.82	-11.47	17.63	11.14	-12.28	17.88	11.06	-11.98
Endset (°C)*	16.97	4.84	-16.12	16.47	2.43	-16.97	16.79	2.59	-16.78

* Values are mean of three trials

P1 -First melting peak P4 -First crystallization peak

P2-Second melting peak P5-Second crystallization peak

P3-Third melting peak P6-Third crystallization peak

In melting curve, shift in the onset, peak and endset temperatures of all the three peaks was observed towards the lower temperatures in fortified butter samples compared to the control butter. Significant shift in the temperatures of the peaks P_1 and P_2 was observed in the melting curve. This is due to the fact that, the fortification of butter by flaxseed oil had increased the proportion of low meting and medium melting fat, thereby initiating the melting of fat earlier as compared to control butter. This further confirms the recovery of flaxseed oil or emulsion into the butterfat. The third peak P_3 indicating the high melting fat also showed slight shift towards lower temperature. It indicates slight decline in the high melting fat i.e. solid fat content in the butter. The results obtained by pNMR also correlate with the DSC results, as solid fat content was decreased in the fortified butter than control.

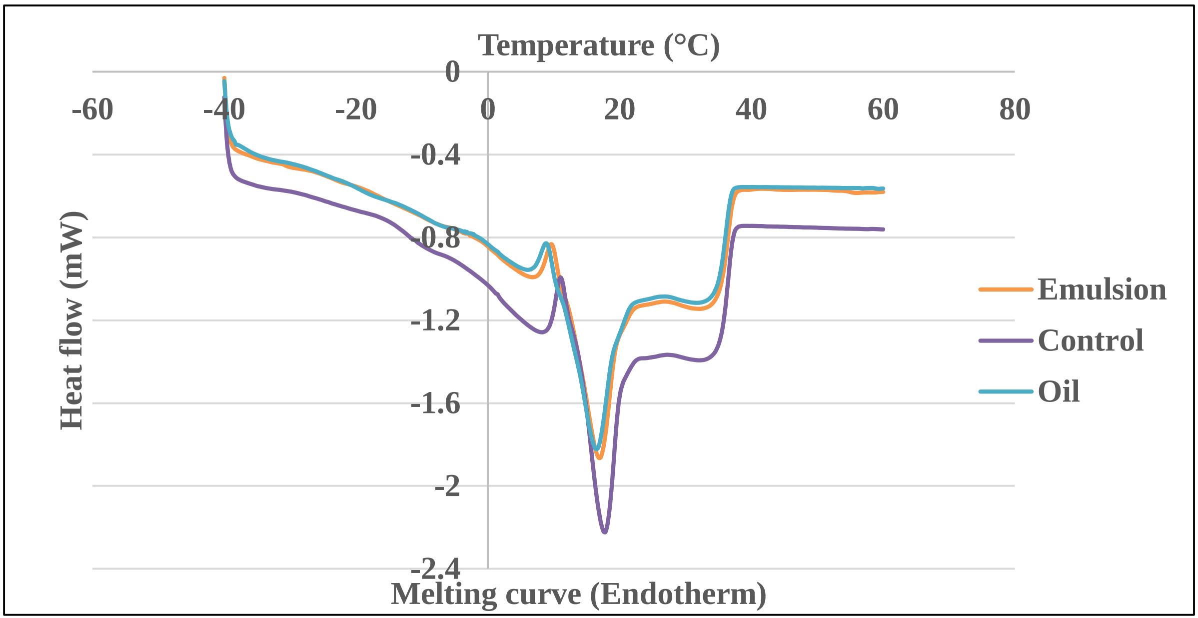


Figure 5.12 Melting curves for control and fortified samples

Similarly, in crystallization curve shift in the all three peaks (P_4, P_5 and P_6) was towards lower temperature. This is because of addition of flaxseed oil or emulsion increases the low meting and medium melting fat in the fortified butter than the control. These low and medium melting fats solidify at lower temperature than the high melting fats. Similar results were reported by Tomaszewska-Gras, (2016) during determination of butter adulteration with palm oil. He observed decrease in the temperature related to the first peak by 2 °C and also found increase in the low melting fraction area and peak height. Similarly, Aktaş and Kaya, (2001) found elevation in the 1st and 2nd peaks in the crystallization curve with the addition of margarine and beef body fat as it contains the vegetable oil which mainly contains liquid fat.

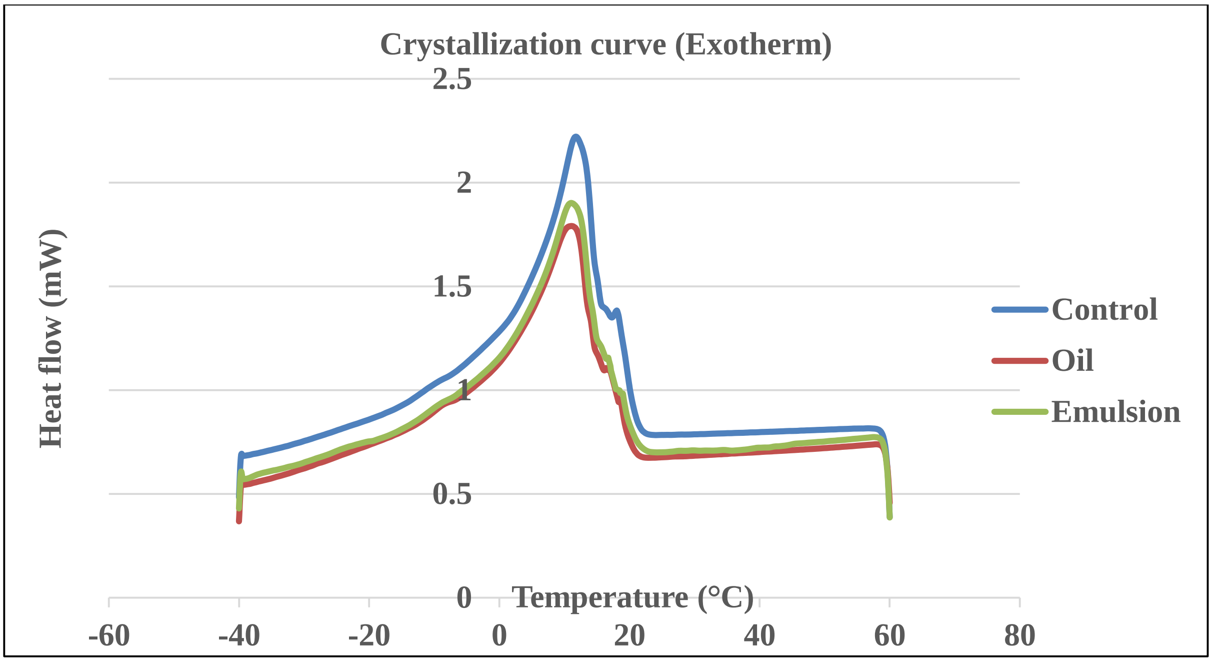


Figure 5.13 Crystallization curves for control and fortified samples

5.5.3 Solid fat content (SFC) in control and fortified butter

Fortification of butter with omega-3 fatty acids using flaxseed oil or emulsion increases the liquid fat content in the fortified butter than control butter. pNMR provides the information regarding the solid fat content (SFC) in the butterfat at a particular temperature. Therefore, it can be used to detect the presence of flaxseed oil in the fortified butter. This method is also used for determination of adulteration with the vegetable fat. In the present study, the temperature was varied from 5 to 40°C with 5°C rise in the temperature. The fortified samples i.e. 4.1% OABA and 6.8% EABA had 50.995 and 51.305% solid fat content respectively at 5°C, which is significantly ($P<0.05$) lower than the control butter (56.63%). The solid fat content decreased significantly with the increase in the temperature up to 30°C, and the values were lower for fortified samples than control at all the temperatures. The decreased SFC content with the rising temperature is shown in the Figure 5.14. This revealed that, addition of flaxseed oil or emulsion significantly reduces the SFC at lower temperature because of partial replacement of butterfat with the vegetable oil and thus enhancement of liquid fat. This decrease in liquid fat in case of fortified butter samples is attributed to the higher unsaturated fat content of flaxseed oil. Similar results were reported by Avramis *et al.* (2003) and observed the decrease in the SFC of the butter with supplementation of cow with the fish meal due to the increase in the unsaturated fatty acid into the butter. Also, Rousseau *et al.* (2003) reported lower value of critical SFC for margarine than the butter that indicates solid fat content in the margarine was reduced due to the presence of vegetable oil. Thus, decrease in the SFC in fortified samples than control butter indicates the recovery of the flaxseed oil or emulsion in the fortified butter.

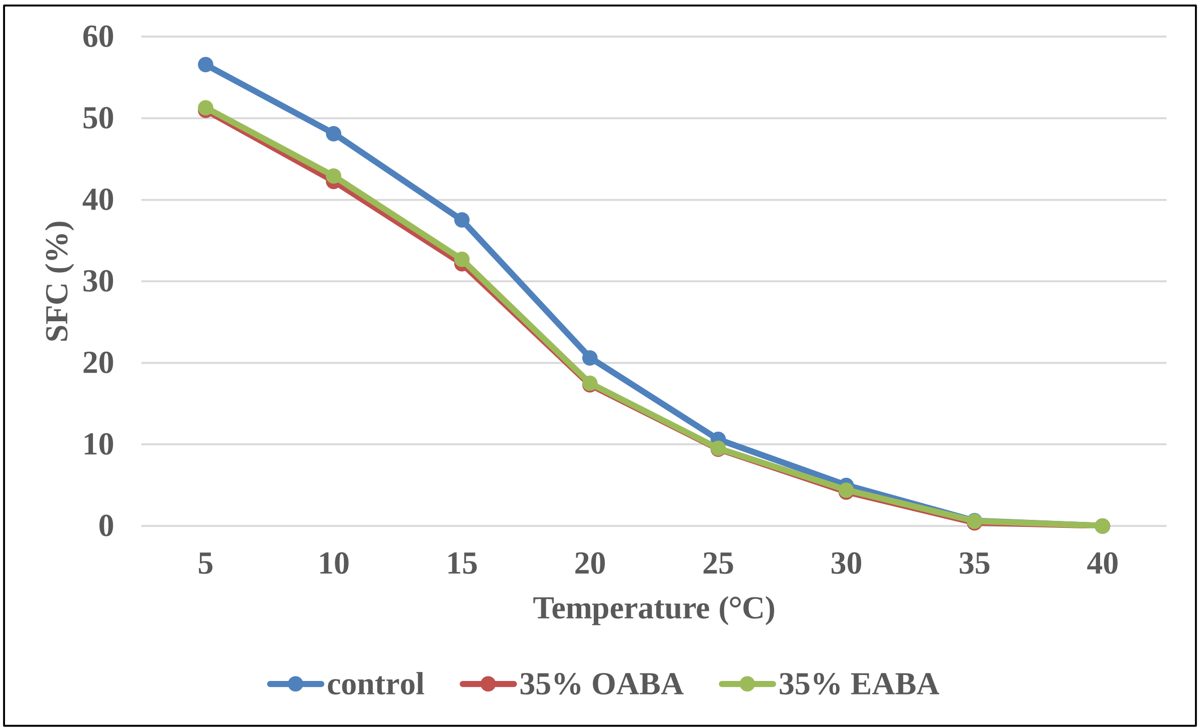


Figure 5.14 Solid fat content (SFC) in the control and fortified samples

5.5.4 Induction period of control and fortified butter

Induction period is the time up to which the secondary reaction products are not detected. Thus, it gives a basic idea about the oxidative stability of the product. In the present study, the induction period for the extracted fat from butter (without heat treatment) was determined. The control butter had higher induction period (4.35 h) than the fortified samples i.e. 4.1% OABA (2.36 h) and 6.8% EABA (2.56 h). Comparatively flaxseed oil emulsion fortification showed higher induction period than direct oil. This may be due the binding property of the residual WPC with the oil. The induction period of the control and fortified samples is shown in the Figure 5.15 to 5.17.

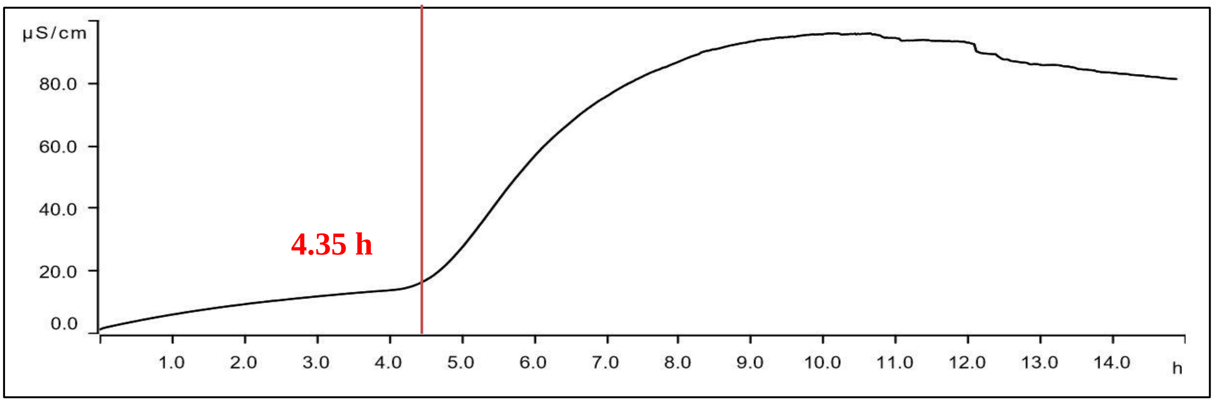


Figure 5.15 Induction period (using Rancimat) of control butter

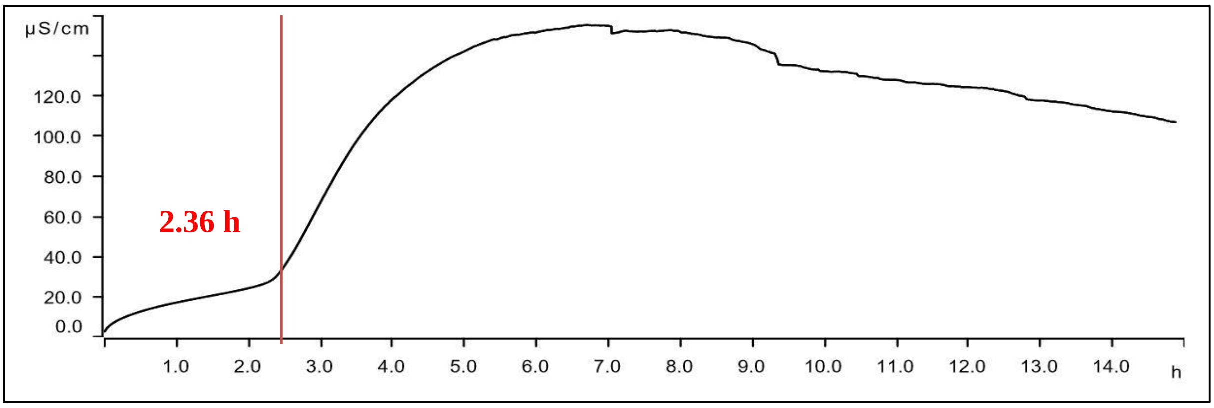


Figure 5.16 Induction period (using Rancimat) of butter prepared by adding flaxseed oil

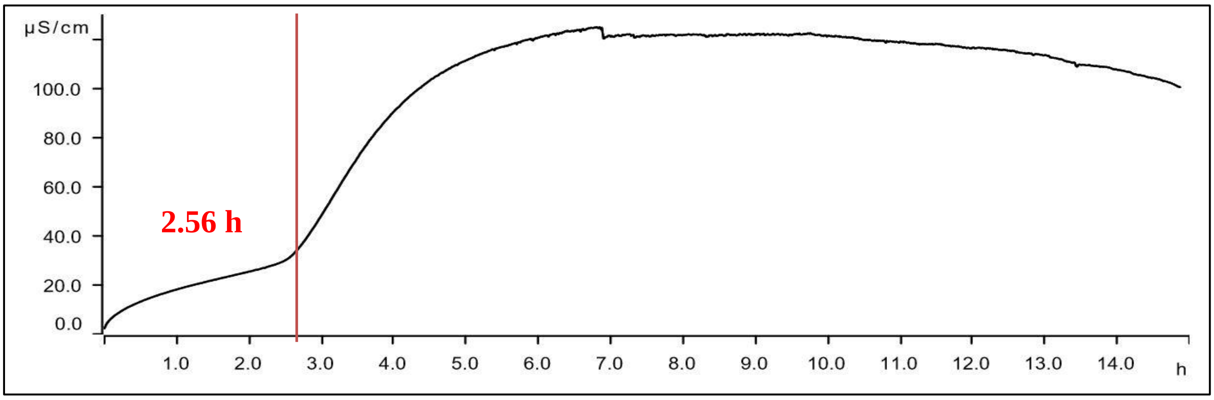


Figure 5.17 Induction period (using Rancimat) of butter prepared by adding flaxseed oil-WPC emulsion

Decline in the induction period for fortified butter samples was due to the increase in unsaturated fatty acids which are more prone to the oxidation through fortification. This may result in lower shelf life of fortified butter as compared to control. This may further help in selecting suitable packaging material with higher barrier properties for oxygen so that oxidation can be prevented in case of fortified samples. Nadeem *et al.* (2013) observed the increase in the induction period (8.91 h) of butter containing *Moringa oleifera* leaf extract than the control butter (6.35 h) owing to the antioxidant potential of the *Moringa oleifera* leaf extract. Similarly, Gramza-Michalowska *et al.* (2007) found that butter added with green tea extract had higher induction period than rosemary extract. This is due to the higher antioxidant potential of green tea extract than rosemary extract.

5.5.5 Water activity of the control and fortified butter

Water activity plays an important role in microbial growth as well as in chemical reaction and indirectly has a significant role on the shelf life the product. In the present investigation, the control (0.9449) butter had higher water activity than fortified samples i.e. 4.1% OABA (0.9074) and 6.8% EABA (0.9126). Normally the water activity of unsalted butter at 24°C is 0.96, which is comparable with the control butter. The lower water activity of fortified samples may help preventing microbial growth upon storage.

5.5.6 Proximate composition of optimized and control butter

According to FSSAI (2011) standard butter should contain minimum 80% fat, maximum 16% moisture, maximum 3% salt and SNF to the extent of maximum 2%. Depending upon the acceptable organoleptic qualities, with maximum addition possible of flaxseed oil and emulsion; and minimum fat loss in the buttermilk, two samples were optimized i.e. sample prepared with addition of 4.1 % flaxseed oil before ageing and 6.8 % emulsion before ageing. The proximate composition of optimized and control butter samples are given in the Table 5.10.

Table 5.10 Proximate composition of control and fortified samples

Constituent (%)	Control	4.1% OABA	6.8% EABA
Fat	87.47±0.395	87.37±0.306	85.31±0.437
Moisture	11.94±0.344	12.15±0.317	14.06±0.529
Protein	0.007±0.0008	0.005±0.0009	0.014±0.005
Ash	0.00055±0.00015	0.0005±0.00015	0.00055±0.00015
Carbohydrate	0.58±0.07	0.47±0.12	0.61±0.124
Curd	0.59±0.072	0.48±0.124	0.63±0.124

The results are expressed as means ± SD of three replicated

Both the optimized and control samples fulfilled the legal requirement of fat, moisture and SNF. The sample; 6.8% EABA had highest moisture content and thus it is the softer than control as well as 4.1% OABA. It also contains highest amount of curd content. It might be due to the residual WPC content in the sample.

5.6 Storage study of the control and fortified butter

Fortification of butter with omega-3 fatty acids using flaxseed oil or emulsion resulted in the elevated level of unsaturated fatty acids especially alpha-linolenic acid in fortified butter. This affects the oxidative stability of butter. Therefore, in the present study, control and fortified samples were packed PP cups and stored at two different temperatures i.e. 5 and -18 °C. The samples were analysed for 90 days with the interval of 15 days. During the storage, the samples were analysed for physico-chemical properties, oxidative stability, textural characteristics, sensory characteristics and microbial quality. The effect of storage period on all these properties is discussed below.

5.6.1 Physico-chemical changes in fortified butter during storage

5.6.1.1 Acidity of control and fortified butter during storage

Acidity plays an important role in the shelf life of product. Storage at higher temperature leads to increase in the acidity. In the present study, control butter stored at 5°C showed significantly ($P<0.05$) higher acidity than the fortified samples (4.1% OABA and 6.8% EABA). The acidity of the samples stored at 5°C showed significant ($P<0.05$) increase in the acidity throughout the storage period (Figure 5.18).

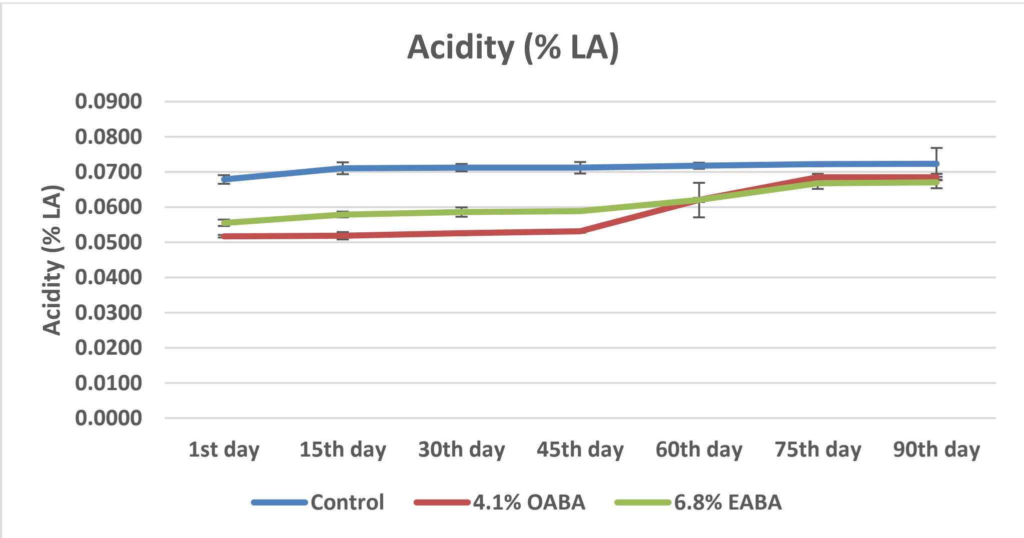


Figure 5.18 Acidity of control and fortified butter during storage at 5°C

In case of samples stored at -18°C, there was slight but statistically significant ($P<0.05$) increase in the acidity of the butter throughout the storage period, in all the samples as shown in the Figure 5.19. The control sample had higher acidity than the fortified butter. Similar results were also reported by Kulkarni and Rama Murthy (1985) for the increase in acidity of the butter during entire storage period. They reported that during first 6 months of storage, acidity of fat increased from 1.2 to 2.3% and then remained constant. The plasma acidity of butter was not increased drastically but uniformly during entire storage period from 2 to 2.6%.

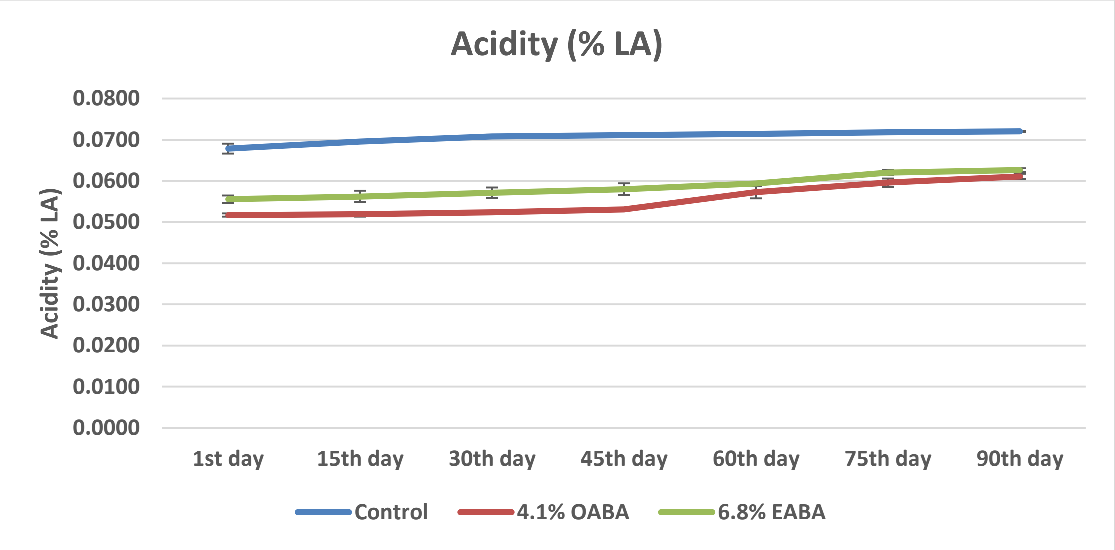


Figure 5.19 Acidity of control and fortified butter during storage at -18°C

5.6.1.2 pH of control and fortified butter during storage

The pH is one of the contributing parameters indicating the storage life of the product as most of the microbial growth and chemical changes occurs at a specific pH. The pH of the samples stored at 5°C, decreased significantly ($P<0.05$) with the storage period as shown in the Figure 5.20. This correlates well with the acidity results mentioned in previous section. The butter sample fortified with oil showed highest, while the control butter depicted lowest pH among the three samples when stored at 5°C. Similar kind of trend was found in butter stored at -18 °C. There was significant ($P<0.05$) decreased in the pH of the butter with the increase in period of storage (Figure 5.21). But storage at -18°C showed lesser degree of change in the pH than at 5°C.

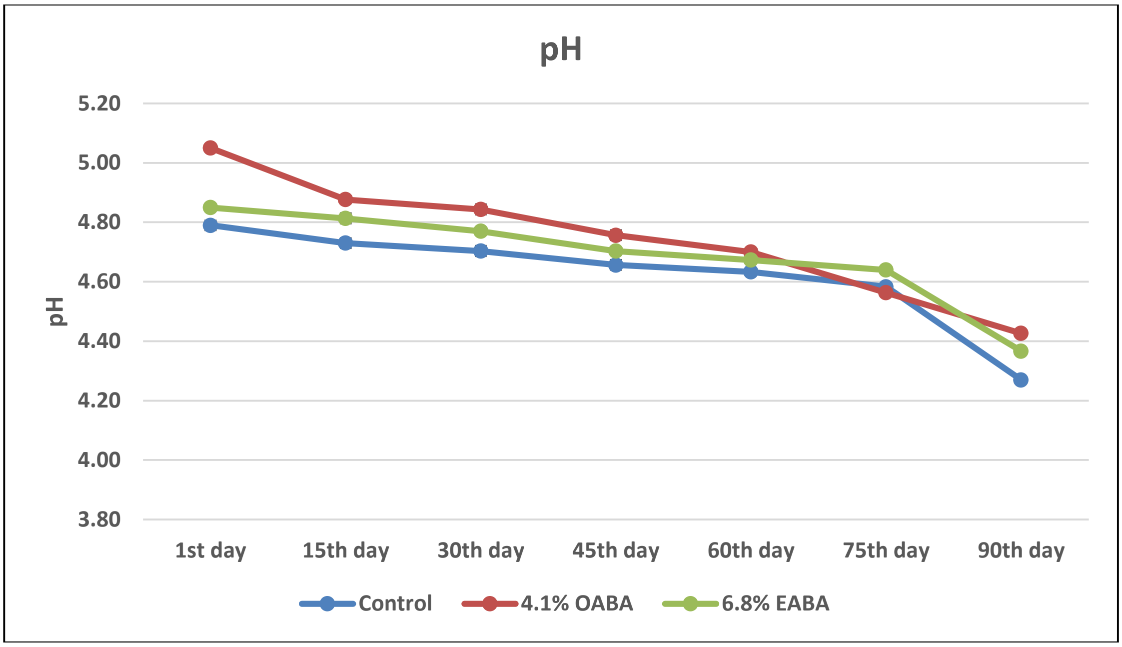


Figure 5.20 pH of control and fortified butter during storage at 5°C

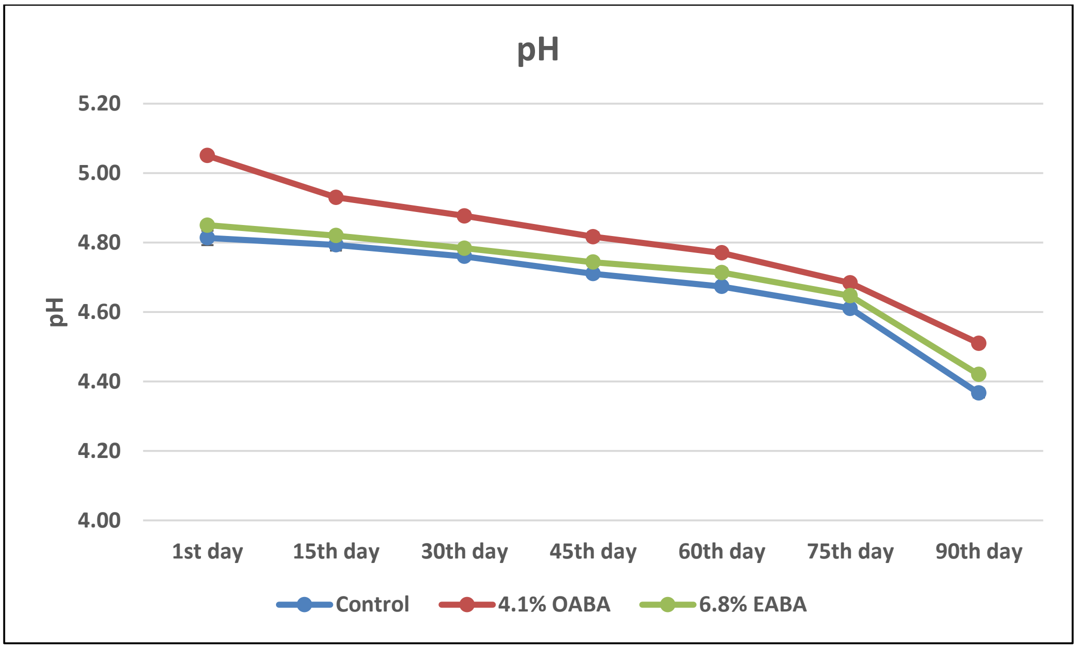


Figure 5.21 pH of control and fortified butter during storage at -18°C

5.6.2 Oxidative stability of control and fortified butter during storage

Butter is the fat rich dairy product and therefore the most common problem associated with the butter is oxidation. Fortification of butter with omega-3 fatty acid using flaxseed oil or emulsion makes it more prone to the oxidation because of elevated level of unsaturated fatty acid. Thus, oxidative stability of the fortified butter in comparison of the control sample is of prime importance in order to predict the shelf life of the product. In oxidative stability three different parameters (Peroxide value, Free fatty acids and TBA value) were analysed for 90 days with the interval of 15 days.

5.6.2.1 Peroxide value (PV) of control and fortified butter during storage

Peroxide value gives an idea about the initiation of the rancidity in the unsaturated fatty acid and it is the measure of autoxidation i.e. oxidative rancidity. Autoxidation is the free radical reaction and involve oxygen for the deteriorative changes in the butter i.e. off odours and off flavour. Peroxides are the intermediate compounds formed during the autoxidation. In the present study, the butter stored at 5°C showed increase in the peroxide value on 15th day of storage and then increased significantly ($P<0.05$) throughout the storage period as shown in the Figure 5.22. Among the three samples, control butter showed lowest peroxide value. The higher value for fortified samples is due to the presence of more unsaturated fatty acids in flaxseed oil. The butter prepared by adding flaxseed oil (4.1% OABA) showed higher peroxide value than the control and sample prepared by adding emulsion (6.8% EABA). This is because of the fact that in emulsion containing samples, emulsion plays some protective role towards oxidation due to the presence of WPC than direct flaxseed oil.

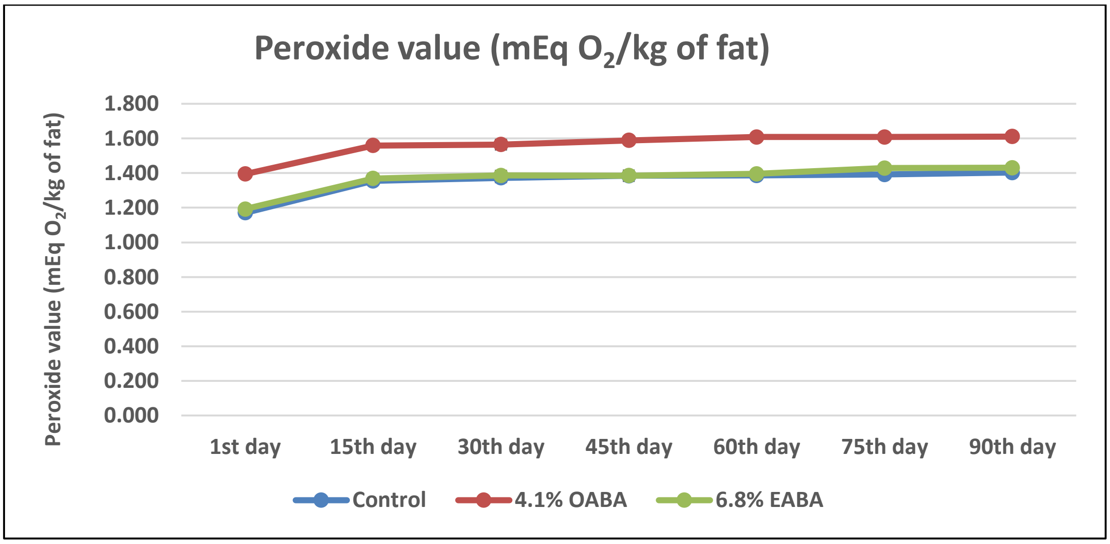


Figure 5.22 Peroxide value of control and fortified butter during storage at 5°C

In case of fortified butter stored at -18°C, the rapid and significant ($P<0.05$) increase in the peroxide value was found till the 30th day of storage and after that slight increase throughout the storage period in all the butter samples as shown in the Figure 5.23. The control butter showed significantly ($P<0.05$) lower peroxide values than fortified butter and the sample 6.8% EABA showed significantly ($P<0.05$) lower values than 4.1% OABA. Results of the Devdhara *et al.* (1991) are in agreement with the findings of the present study. They found increased peroxide value with the period of storage and storage at higher temperature (10°C) showed significant higher value of peroxide value than lower temperature storage (4°C) in case of butter. Thus, it can be concluded that temperature and period of storage significantly affects the peroxide value of butter i.e. oxidative stability.

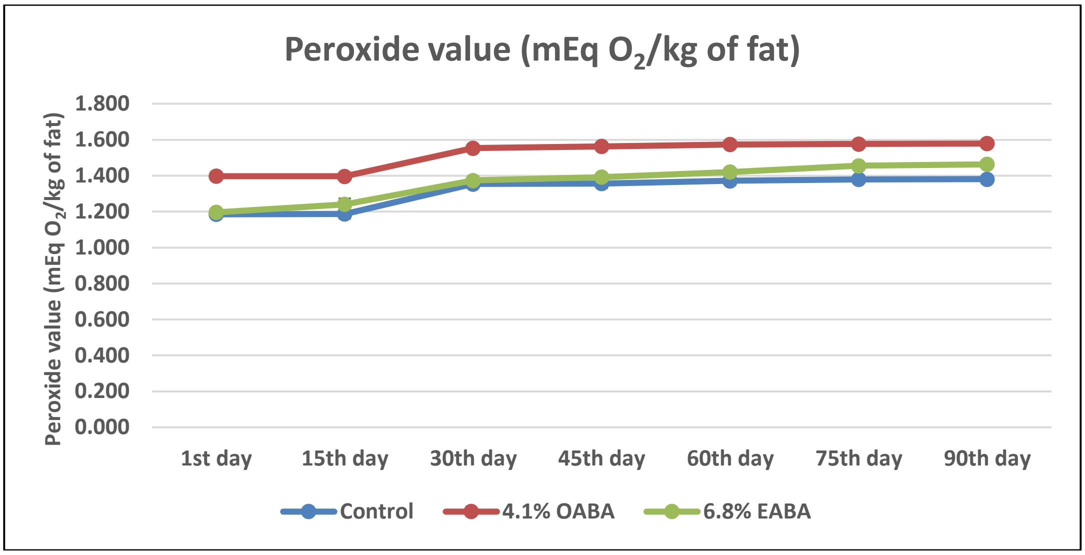


Figure 5.23 Peroxide value of control and fortified butter during storage at -18°C

5.6.2.2 Free fatty acids (FFA) of control and fortified butter during storage

Free fatty acid content is the indicator of hydrolytic rancidity. The free fatty acids are formed in the products due to the action of lipase enzyme. The lipase enzyme acts on the fat present in the product, bring down the lipolysis which finally results in off flavour. In the present study, the samples stored at 5°C showed significant ($P<0.05$) increase in the free fatty acid (FFA) content during the storage period as shown in the Figure 5.24.

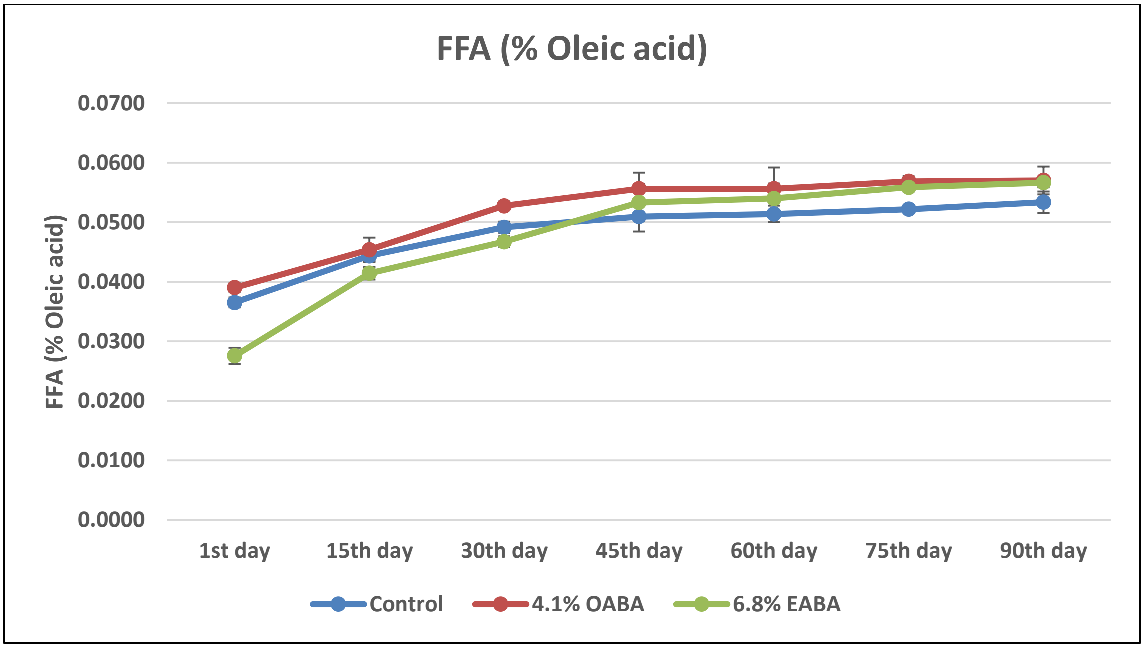


Figure 5.24 Free fatty acid (FFA) content of control and fortified butter during storage at 5°C

The free fatty acid content of the 4.1% OABA sample was significantly (P<0.05) higher than control and 6.8% EABA over the entire storage period. Initially FFA content of the 6.8% EABA was significantly lower than the control sample upto 45th day of storage and then increased throughout the period of storage but statistically there was no significant (p>0.05) difference in the FFA content of control and 6.8% EABA.

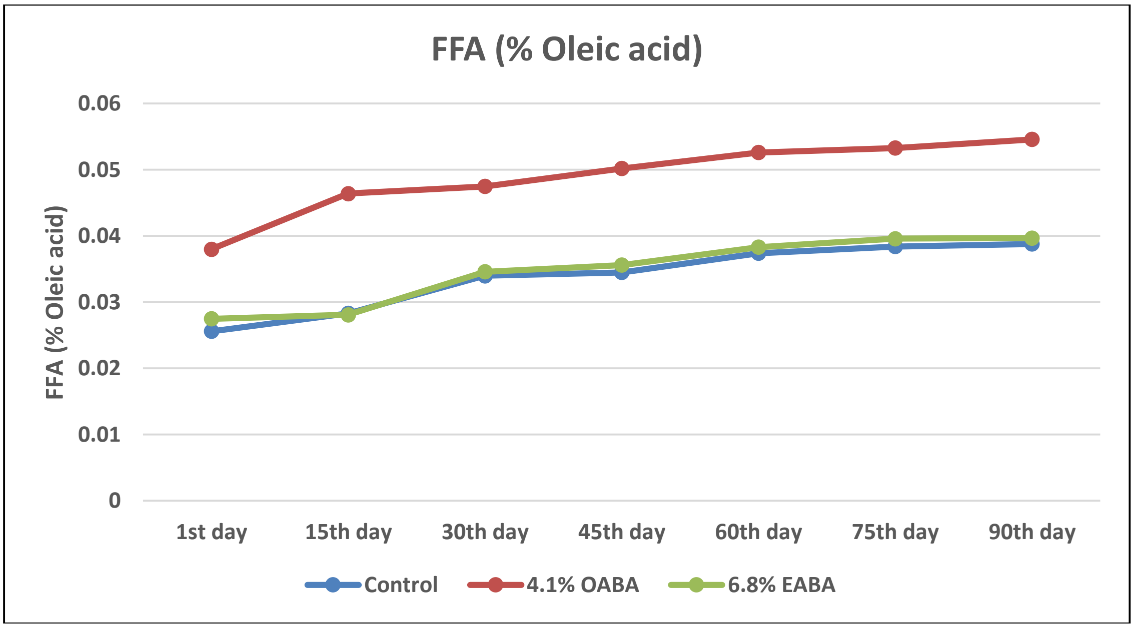


Figure 5.25 Free fatty acid (FFA) content of control and fortified butter during storage at -18°C

Similar trend was found for samples stored at -18°C, the free fatty acid content of the samples increased significantly (P<0.05) over the storage period (Figure 5.25). The sample containing oil (4.1% OABA) had significantly (P<0.05) higher FFA content than control as well as emulsion containing butter sample (6.8% EABA) and increased over the period of storage. There was no significant difference in the FFA content of control and emulsion containing butter sample during storage. This is because of the protective action of the emulsion over direct oil. In the present study the fortification using flaxseed oil elevated unsaturated fatty acid content of the butter and thus FFA content was increased throughout the storage period at both the temperatures. The results of the present study are similar to those reported by Devdhara *et al.* (1991). They found increased FFA content with the period of storage and at higher temperature (10°C) than lower temperature storage (4°C). Thus, concluded that temperature and period of storage significantly affects the FFA content of butter.

5.6.2.3 Thiobarbituric acid value (TBA Value) of control and fortified butter during storage

TBA value is also an indicator of oxidative rancidity. The secondary oxidation of polyunsaturated fatty acids resulted into the formation of malonaldehyde (MDA). The MDA reacts with the two molecules of TBA reagent forming a red coloured compound which can be quantified using spectrophotometer at 532 nm wavelength. In the present study, the TBA value of the samples stored at 5°C increased significantly (P<0.05) over a period of storage as shown in Figure 5.26. The sample 4.1% OABA showed significantly (P<0.05) higher and control significantly (P<0.05) lower TBA value among the samples. Similarly, TBA value of the samples stored at -18°C increased significantly (P<0.05) throughout the storage period (Figure 5.27). The TBA value of the sample having flaxseed oil was significantly (P<0.05) higher and control sample had significantly (P<0.05) lower value among the samples and it increased throughout the storage period.

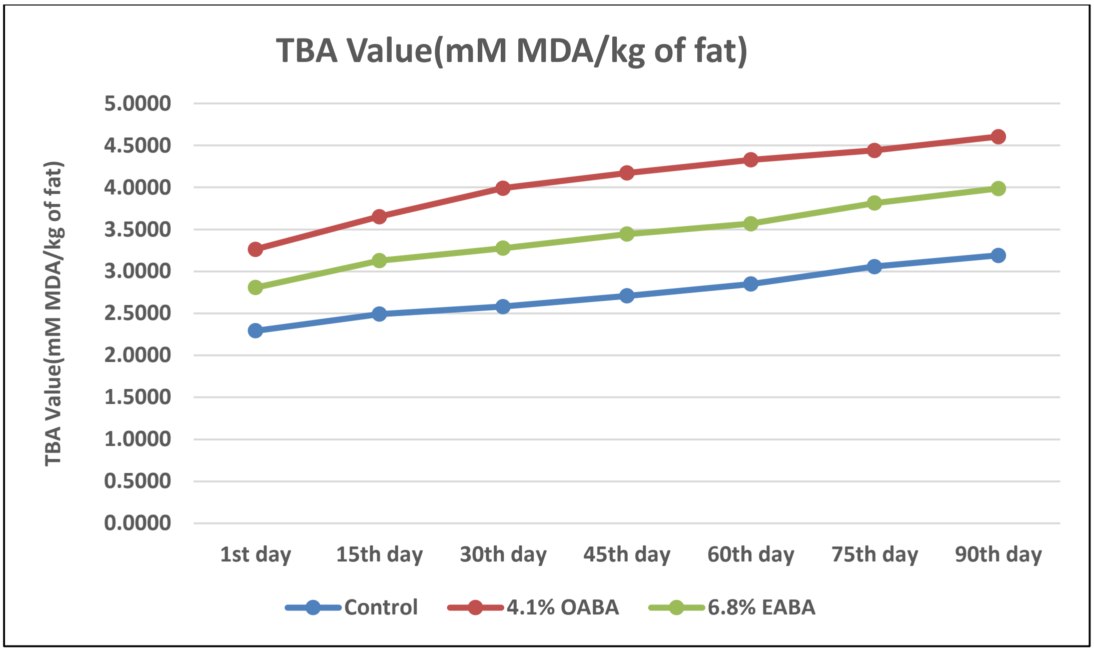


Figure 5.26 TBA value of control and fortified butter during storage at 5°C

At both the temperature increase in the TBA value was observed but at -18°C storage temperature the extent of increase was lesser than 5°C. In the present study, increased level of unsaturated fatty acids leads to increase in the TBA value throughout the storage at both the temperatures.

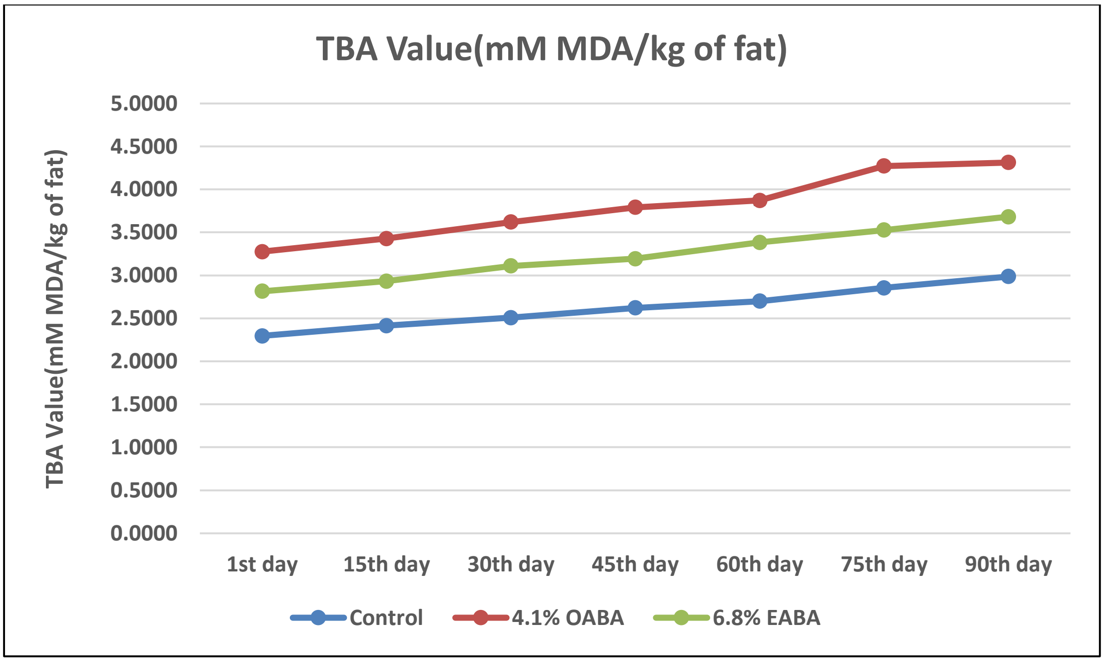


Figure 5.27 TBA value of control and fortified butter during storage at -18°C

5.6.3 Textural properties of control and fortified butter during storage

Texture is one of the important parameters from consumers' point of view. In case of butter, spreadability is most important factor. Texture of the product can be analysed by various method such as sensory analysis, instrumental method etc. Two tests were carried out to determine the texture of butter i.e. spreadability test and creep test.

In spreadability test, the firmness and work of shear of the control butter stored at 5°C increased significantly ($P<0.05$) throughout the storage period. The firmness and work of shear of the sample prepared from addition of flaxseed oil (4.1% OABA) showed significant ($P<0.05$) increase during the storage period. Similarly, sample made using flaxseed oil-WPC emulsion (6.8% EABA) showed significant ($P<0.05$) increment in the firmness and work of shear over a period of storage. Among the samples stored at 5°C, control butter showed significantly higher, while the sample prepared using emulsion lower firmness and work of shear throughout the storage period (Table 5.11).

Table 5.11 Effect of storage time on firmness (N) and work of shear (N.sec) of butter fortified with flaxseed oil and flaxseed oil-WPC emulsion when stored at 5 °C

Period of storage (Days)	Control		4.1% OABA		6.8% EABA	
	Firmness (N)	Work of shear (N.sec)	Firmness (N)	Work of shear (N.sec)	Firmness (N)	Work of shear (N.sec)
1	7.316±0.380^{eA}	7.730±0.272^{dA}	5.764±0.197^{eB}	6.139±0.168^{dB}	4.501±0.208^{eC}	4.676±0.224^{dC}
15	7.463±0.415^{eA}	7.887±0.241^{dA}	5.911±0.172^{eB}	6.300±0.176^{dB}	4.718±0.279^{eC}	4.834±0.247^{dC}
30	7.581±0.382^{dA}	7.997±0.228^{cA}	6.068±0.112^{dB}	6.400±0.203^{cB}	4.816±0.266^{dC}	4.899±0.166^{cC}
45	7.653±0.313^{cdA}	8.057±0.262^{cA}	6.130±0.082^{cdB}	6.525±0.214^{cB}	4.833±0.394^{cdC}	5.081±0.313^{cC}
60	7.764±0.297^{bcA}	8.202±0.221^{bA}	6.195±0.136^{bcB}	6.590±0.263^{bB}	4.924±0.459^{bcC}	5.222±0.306^{bC}
75	7.892±0.346^{abA}	8.471±0.225^{abA}	6.247±0.215^{abB}	6.718±0.259^{abB}	5.074±0.304^{abC}	5.435±0.307^{abC}
90	7.952±0.284^{aA}	8.506±0.215^{aA}	6.516±0.288^{aB}	6.930±0.210^{aB}	5.209±0.181^{aC}	5.589±0.298^{aC}

n=5, results are expressed as Mean± SD, with different small letters superscript (a, b, c) within row and capital letters (A, B, C) within column differ significantly P<0.05 among the samples; OABA: Oil addition before ageing, EABA: Emulsion addition before ageing

Table 5.12 Effect of storage time on Stickiness (N) and work of adhesion (N.sec) of butter fortified with flaxseed oil and flaxseed oil-WPC emulsion when stored at 5 °C

Period of storage (Days)	Control		4.1% OABA		6.8% EABA	
	Stickiness (N)	Work of adhesion (N.sec)	Stickiness (N)	Work of adhesion (N.sec)	Stickiness (N)	Work of adhesion (N.sec)
1	-6.512±0.394^{aC}	-1.945±0.094^{aC}	-5.300±0.217^{aB}	-1.649±0.016^{aB}	-4.273±0.253^{aA}	-1.282±0.035^{aA}
15	-6.699±0.457^{abC}	-2.101±0.178^{bC}	-5.424±0.313^{abB}	-1.676±0.016^{bB}	-4.380±0.279^{abA}	-1.297±0.038^{bA}
30	-6.837±0.487^{bC}	-2.162±0.096^{cC}	-5.424±0.313^{bB}	-1.693±0.013^{cB}	-4.393±0.180^{bA}	-1.318±0.042^{cA}
45	-6.934±0.478^{bcC}	-2.169±0.088^{cC}	-5.569±0.276^{bcB}	-1.701±0.015^{cB}	-4.505±0.224^{bcA}	-1.329±0.039^{cA}
60	-7.092±0.464^{cC}	-2.198±0.073^{dC}	-5.707±0.199^{cB}	-1.743±0.032^{dB}	-4.663±0.203^{cA}	-1.341±0.032^{dA}
75	-7.404±0.396^{cC}	-2.245±0.060^{deC}	-5.826±0.147^{cB}	-1.752±0.050^{deB}	-4.791±0.162^{cA}	-1.358±0.028^{deA}
90	-7.383±0.401^{cC}	-2.285±0.046^{eC}	-5.869±0.36^{cB}	-1.776±0.030^{eB}	-4.855±0.269^{cA}	-1.393±0.041^{eA}

n=5, results are expressed as Mean± SD, with different small letters superscript (a, b, c) within row and capital letters (A, B, C) within column differ significantly P<0.05 among the samples; OABA: Oil addition before ageing, EABA: Emulsion addition before ageing

The stickiness and work of adhesion of control butter increased significantly ($P<0.05$) over the storage period (Table 5.12). The butter sample prepared by fortifying with flaxseed oil (4.1% OABA) showed a significant ($P<0.05$) increase in the stickiness and work of adhesion during storage. Similarly, a significant ($P<0.05$) increase in the stickiness and work of adhesion was observed throughout the storage period. Control sample had highest and 6.8% EABA had lowest value of stickiness and work of adhesion.

In case of butter samples stored at -18°C, there was significant increase in the textural properties such firmness, work of shear, stickiness and work of adhesion throughout the storage period. Control butter had higher and 6.8% EABA had lower values of textural properties. Among the samples, all the samples are significantly different from each other in every property as shown in the Table 5.13 and 5.14. But storage at -18°C showed higher values of textural properties than 5 °C storage. Thus, it is concluded that during storage at sub zero temperature, there is probably more solid fat in the butter samples which leads to increase in firmness, work of shear, stickiness and work of adhesion.

Texture of the butter was also analysed by creep test. In creep test, constant stress is applied for particular period of time and recovery of the product was analysed. Creep test gives an idea regarding the stacking strength of the butter. Creep test provides information of yield stress and retardation time. Further, yield stress is the minimum stress required to start flow behaviour and retardation time is the time required to recover 63% of the total deformation.

Table 5.13 Effect of storage time on firmness (N) and work of shear (N.sec) of butter fortified with flaxseed oil and flaxseed oil-WPC emulsion when stored at -18 °C

Period of storage (Days)	Control		4.1% OABA		6.8% EABA	
	Firmness (N)	Work of shear (N.sec)	Firmness (N)	Work of shear (N.sec)	Firmness (N)	Work of shear (N.sec)
1	7.527±0.422^{dA}	8.096±0.480^{fA}	6.016±0.225^{dB}	6.203±0.116^{fB}	4.905±0.166^{dC}	4.841±0.143^{fC}
15	7.697±0.422^{cdA}	8.137±0.246^{efA}	6.190±0.236^{cdB}	6.391±0.169^{efB}	5.105±0.206^{cdC}	5.076±0.080^{efC}
30	7.701±0.295^{bcA}	7.997±0.228^{deA}	6.216±0.265^{bcB}	6.500±0.174^{deB}	5.261±0.201^{bcC}	5.378±0.171^{deC}
45	7.802±0.313^{bcA}	8.282±0.313^{cdA}	6.349±0.250^{bcB}	6.706±0.161^{cdB}	5.251±0.306^{bcC}	5.374±0.304^{cdC}
60	7.913±0.277^{abcA}	8.403±0.228^{bcA}	6.415±0.313^{abcB}	6.823±0.284^{bcB}	5.307±0.285^{abcC}	5.455±0.248^{bcC}
75	8.022±0.217^{abA}	8.570±0.193^{abA}	6.442±0.217^{abB}	6.919±0.226^{abB}	5.359±0.267^{abC}	5.646±0.231^{abC}
90	8.194±0.298^{aA}	8.624±0.206^{aA}	6.729±0.247^{aB}	7.145±0.196^{aB}	5.411±0.243^{aC}	5.739±0.278^{aC}

n=5, results are expressed as Mean± SD, with different small letters superscript (a, b, c) within row and capital letters (A, B, C) within column differ significantly P<0.05 among the samples; OABA: Oil addition before ageing, EABA: Emulsion addition before ageing

Table 5.14 Effect of storage time on Stickiness (N) and work of adhesion (N.sec) of butter fortified with flaxseed oil and flaxseed oil-WPC emulsion when stored at -18 °C

Period of storage (Days)	Control		4.1% OABA		6.8% EABA	
	Stickiness (N)	Work of adhesion (N.sec)	Stickiness (N)	Work of adhesion (N.sec)	Stickiness (N)	Work of adhesion (N.sec)
1	-6.769±0.359^{a}	-2.001±0.179^{a}	-5.348±0.129^{a}	-1.592±0.095^{a}	-4.681±0.179^{a}	-1.339±0.046^{a}
15	-6.883±0.359^{ab}	-2.162±0.081^{b}	-5.634±0.349^{ab}	-1.684±0.025^{b}	-4.740±0.279^{ab}	-1.351±0.018^{b}
30	-6.947±0.296^{ab}	-2.199±0.062^{bc}	-5.673±0.283^{ab}	-1.705±0.016^{bc}	-4.711±0.249^{ab}	-1.347±0.063^{bc}
45	-7.196±0.377^{bc}	-2.209±0.051^{cd}	-5.757±0.265^{bc}	-1.732±0.07^{cd}	-4.842±0.257^{bc}	-1.386±0.057^{cd}
60	-7.398±0.387^{cd}	-2.258±0.071^{cde}	-5.903±0.296^{cd}	-1.754±0.032^{cde}	-4.856±0.242^{cd}	-1.393±0.016^{cde}
75	-7.522±0.316^{de}	-2.280±0.063^{de}	-6.084±0.143^{de}	-1.768±0.035^{de}	-4.894±0.144^{de}	-1.409±0.042^{de}
90	-7.706±0.317^{e}	-2.317±0.046^{e}	-6.165±0.153^{e}	-1.784±0.017^{e}	-5.000±0.101^{e}	-1.421±0.046^{e}

n=5, results are expressed as Mean± SD, with different small letters superscript (a, b, c) within row and capital letters (A, B, C) within column differ significantly P<0.05 among the samples; OABA: Oil addition before ageing, EABA: Emulsion addition before ageing

In the samples stored at 5°C as well as -18°C, exhibited a significant ($P<0.05$) increase in the yield stress throughout the period of storage (Figure 5.15). Thus, it can be said that the butter samples were firmer upon storage. The control sample stored at both the temperatures showed higher and 6.8% EABA showed lower yield stress. These results also correlate with the increasing firmness values. Among the samples at each temperature, all the samples were significantly ($P<0.05$) different from each other. The samples stored at -18°C showed higher values of yield stress than 5°C storage. Similarly, a significant ($P<0.05$) increase retardation time of the butter samples stored at 5°C as well as -18°C was observed during the period of storage is shown in the Table 5.16. Control butter showed higher and 6.8% EABA showed lower retardation time at both the temperature.

The stored samples were significantly ($P<0.05$) different from each other at every temperature (Table 5.16). The samples stored at -18°C had higher retardation time than 5°C storage. Similar studies have been reported by Schaap *et al.* (1981) for increase in the firmness of butter up to 21 days when stored at 15°C for 40 days. Nabar *et al.* (1969) manufactured butter from buffalo milk cream and observed an increase in the firmness of the butter during storage. Kulkarni and Rama Murthy (1985) also found that maximum increase in the firmness occurs in the 1st week of storage. Similarly, Desai *et al.* (1994) observed that during 1st week firmness of butter increased drastically and later slightly throughout the storage period.

Table 5.15 Effect of storage time on Yield stress (N) of butter fortified with flaxseed oil and flaxseed oil-WPC emulsion

Period of storage (Days)	5 °C			-18 °C		
	Control	35% OABA	35% EABA	Control	35% OABA	35% EABA
1	$0.744±0.007^{dA}$	$0.705±0.004^{dB}$	$0.666±0.002^{dC}$	$0.761±0.003^{fA}$	$0.712±0.002^{fB}$	$0.703±0.008^{fC}$
15	$0.754±0.006^{cA}$	$0.713±0.008^{cB}$	$0.690±0.009^{cC}$	$0.768±0.005^{efA}$	$0.719±0.008^{efB}$	$0.705±0.012^{efC}$
30	$0.760±0.004^{cA}$	$0.717±0.008^{cB}$	$0.691±0.003^{cC}$	$0.772±0.002^{deA}$	$0.720±0.006^{deB}$	$0.710±0.009^{deC}$
45	$0.763±0.005^{bA}$	$0.728±0.004^{bB}$	$0.708±0.006^{bC}$	$0.778±0.001^{cdA}$	$0.730±0.004^{cdB}$	$0.715±0.010^{cdC}$
60	$0.769±0.004^{bA}$	$0.729±0.012^{bB}$	$0.711±0.009^{bC}$	$0.781±0.002^{cA}$	$0.735±0.003^{cB}$	$0.718±0.009^{cC}$
75	$0.775±0.004^{bA}$	$0.732±0.002^{bB}$	$0.714±0.008^{bC}$	$0.786±0.002^{abA}$	$0.736±0.005^{abB}$	$0.719±0.007^{abC}$
90	$0.775±0.004^{aA}$	$0.746±0.001^{aB}$	$0.716±0.007^{aC}$	$0.789±0.002^{aA}$	$0.746±0.011^{aB}$	$0.723±0.005^{aC}$

n=3, results are expressed as Mean± SD, with different small letters superscript (a, b, c) within row and capital letters (A, B, C) within column differ significantly $P<0.05$ among the samples; OABA: Oil addition before ageing, EABA: Emulsion addition before ageing

Table 5.16 Effect of storage time on Retardation time (seconds) of butter fortified with flaxseed oil and flaxseed oil-WPC emulsion

Period of storage (Days)	5 °C			-18 °C		
	Control	35% OABA	35% EABA	Control	35% OABA	35% EABA
1	$2.070±0.107^{cA}$	$1.726±0.083^{cB}$	$1.542±0.134^{cC}$	$2.299±0.138^{cA}$	$1.965±0.100^{cB}$	$1.702±0.104^{cC}$
15	$2.093±0.175^{bcA}$	$1.727±0.092^{bcB}$	$1.648±0.151^{bcC}$	$2.312±0.179^{bcA}$	$1.975±0.135^{bcB}$	$1.852±0.159^{bcC}$
30	$2.233±0.141^{abcA}$	$1.868±0.110^{abcB}$	$1.663±0.123^{abcC}$	$2.426±0.139^{abcA}$	$2.105±0.120^{abcB}$	$1.862±0.151^{abcC}$
45	$2.270±0.141^{abA}$	$1.868±0.094^{abB}$	$1.696±0.116^{abC}$	$2.484±0.135^{abA}$	$2.111±0.112^{abB}$	$1.909±0.119^{abC}$
60	$2.281±0.143^{abA}$	$1.875±0.135^{abB}$	$1.703±0.117^{abC}$	$2.490±0.147^{abA}$	$2.135±0.157^{abB}$	$1.910±0.115^{abC}$
75	$2.291±0.192^{abA}$	$1.900±0.094^{abB}$	$1.703±0.083^{abC}$	$2.512±0.154^{abA}$	$2.159±0.139^{abB}$	$1.934±0.106^{abC}$
90	$2.304±0.125^{aA}$	$2.004±0.119^{aB}$	$1.786±0.136^{aC}$	$2.520±0.122^{aA}$	$2.215±0.111^{aB}$	$2.007±0.146^{aC}$

n=3, results are expressed as Mean± SD, with different small letters superscript (a, b, c) within row and capital letters (A, B, C) within column differ significantly $P<0.05$ among the samples; OABA: Oil addition before ageing, EABA: Emulsion addition before ageing

5.6.4 Sensory characteristics of control and fortified butter during storage

Sensory evaluation is one of the important properties to predict the quality of product using human sense organs. It gives an idea about all the attributes of the product. In the present study, during storage sensory evaluation of the control and fortified samples was carried out after every 15 days of interval for 90 days.

There was no significant ($P>0.05$) difference in the scores for colour and appearance, body and texture of the samples stored at 5°C cover the entire storage period as shown in the Table 5.17. This may be because of the slight difference in the colour of the sample which cannot be detected by sensory panelist. But during optimization significant change was observed even at the 2.9% and 4.8% addition of flaxseed oil and emulsion respectively, when measured by instrumental method. Similarly, no significant difference found in the spreadability and flavour scores of the samples stored at 5°C cover the entire storage period as shown in the Table 5.18. This may be because of only slight perceivable changes might have occurred during storage which could be detected by the instrumental methods but cannot be perceived by the human senses. Thus, in the instrumental textural analysis of the control and fortified butter, significant ($P<0.05$) increase in the textural parameters was found during the storage period.

But statistically significant ($P<0.05$) difference was observed in the flavour score among the samples stored at 5°C with control butter having highest flavour score than fortified butter samples. Thus, the addition of the flaxseed oil affected the flavour of the butter significantly. Overall acceptability of the samples stored at 5°C decreased significantly ($P<0.05$) during storage period as shown in the Table 5.19. This is may be due the oxidative changes occurring in the control and fortified butter during storage. The control butter had higher and 4.1% OABA had lower overall acceptability score. The overall acceptability scores of all butter samples stored at 5°C was significantly ($P<0.05$) different among the samples. In a study conducted by Kulkarni and Rama Murthy (1985), little effect was observed in sensory quality of the butter during storage. They stored butter samples at two different (4°C and 10°C) temperatures and observed the occurrence of rancid flavour by sensory analysis. Samples were stored minimum for 90 days and 105 days at 10°C and 4°C respectively. Rancid flavour in the sample (without preservative) was perceived only after 60 days and 75 days at 10°C and 4°C, respectively. However, in the present study, no rancid flavour was observed till 90 days of storage in control as well as fortified butter samples.

In case of butter samples stored at -18°C significant ($P<0.05$) decline was observed in the scores of colour and appearance and body and texture of the control and fortified butter throughout the storage period as shown in the Table 5.20. Similarly, spreadability and flavour scores of the control and fortified samples decreased significantly ($P<0.05$) throughout the storage period (Table 5.21). Addition of flaxseed oil and emulsion improved the spreadability of butter which is a desirable attribute of the butter. In addition to this, when determined by textural analysis, firmness and work of shear increased significantly throughout the storage period resulting in the increase firmness and decrease in spreadability. So, the results of sensory evaluation are similar to those of textural analysis.

There was significant ($P<0.05$) difference in the flavour scores of the control and fortified butter among the samples (Table 5.21). The control butter showed highest and 4.1% OABA showed lowest flavour scores among the samples. This result correlates with the sensory analysis of the control and fortified butter during optimisation as the flavour is the mostly affected attribute by the addition of flaxseed oil or emulsion. Further, there was a decline in the flavour scores observed during the storage which may be due increase in the oxidative reaction which further leads to the off flavour development in the butter. The overall acceptability scores of the samples stored at -18°C was found to decrease significantly ($P<0.05$) as shown in the Table 5.22. Similar results were reported for sensory parameters by Kulkarni and Rama Murthy (1985). Furthermore, the sample stored at -18°C had higher sensory scores than butter samples stored at 5°C. This may be due to more rates of oxidative changes occurring at higher temperature as these reactions have Q_{10} values of 2 to 3, thereby decreasing the overall acceptability of the butter.

Table 5.17 Effect of storage time on Colour & appearance and Body & texture of butter fortified with flaxseed oil and flaxseed oil-WPC emulsion when stored at 5°C

Period of storage (Days)	Control		4.1% OABA		6.8% EABA	
	Colour and appearance	Body and texture	Colour and appearance	Body and texture	Colour and appearance	Body and texture
1	8.25±0.250	8.10±0.279	7.93±0.233	7.65±0.450	8.14±0.180	7.90±0.200
15	8.02±0.352	7.97±0.478	7.91±0.234	7.68±0.319	7.95±0.269	7.85±0.229
30	8.00±0.387	7.92±0.445	7.85±0.320	7.72±0.325	7.90±0.200	7.82±0.389
45	7.93±0.272	7.92±0.218	7.81±0.217	7.69±0.255	7.93±0.155	7.80±0.245
60	7.93±0.272	7.82±0.286	7.80±0.245	7.68±0.227	7.91±0.158	7.80±0.245
75	7.86±0.196	7.83±0.224	7.77±0.293	7.66±0.301	7.88±0.199	7.75±0.250
90	7.83±0.224	7.80±0.261	7.75±0.250	7.64±0.237	7.80±0.245	7.69±0.255

n=10, results are expressed as Mean± SD, with different small letters superscript (a, b, c) within row and capital letters (A, B, C) within column differ significantly P<0.05 among the samples; OABA: Oil addition before ageing, EABA: Emulsion addition before ageing

Table 5.18 Effect of storage time on spreadability and flavour of butter fortified with flaxseed oil and flaxseed oil-WPC emulsion when stored at 5°C

Period of storage (Days)	Control		4.1% OABA		6.8% EABA	
	Spreadability	Flavour	Spreadability	Flavour	Spreadability	Flavour
1	7.77±0.420	$8.08±0.354^{A}$	8.10±0.371	$7.80±0.245^{B}$	8.15±0.196	$7.84±0.287^{B}$
15	7.76±0.405	$8.00±0.297^{A}$	7.94±0.498	$7.70±0.332^{B}$	8.08±0.189	$7.80±0.245^{B}$
30	7.73±0.417	$7.90±0.374^{A}$	7.81±0.345	$7.68±0.319^{B}$	7.96±0.169	$7.75±0.250^{B}$
45	7.71±0.262	$7.88±0.199^{A}$	7.78±0.236	$7.68±0.244^{B}$	7.90±0.200	$7.78±0.236^{B}$
60	7.70±0.245	$7.87±0.313^{A}$	7.73±0.475	$7.67±0.283^{B}$	7.90±0.200	$7.77±0.249^{B}$
75	7.68±0.227	$7.86±0.262^{A}$	7.70±0.245	$7.65±0.229^{B}$	7.88±0.199	$7.70±0.245^{B}$
90	7.66±0.224	$7.80±0.245^{A}$	7.69±0.255	$7.64±0.237^{B}$	7.87±0.335	$7.71±0.221^{B}$

n=10, results are expressed as Mean± SD, with different small letters superscript (a, b, c) within row and capital letters (A, B, C) within column differ significantly P<0.05 among the samples; OABA: Oil addition before ageing, EABA: Emulsion addition before ageing

Table 5.19 Effect of storage time on overall acceptability butter fortified with flaxseed oil and flaxseed oil-WPC emulsion when stored at 5°C

Period of storage (Days)	Control	4.1% OABA	6.8% EABA
1	8.05 ± 0.269^{aA}	7.86 ± 0.223^{aB}	8.01 ± 0.147^{aAB}
15	7.94 ± 0.263^{abA}	7.81 ± 0.264^{abB}	7.92 ± 0.187^{abAB}
30	7.89 ± 0.284^{abA}	7.77 ± 0.214^{abB}	7.86 ± 0.179^{abAB}
45	7.86 ± 0.140^{abA}	7.74 ± 0.170^{abB}	7.85 ± 0.176^{abAB}
60	7.83 ± 0.198^{bA}	7.72 ± 0.210^{bB}	7.85 ± 0.142^{bAB}
75	7.81 ± 0.109^{bA}	7.70 ± 0.150^{bB}	7.80 ± 0.096^{bAB}
90	7.77 ± 0.140^{bA}	7.68 ± 0.109^{bB}	7.77 ± 0.167^{bAB}

n=10, results are expressed as Mean± SD, with different small letters superscript (a, b, c) within row and capital letters (A, B, C) within column differ significantly $P<0.05$ among the samples; OABA: Oil addition before ageing, EABA: Emulsion addition before ageing

Table 5.20 Effect of storage time on Colour & appearance and Body & texture of butter fortified with flaxseed oil and flaxseed oil-WPC emulsion when stored at -18°C

Period of storage (Days)	Control		4.1% OABA		6.8% EABA	
	Colour and appearance	Body and texture	Colour and appearance	Body and texture	Colour and appearance	Body and texture
1	8.05 ± 0.415^{a}	8.05 ± 0.269^{a}	8.00 ± 0.387^{a}	7.85 ± 0.320^{a}	7.93 ± 0.155^{a}	7.90 ± 0.200^{a}
15	7.96 ± 0.080^{ab}	7.85 ± 0.229^{ab}	7.90 ± 0.200^{ab}	7.80 ± 0.332^{ab}	8.00 ± 0.227^{ab}	7.85 ± 0.320^{ab}
30	7.90 ± 0.211^{abc}	7.81 ± 0.228^{ab}	7.86 ± 0.207^{abc}	7.80 ± 0.258^{ab}	7.88 ± 0.210^{abc}	7.81 ± 0.228^{ab}
45	7.87 ± 0.205^{abc}	7.80 ± 0.245^{ab}	7.84 ± 0.191^{abc}	7.78 ± 0.236^{ab}	7.87 ± 0.461^{abc}	7.80 ± 0.245^{ab}
60	7.85 ± 0.229^{bc}	7.77 ± 0.249^{b}	7.82 ± 0.268^{bc}	7.75 ± 0.335^{b}	7.83 ± 0.283^{bc}	7.75 ± 0.250^{b}
75	7.81 ± 0.217^{bc}	7.76 ± 0.301^{b}	7.80 ± 0.245^{bc}	7.73 ± 0.326^{b}	7.78 ± 0.325^{bc}	7.74 ± 0.229^{b}
90	7.78 ± 0.236^{c}	7.70 ± 0.400^{b}	7.70 ± 0.332^{c}	7.65 ± 0.320^{b}	7.74 ± 0.344^{c}	7.67 ± 0.283^{b}

n=10, results are expressed as Mean± SD, with different small letters superscript (a, b, c) within row and capital letters (A, B, C) within column differ significantly $P<0.05$ among the samples; OABA: Oil addition before ageing, EABA: Emulsion addition before ageing

Table 5.21 Effect of storage time on spreadability and flavour of butter fortified with flaxseed oil and flaxseed oil-WPC emulsion when stored at -18°C

Period of storage (Days)	Control		4.1% OABA		6.8% EABA	
	Spreadability	Flavour	Spreadability	Flavour	Spreadability	Flavour
1	8.00±0.316^{a}	7.95±0.350^{aA}	8.05±0.150^{a}	7.85±0.391^{aC}	8.12±0.199^{a}	7.86±0.262^{aB}
15	7.97±0.358^{ab}	8.00±0.224^{abA}	8.03±0.329^{ab}	7.61±0.176^{abC}	8.00±0.447^{ab}	7.68±0.227^{abB}
30	7.95±0.158^{ab}	7.90±0.294^{abcA}	8.07±0.287^{ab}	7.60±0.316^{abcC}	7.99±0.307^{ab}	7.68±0.239^{abcB}
45	7.85±0.229^{abc}	7.85±0.229^{bcA}	8.06±0.405^{abc}	7.54±0.353^{bcC}	7.95±0.401^{abc}	7.64±0.434^{bcB}
60	7.81±0.217^{abc}	7.83±0.361^{bcA}	7.90±0.374^{abc}	7.35±0.450^{bcC}	7.91±0.274^{abc}	7.60±0.374^{bcB}
75	7.80±0.245^{bc}	7.81±0.356^{cA}	7.85±0.391^{bc}	7.33±0.316^{cC}	7.90±0.300^{bc}	7.58±0.397^{cB}
90	7.75±0.250^{c}	7.80±0.245^{cA}	7.83±0.347^{c}	7.33±0.224^{cC}	7.78±0.349^{c}	7.56±0.254^{cB}

n=10, results are expressed as Mean± SD, with different small letters superscript (a, b, c) within row and capital letters (A, B, C) within column differ significantly P<0.05 among the samples; OABA: Oil addition before ageing, EABA: Emulsion addition before ageing

Table 5.22 Effect of storage time on overall acceptability butter fortified with flaxseed oil and flaxseed oil-WPC emulsion when stored at -18°C

Period of storage (Days)	Control	4.1% OABA	6.8% EABA
1	8.01±0.253^{aA}	7.94±0.170^{aB}	7.95±0.141^{aAB}
15	7.95±0.118^{abA}	7.84±0.141^{abB}	7.88±0.145^{abAB}
30	7.89±0.099^{bcA}	7.83±0.141^{bcB}	7.84±0.121^{bcAB}
45	7.84±0.130^{bcdA}	7.81±0.165^{bcdB}	7.82±0.280^{bcdAB}
60	7.82±0.108^{cdeA}	7.71±0.173^{cdeB}	7.77±0.225^{cdeAB}
75	7.80±0.157^{deA}	7.68±0.213^{deB}	7.75±0.199^{deAB}
90	7.76±0.160^{eA}	7.63±0.142^{eB}	7.69±0.104^{eAB}

n=10, results are expressed as Mean± SD, with different small letters superscript (a, b, c) within row and capital letters (A, B, C) within column differ significantly P<0.05 among the samples; OABA: Oil addition before ageing, EABA: Emulsion addition before ageing

5.6.5 Alpha-linolenic acid (ALA) content of control and fortified butter during storage

The quantification of alpha-linolenic acid in the fresh and stored butter samples was done by GC-MS. It is found that the initial alpha-linolenic acid present in control (0.872%) butter was increased to 3.149% in 4.1% OABA and to 3.174% in 6.8% EABA. The highest recovery of ALA was found in butter sample prepared by addition of emulsion in cream (6.8% EABA. This may be due to the binding property of the emulsifier i.e. WPC present in the emulsion. During 90 days of storage, the ALA content decreased in the control and fortified butter (Figure 5.28). Similar results were obtained by Gowda *et al.* (2018) for decrease in the α-linolenic acid acid content of the omega-3 fatty acid fortified ice-cream during storage of 120 days. However, in case of lesser storage life product i.e. curd for 8 days, no change is observed in the ALA content during storage by Veena *et al.* (2017).

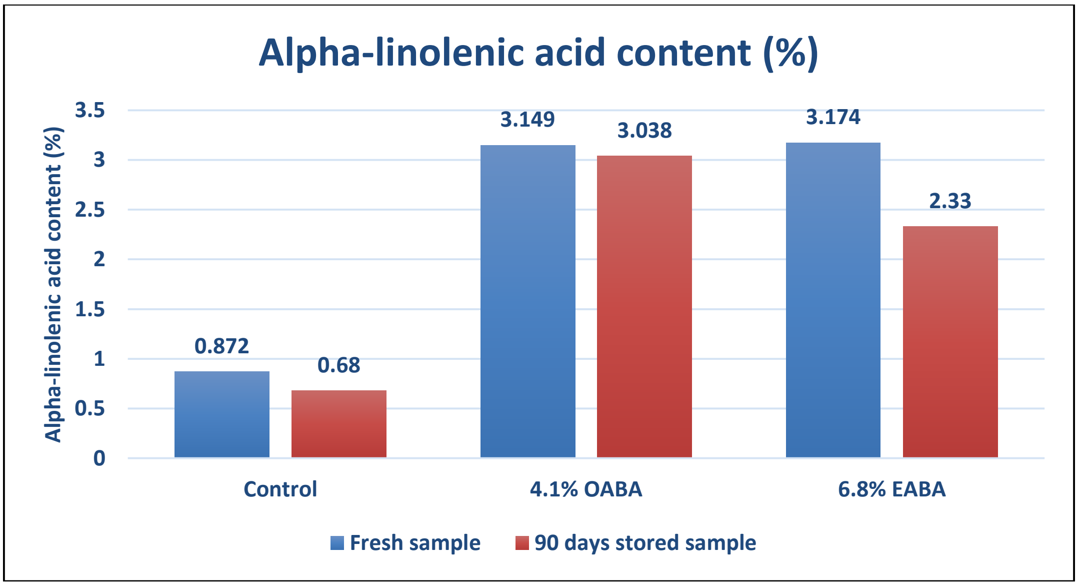


Figure 5.28 Effect of storage on alpha-linolenic acid content in control and fortified butter

5.6.6 Microbial quality of control and fortified butter during storage

The shelf life of the product also depends on the microbial quality of butter. In the present study, the microbial quality of the control and fortified samples was checked during storage after every 15 days interval. There was no microbial growth i.e. coliform count as well as yeast and mould count was observed throughout the period of storage as shown in the Table 5.23. According to FSSAI, the maximum limit for coliform count is

20 cfu /g and for yeast and mould count is 50 cfu /g for butter. Thus, from the results it is concluded that the microbial quality of the product was acceptable till the end of storage study.

Table 5.23 Effect storage time on microbial quality of control and fortified butter

Period of storage (Days)	Control		4.1% OABA		6.8% EABA	
	Coliform count	Yeast and mould count	Coliform count	Yeast and mould count	Coliform count	Yeast and mould count
1	ND	ND	ND	ND	ND	ND
15	ND	ND	ND	ND	ND	ND
30	ND	ND	ND	ND	ND	ND
45	ND	ND	ND	ND	ND	ND
60	ND	ND	ND	ND	ND	ND
75	ND	ND	ND	ND	ND	ND
90	ND	ND	ND	ND	ND	ND

n=3, results are expressed as Mean± SD

Conclusion

Thus, from the above results it can be concluded that addition of flaxseed oil or flaxseed oil-whey protein concentrate emulsion in cream before ageing could be used as a suitable approach for developing omega-3 fatty acids fortified butter. The developed product is stable under both refrigeration and deep freezer conditions with acceptable sensory and textural properties. The developed butter is also acceptable in terms of oxidative stability. Further, most importantly, the alpha-linolenic acid content was higher in the butter fortified with flaxseed oil (3.149%) as well as with emulsion (3.174%) than control (0.86%) as determined by gas chromatography-mass spectroscopy. Thus, the developed fortified butter can provide nearly 27% recommended dietary allowance (RDA) of alpha linolenic acid in one serving.

6.0 SUMMARY AND CONCLUSION

Butter is a fat rich dairy product containing more than 80% fat. India is the largest producer of butter and butter consumption is increasing day by day. Butter is rich source of fat and fat soluble vitamins A, E, K. But, it's a poor source of omega -3 fatty acids, which are considered as essential fatty acids. Omega-3 fatty acids provide several health benefits such as prevention of CVDs, improved eye health, reduced risk of hypertension, neurogenerative disorder, cancers and diabetes, anti-inflammatory and hypocholesterolemic effect. Also, high level of fortification with omega-3 fatty acids is possible in fat rich products with added flavourings. In addition to this, supplementation of α-linolenic acid (ALA) with dairy lipid increases the level of DHA in the tissues than ALA alone. Flaxseed oil is one of the richest vegetarian source of omega-3 fatty acids and contains more than 55% ALA. Higher amount of unsaturated fatty acids makes oil more prone to the oxidation. Further, flaxseed oil can be added in the form of emulsion as it improves organoleptic properties, bioavailability of ALA and provides the protective effect towards oxidation.

The study was conducted by keeping in mind that minimum 25% of the RDA level of alpha-linolenic acid can be fulfilled per serving of the butter. The salient findings of the present study are summarized in this section.

1. Flaxseed oil emulsions prepared with 45, 60, and 75% oil load and WPC and WPI had maximum physical stability and no visible oil separation among other studied emulsifiers and stabilizers. Further, three levels of WPC (5, 7.5 and 10%) have been studied and 7.5% WPC was selected for making emulsion. Finally, based upon the higher physical stability, emulsion containing 7.5% WPC and 60% oil load was selected for incorporation in the cream for butter making.
2. Two different ageing times (16 and 20 h) were selected for the optimization and ageing was carried out at refrigerated temperature i.e. 5-7 °C. Fat loss in the buttermilk was lesser when cream aged for 20 h than 16 h ageing. So, 20 h ageing time was selected for preparation of omega-3 fatty acids fortified butter.
3. The effect of type of fortificant (oil or emulsion), stage of addition of fortificant (before and after ageing) and level of fortificant (2.9, 4.1 & 5.1% for flaxseed oil and 4.8, 6.8 & 8.6% for flaxseed oil emulsion) on various physico-chemical,

sensory, textural and sensory characteristics was evaluated. The level of fortificant was selected in such a way so as to provide at least 25, 35 and 45% RDA of ALA in one serving of butter.

4. Addition of flaxseed oil and emulsion before ageing showed lesser fat loss than when added after ageing. The fat loss increased with the level of fortificant addition but it was still below 1.5% for all the levels of oil studied. There was no significant ($p>0.05$) change in the yield of the butter due to the stage and level of addition of fortificant. The churning time of cream significantly ($p< 0.05$) reduced with increase in the level of flaxseed oil and emulsion.
5. The effect of stage and level of fortificant on the sensory quality of the butter was studied. The butter prepared by adding 4.1 and 6.8% flaxseed oil and flaxseed oil emulsion, respectively before ageing had comparable sensory score with the control butter and thus selected for optimised process.
6. Significant ($p< 0.05$) increase was found in the yellowness index of fortified butter prepared with 4.1 and 6.8% flaxseed oil and flaxseed oil emulsion, respectively.
7. The textural properties such as firmness, work of shear, stickiness, work of adhesion, yield stress and retardation time decreased significantly ($p< 0.05$) with the level of flaxseed oil and emulsion addition. The butter prepared by adding 4.1 and 6.8% flaxseed oil and flaxseed oil emulsion respectively before ageing showed comparable textural properties with the control butter.
8. The alpha-linolenic acid (ALA) content in the control, butter prepared from oil fortified cream (4.1% OABA), butter prepared from cream containing emulsion (6.8% EABA) and flaxseed oil was 0.872, 3.149, 3.174 and 61.17%, respectively. Thus, significant increase in in the ALA content was found in fortified butters than control.
9. In Differential scanning calorimetry (DSC) thermal analysis, shift in the peaks of high melting fraction (HMF), medium melting fraction (MMF) and low melting fraction (LMF) was observed towards the lower temperature in both melting and crystallization curves. This is because of the increase in the LMF and MMF content due to the fortification of butter using flaxseed oil.
10. Solid fat content (SFC) in control and fortified butter was determined by Pulsed nuclear magnetic resonance (pNMR) technique. The fortified samples i.e. 4.1%

OABA and 6.8% EABA had 50.995 and 51.305% solid fat content, respectively at 5°C, which is significantly ($p < 0.05$) lower than the control butter (56.63%).

11. Induction period of control and fortified butter was determined by rancimat. The control butter had higher induction period (4.35 h) than the fortified samples i.e. 4.1% OABA (2.36 h) and 6.8% EABA (2.56 h). Decline in the induction period was due to the increase in the unsaturated fatty acids which are more prone to the oxidation through fortification.
12. In the present investigation, the control (0.9449) butter had higher water activity than fortified samples i.e. 0.9074 for sample prepared by adding oil in cream (4.1% OABA) and 0.9126 for the butter prepared from cream containing emulsion (6.8% EABA).
13. In proximate composition of control butter, fat, moisture, protein, ash, carbohydrate and curd content were 87.47, 11.94, 0.007, 0.00055, 0.58 and 0.59% respectively. In case of 4.1% OABA butter sample, fat, moisture, protein, ash, carbohydrate and curd content were 87.37, 12.15, 0.005, 0.0005, 0.47 and 0.48% respectively. Similarly, 6.8% EABA butter sample had 85.31, 14.06, 0.014, 0.00055, 0.61 and 0.63% of fat, moisture, protein, ash, carbohydrate and curd content, respectively.
14. The three samples were also kept for storage study at 5 and -18˚C. Control butter stored at 5 as well as -18°C had higher acidity than the fortified samples (4.1% OABA and 6.8% EABA). The acidity of the samples showed significant ($p < 0.05$) increase throughout the storage period
15. The oxidative stability parameters such as Peroxide value, Free fatty acids and TBA value of control and fortified butter increased significantly ($p < 0.05$) throughout the storage period in the samples stored at 5 as well as -18°C.
16. During storage at 5 and -18°C, the textural properties such as firmness, work of shear, stickiness, work of adhesion, yield stress and retardation time increased significantly ($p < 0.05$) throughout the storage period. Among the samples the fortified samples had lower firmness and other parameters than control butter. But lesser extent of increase was found in the samples stored 5°C than -18°C storage.
17. In sensory evaluation during storage, significant decrease in the overall acceptability of the control and fortified butter was observed when stored at 5 as well as -18°C. Among the samples the control butter showed higher overall

acceptability than control sample. The microbial quality of the control and fortified butter was good throughout period of storage.

18. Alpha-linolenic acid (ALA) content of control, oil fortified and emulsion fortified butter decreased from 0.872 to 0.680%, 3.149 to 3.038% and 3.174 to 2.33% over the storage period respectively.

Conclusion

Therefore, it can be inferred that the addition of flaxseed oil or flaxseed oil-whey protein concentrate emulsion in cream before ageing could be used as a suitable approach for developing omega-3 fatty acids fortified butter. The developed product is stable under both refrigeration and deep freezer conditions with acceptable sensory and textural properties. The developed butter is also acceptable in terms of oxidative stability. Further, most importantly, the alpha-linolenic acid content was higher in the butter fortified with flaxseed oil (3.149%) as well as with emulsion (3.174%) than control (0.86%). Thus, in the present study, the process for developing omega- 3 fatty acid fortified butter has been successfully optimized resulting in the fortified butter which can provide nearly 27% recommended dietary allowance (RDA) of alpha linolenic acid in one serving.

BIBLIOGRAPHY

Adarme-Vega, T. C., Thomas-Hall, S. R. and Schenk, P. M. (2014). Towards sustainable sources for omega-3 fatty acids production. *Current Opinion in Biotechnology*, ***26,*** 14-18.

Agostoni, C. (2008). Role of long-chain polyunsaturated fatty acids in the first year of life. *Journal of Pediatric Gastroenterology and Nutrition*, ***47,*** S41-S44.

Ahmad, A., Moriguchi, T. and Salem Jr, N. (2002). Decrease in neuron size in docosahexaenoic acid-deficient brain. *Pediatric Neurology*, ***26*(3)**, 210-218.

Ahmed N. S., Helel, F. R. and El-Nimr, A. A. (1979). Modified butter containing vegetable oils. *Milchwissenschaft*, ***34***, 218-219.

Aktaş, N., and Kaya, M. (2001). Detection of beef body fat and margarine in butterfat by differential scanning calorimetry. *Journal of Thermal Analysis and Calorimetry*, ***66*(3),** 795-801.

Amer, M. A., and Myhr, A. N. (1973). Modification of butter to improve low temperature spreadability. *Canadian Institute of Food Science and Technology Journal*, ***6(4),*** 261-265.

Amr, A. S. (1991). Effectiveness of synthetic and potential natural antioxidants in improving the stability of sheep's anhydrous butter fat during long-term storage. *Journal of the Science of Food and Agriculture*, ***55,*** 75-85.

An, W. S., Kim, H. J., Cho, K. H. and Vaziri, N. D. (2009). Omega-3 fatty acid supplementation attenuates oxidative stress, inflammation, and tubulointerstitial fibrosis in the remnant kidney. *American Journal of Physiology-Renal Physiology*, ***297(4),*** F895–F903.

Anonymous (2013). Process Development for the production of Functional butter. In Annual report 2012-13, National Dairy Research Institute, Karnal Haryana. pp44-45.

Anonymous (2018). Obesity and overweight. WHO. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight, as accessed on 23-7-18.

Anonymous (2018). India Dairy, Butter Production by Year. USDA. https://www.indexmundi.com/agriculture/?country=in&commodity=butter&graph=production, as accessed on 21-7-2018.

AOAC (2005). Official methods of Analysis of the Association Analytical Chemists 17th edition Washington, D. C USA.

AOCS (1993). Official Methods and Recommended Practices of the AOCS. 4th Ed. AOCS Press, Champaign, IL.

AOCS (1993). Official methods and recommended practices of the American oil chemists' society. Vol. 1, 4"' Ed., 2"d print. Method cd. 8.53 (including 1990 and 1992 additions and revisions). American Oil Chemists' Society, Champaign, Ill.

Arbuckle, L. D. and Innis, S. M. (1992). Docosahexaenoic acid in developing brain and retina of piglets fed high or low α-linolenate formula with and without fish oil. *Lipids*, ***27*(2),** 89-93.

Avramis, C. A., Wang, H., McBride, B. W., Wright, T. C. and Hill, A. R. (2003). Physical and processing properties of milk, butter, and cheddar cheese from cows fed supplemental fish meal. *Journal of Dairy Science*, ***86*(8),** 2568-2576.

Baer, R., Ryali, J., Schingoethe, D., Kasperson, K., Donovan, D., Hippen, A. and Franklin, S. (2001). Composition and properties of milk and butter from cows fed fish oil. *Journal of Dairy Science*, ***84,*** 345–353.

Banning, M. (2005). The role of omega-3-fatty acids in the prevention of cardiac events. *British Journal of Nursing*, **25,** 503–508.

Barakat, L. A. A. and Mehmoud, R. H. (2011). The antiatherogenic, renal protective and immunomodulatory effects of purslane, pumpkin and flax seeds on Hypercholesterolemic rats. *North American Journal of Medical Sciences*, ***3(9),*** 351-357.

Bermúdez-Aguirre, D. and Barbosa-Cánovas, G. V. (2011). Quality of selected cheeses fortified with vegetable and animal sources of omega-3. *LWT-Food Science and Technology*, ***44*(7)**, 1577-1584.

Bibus, D. and Lands, B. (2015). Balancing proportions of competing omega-3 and omega-6 highly unsaturated fatty acids (HUFA) in tissue lipids. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, ***99,*** 19-23.

Bobe, G., Zimmerman, S., Hammond, E. G., Freeman, A. E., Porter, P. A., Luhman, C. M. and Beitz, D. C. (2007). Butter composition and texture from cows with different milk fatty acid compositions fed fish oil or roasted soybeans. *Journal of Dairy Science*, ***90*****(6)**, 2596-2603.

Bornaz, S., Fanni, J. and Parmentier, M. (1994). Limit of the solid fat content modification of butter. *Journal of the American Oil Chemists' Society*, ***71*****(12),** 1373-1380.

Brenna, J. T., Salem Jr, N., Sinclair, A. J. and Cunnane, S. C. (2009). α-Linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. *Prostaglandins, Leukotrienes and Essential fatty acids*, ***80*****(2-3),** 85-91.

Calder, P. C. (2015). Marine omega-3 fatty acids and inflammatory processes: effects, mechanisms and clinical relevance. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, ***1851*****(4),** 469-484.

Cantor, A. H., Decker, E. A. and Collins, V. P. (2000). Fatty acids in poultry and egg products. *In: Fatty Acids in Foods and Their Health Implications,* (pp. 153–174). Chow, C. K., Ed., Marcel Drekker, Inc, New York.

Cardozo, L. F. M. D. F., Chagas, M. A., Soares, L. L. and Bonaventura, G. T. (2010). Exposure to flaxseed during lactation does not alter prostate area or epithelium height but changes lipid profile in rats. *Nutrición Hospitalaria*, ***25(2),*** 250-255.

Chee, C. P., Gallaher, J. J., Djordjevic, D., Faraji, H., McClements, D. J., Decker, E. A. and Coupland, J. N. (2005). Chemical and sensory analysis of strawberry flavoured yogurt supplemented with an algae oil emulsion. *Journal of Dairy Research*, ***72*****(3),** 311-316.

Chen, B., Mc Clements, D. J. and Decker, E. A. (2013). Design of foods with bioactive lipids for improved health. *Annual Review of Food Science and Technology*, ***4,*** 35-56.

Chen, J., Power, K. A., Mann, J., Cheng, A. and Thompson, L. U. (2007). Flaxseed alone or in combination with tamoxifen inhibits MCF-7 breast tumor growth in ovariectomized athymic mice with high circulating levels of estrogen. *Experimental Biology and Medicine,* ***232,*** 1071–1080.

Choe, E. and Min, D. B. (2007). Chemistry of deep-fat frying oils. *Journal of Food Science.* ***72(5),*** R77–R86.

Choe, E. and Min, D. B. (2006). Mechanisms and factors for edible oil oxidation. *Comprehensive Reviews in Food Science and Food Safety*, ***5(4),*** 169-186.

Christie W. W. (1995). Composition and structure of milk lipids. *In: Advances in Dairy Chemistry, Lipids,* (Vol. 2, pp. 1–36). Fox, P. F., Ed., Chapman and Hall, London, UK.

Cicero, A. F., Derosa, G., Di, G., Bove, M., Gaddi, A.V. and Borghi, C. (2010). Omega-3 polyunsaturated fatty acids supplementation and blood pressure levels in hyper triglyceridemic patients with untreated normal-high blood pressure and with or without metabolic syndrome: a retrospective study. *Clinical and Experimental Hypertension,* ***32(2),*** 137–144.

Cleland, L. G., James, M. J. and Proudman, S. M. (2003). The role of fish oils in the treatment of rheumatoid arthritis. *Drugs*, ***63,*** 845–853.

Connor, W. E. (2000). Importance of n-3 fatty acids in health and disease. *American Journal of Clinical Nutrition*, ***71(S),*** 171–179.

Couedelo, L., Boue-Vaysse, C., Fonseca, L., Montesinos, E., Djoukitch, S., Combe, N. and Cansell, M. (2011). Lymphatic absorption of α-linolenic acid in rats fed flaxseed oil-based emulsion. *British Journal of Nutrition*, ***105***, 1026–1035

Covington, M. B. (2004). Omega-3 fatty acids. *Atlantic*, *1* (2.0).

Cox, C., Mann, J., Sutherland, W., Chisholm, A. and Skeaff, M. (1995). Effects of coconut oil, butter, and safflower oil on lipids and lipoproteins in persons with moderately elevated cholesterol levels. *Journal of Lipid Research*, ***36*****(8),** 1787-1795.

Dawczynski, C., Martin, L., Wagner, A. and Jahreis, G. (2010). n-3 LC-PUFA-enriched dairy products are able to reduce cardiovascular risk factors: a double-blind, cross-over study. *Clinical Nutrition*, ***29,*** 592–99.

De S (1980). Butter. *Outlines of Dairy Technology*, (pp. 143-173). Oxford University Press, New Delhi, India

Deffense, E. (1987). Multistep butteroil fractionation and spreadable butter. *Fat Science Technology*, ***89***, 502-407.

DePeter, E. J., Taylor, S. J., Franke, A. A. and Aguirre, A. (1985). Effects of feeding whole cottonseed on composition of milk. *Journal of Dairy Science, **68***, 897-902.

Desai, N. B., Thaker, P. N. and Prajapati, P. S. (1994). Rheology of commercial table butter determined with cone penetrometer. *Indian Journal of Dairy Science*, ***47,*** 352-352.

Devdhara, V. D., Murthi, T. N., Punjrath, J. S. and Aneja, R. P. (1991). Low-calorie butter spreads from cream: Part I. Manufacture, physical properties and keeping quality. *Indian Journal of Dairy Science*, ***44*****(1),** 111-115.

Dickinson, E. (2001). Milk protein interfacial layers and the relationship to emulsion stability and rheology. *Colloids and Surfaces B: Biointerfaces*, ***20*****(3),** 197-210.

Divya, K. B, Kumar, S., Mittal, V. and Sabikhi, L. (2013). Sensory attributes, fatty acid profile and oxidative stability of low fat buffalo milk supplemented with omega 3 fatty acids and dietary fiber. *Indian Journal of Dairy Science*, ***66(6),*** 469- 476.

Douaud, C. (2006). First DHA/ARA-fortified organic baby formula launched. Available from www.nutraingredients-usa.com/Industry/First-DHA-ARAfortified-organic-baby-formula-launched. Accessed on January 30, 2019.

Drouin, G., Catheline, D., Sinquin, A., Baudry, C., Le Ruyet, P., Rioux, V. and Legrand, P. (2018). Incorporation of dairy lipids in the diet increased long-chain Omega-3 fatty acids status in post-weaning rats. *Frontiers in Nutrition*, ***5.***

Dyerberg, J., Eskesen, D. C., Andersen, P. W., Astrup, A., Buemann, B. and Christensen, J. H. (2004). Effects of trans- and n-3 unsaturated fatty acids on cardiovascular risk markers in healthy males. An 8 weeks dietary intervention study. *European Journal of Clinical Nutrition**, 58,*** 1062–1070.

Estrada, J. D., Boeneke, C., Bechtel, P. and Sathivel, S. (2011). Developing a strawberry yogurt fortified with marine fish oil. *Journal of Dairy Science*, ***94*****(12),** 5760-5769.

Eunok, C. and David, B. M. (2006). Mechanisms and factors for edible oil oxidation. *Comprehensive Reviews on Food Science and Food Safety*, ***5(4)****,* 169–186.

FAO/WHO (1996). Preparation and use of food-based dietary guidelines Nutrition Programme, Geneva: WHO.

FAO/WHO (2010). Fats and Fatty Acids in Human Nutrition Rome: FAO Food and nutrition paper # 697 91 Report of an expert consultation Geneva, November 10–14, 2008.

Farrell, D. J. (1998). Enrichment of hen eggs with n-3 long-chain fatty acids and evaluation of enriched eggs in humans. *The American Journal of Clinical Nutrition*, ***68*****(3),** 538-544.

Fearon, A. M. (2001). Optimizing milkfat composition and processing properties. *Australian Journal of Dairy Technology*, ***2***, 104–108.

Fewtrell, M. S. (2006). Long-chain polyunsaturated fatty acids in early life: effects on multiple health outcomes. *In: Primary Prevention by Nutrition Intervention in Infancy and Childhood* (Vol**.** 57, pp. 203-221). Karger Publishers, Switzerland.

FSSAI (2011) https://www.archives.fssai.gov.in, as accessed on 12/02/2019

Gallaher, J. J., Djordjevic, D., Faraji, H., McClements, D. J., Decker, E. A., Hollender, R., Peterson, D. G., Roberts, R. F. and Coupland, J. N. (2005). Chemical and sensory analysis of strawberry flavoured yogurt supplemented with an algae oil emulsion. *Journal of Dairy Research*, ***72(3)***, 311-316.

Garcia, O., Mahmood K. and Hemme, T. (2003). A review of milk production in Pakistan with particular emphasis on small scale producers. PPLPI Working Paper No. 3. IFCN, Rome.

GBD, (2013). Mortality and Causes of Death Collaborators (17 December 2014). Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. ***385*** (9963), 117–71.

Ganesan, B., Brothersen, C. and McMahon, D. J. (2014). Fortification of foods with omega-3 polyunsaturated fatty acids. *Critical Reviews in Food Science and Nutrition*, ***54*****(1),** 98-114.

Ghafoorunissa. (1998). Requirements of dietary fats to meet nutritional needs & prevent the risk of atherosclerosis-An Indian perspective. *Indian Journal of Medical Research*, ***108,*** 191-202.

Glibowski, P., Zarzycki, P. and Krzepkowska, M. (2008). The Rheological and Instrumental Textural Properties of Selected Table Fats. *International Journal of Food Properties*, ***11(3),*** *678–686.*

Gogus, U. and Smith, C. (2010). n-3 Omega fatty acids: a review of current knowledge. *International Journal of Food Science and Technology*, ***45*****(3),** 417-436.

Gökmen, V., Mogol, B. A., Lumaga, R. B., Fogliano, V., Kaplun, Z. and Shimoni, E. (2011). Development of functional bread containing nanoencapsulated omega-3 fatty acids. *Journal of Food Engineering*, ***105*****(4),** 585-591.

Gonzalez, S., Duncan, S. E., O'Keefe, S. F., Sumner, S. S. and Herbein, J. H. (2003). Oxidation and Textural Characteristics of Butter and Ice Cream with Modified Fatty Acid Profiles. *Journal of Dairy Science*, ***86(1),*** *70–77.*

Gopinath, B., Harris, D. C., Flood, V. M., Burlutsky, G. and Mitchell, P. (2011). Consumption of long-chain n-3 PUFA, α-linolenic acid and fish is associated with the prevalence of chronic kidney disease. *British Journal of Nutrition*, ***105*****(9),** 1361-1368.

Gowda, A., Sharma, V., Goyal, A., Singh, A. K. and Arora, S. (2018). Process optimization and oxidative stability of omega-3 ice cream fortified with flaxseed oil microcapsules. *Journal of Food Science and Technology*, ***55*****(5),** 1705-1715.

Goyal, A., Sharma, V., Sihag, M. K., Singh, A. K., Arora, S. and Sabikhi, L. (2016). Fortification of dahi (Indian yoghurt) with omega-3 fatty acids using microencapsulated flaxseed oil microcapsules. *Journal of Food Science and Technology*, ***53*****(5),** 2422-2433.

Goyal, A., Sharma, V., Sihag, M. K., Tomar, S. K., Arora, S., Sabikhi, L. and Singh, A. K. (2015). Development and physico-chemical characterization of microencapsulated flaxseed oil powder: A functional ingredient for omega-3 fortification. *Powder Technology*, ***286,*** 527-537.

Gramza-Michalowska, A., Korczak, J. and Regula, J. (2007). Use of plant extracts in summer and winter season butter oxidative stability improvement. *Asia Pacific Journal of Clinical Nutrition*, ***16*****(S1),** 85-88.

Gruenwald, J., Petzold, E., Busch, R., Petzold, H. P. and Graubaum, H. J. (2009). Effect of glucosamine sulfate with or without omega-3 fatty acids in patients with osteoarthritis. *Advances in Therapy*, ***26*****(9),** 858.

Gunstone, F. D. (1996). Fatty acids-Nomenclature, structure, isolation and structure determination, biosynthesis and chemical synthesis. *In: Fatty Acid and Lipid Chemistry*, (pp. 1-35) Blackie Academic and Professional, Glasgow, UK.

Gunstone, F. D. (Ed.). (2006). Vegetable sources of lipids. *In: Modifying lipids for use in food*, (pp. 11-27) Wood head Publishing, Swaston, Cambridge.

Han, E., Yun, Y., Kim, G., Lee, Y. H., Wang, H. J., Lee, B. W. and Kang, E. S. (2016). Effects of omega-3 fatty acid supplementation on diabetic nephropathy progression in patients with diabetes and hypertriglyceridemia. *PLoS One*, ***11*****(5),** e0154683.

Harris, W. S., Mozaffarian, D., Rimm, E., Kris-Etherton, P., Rudel, L. L., Appel, L. J. and Sacks, F. (2009). Omega-6 fatty acids and risk for cardiovascular disease: A science advisory from the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. *Circulation*, ***119*****(6),** 902-907.

Hassan, A. A., Rasmy, N. M., Foda, M. I. and Bahgaat, W. K. (2012). Production of functional biscuits for lowering blood lipids. *World Journal of Dairy and Food Science*, ***7(1),*** 01-20.

Herrera, E. (2002). Implications of dietary fatty acids during pregnancy on placental, fetal and postnatal development—A review. *Placenta*, ***23***(Suppl. A), S9–S19.

Herrera, E., Amusquivar, E., Lopez-Soldado, I. and Ortega, H. (2006). Maternal lipid metabolism and placental lipid transfer. *Hormone Research in Paediatrics*, ***65*** (Suppl. 3), 59-64.

Haque, M. R. and Bradbury, J. H. (2002). Total cyanide determination of plants and foods using the picrate and acid hydrolysis methods. *Food Chemistry*, ***77*****(1),** 107-114.

Horn, A. F., Nielsen, N. S., Andersen, U., Søgaard, L. H., Horsewell, A. and Jacobsen, C. (2011). Oxidative stability of 70% fish oil in water emulsions: Impact of emulsifiers and pH. *European Journal of Lipid Science and Technology*, ***113*****(10),** 1243-1257.

Hulbert, A. J., Turner, N., Storlien, L. H. and Else, P. L. (2005). Dietary fats and membrane function: implications for metabolism and disease. *Biological Reviews*, ***80*****(1),** 155-169.

Hwang, D. and Rhee, S. H. (1999). Receptor-mediated signaling pathways: Potential targets of modulation by dietary fatty acids. *American Journal of Clinical Nutrition,* ***70(4),*** 545–556.

ICMR (2010). Nutrient Requirements and Recommended Dietary Allowances for Indians. A Report of the Expert Group of the Indian Council of Medical Research 2009. Available from: http://icmr.nic.in/final/RDA-2010.pdf

ISSFAL (2004). Recommendations for intake of polyunsaturated fatty acids in healthy adults. (International Society for the Study of Fatty acids and Lipids (ISSFAL) News, 11: 12-18.

Indian standards, IS:3507-1966. Methods for sampling and test for butter. Indian Standard Institution, New Delhi, India.

Innis, S. M., Sprecher, H., Hachey, D., Edmond, J. and Anderson, R. E. (1999). Neonatal polyunsaturated fatty acid metabolism. *Lipids,* ***34(2),*** 139–149.

Innis, S. M. and Jacobson, K. (2007). Dietary lipids in early development and intestinal inflammatory disease. *Nutrition Review,* ***65****,* S188–S193.

Institute of Medicine, Food and Nutrition Board (2005). Dietary reference intakes for energy, carbohydrate, fibre, fat, fatty acids, cholesterol, protein, and amino acids (macronutrients). Washington, DC: National Academy Press.

Ivanov, S., Rashevskaya, T. and Makhonina, M. (2011). Flaxseed additive application in dairy products production. *Procedia Food Science,* ***1,*** 275-280.

Jennifer, L. A., Susan, J. W., Bernhard, H. J., Lilian, U. T. and Jane, A. (2010). Health effects with consumption of the flax lignan secoisolariciresinol diglucoside. *British Journal of Nutrition*, ***103(7),*** 929-939.

Jensen, R. G., Ferris, A. M., and Lammi-Keefe, C. J. (1992). Lipids in human milk and infant formulas. *Annual Review of Nutrition*, ***12*****(1),** 417-441.

Kaithwas, G., Mukherjee, A., Chaurasia, A. K. and Majumdar, D. K. (2011). Antiinflammatory, analgesic and antipyretic activities of Linum usitatissimum L.(flaxseed/linseed) fixed oil, *Indian Journal of Experimental Biology, **49***, 932-938.

Kalo, P., Huorati, H. and Antila, M. (1990). Pseudomonas fluorescens lipase-catalysed interesterification of butter fat in the absence of a solvent. *Milchwissenschaft*, ***45***, 281-285.

Kalo, P., Parviainen, P., Vaara, K., Ali-Yrrko¨ S. and Antila, M. (1986a). Changes in the triglyceride composition of butter fat induced by lipase and sodium methoxide catalysed inter-esterification reactions. *Milchwissenschaft, **41***, 82-85.

Kalo, P., Vaara, K. and Antila, M. (1986b) Changes in triglyceride composition and melting properties of butter fat fraction/ rapeseed oil mixtures induced by lipase catalyzed interesterification. *Fette Seifen Anstrichtm, **88***, 362365.

Kapoor, S., Sachdeva, R. and Kochhar, A. (2011). Efficacy of flaxseed supplementation on nutrient intake and other lifestyle pattern in menopausal diabetic females. *Thnomedicine, **5(3),*** 153-160.

Kartal, C., Unal, M. K. and Otles, S. (2016). Flaxseed oil-in-water emulsions stabilized by multilayer membranes: oxidative stability and the effects of pH. *Journal of Dispersion Science and Technology*, ***37*****(12),** 1683-1691.

Kaylegian, K. E. and Lindsay, R. C. (1992). Performance of selected milk fat fractions in cold-spreadable butter. *Journal of Dairy Science*, ***75(12)***, 3307-3317.

Kelley, D. S., Vemuri, M., Adkins, Y., Gill, S. H. S., Fedor, D. and Mackey, B. E. (2009). Flaxseed oil prevents trans-10, cis-12-conjugated linoleic acid-induced insulin resistance in mice. *British Journal of Nutrition*, ***101,*** 701–708.

Khalesi, S., Jamaluddina, R. and Ismailab, A. (2011). Effect of raw and heated flaxseed (*Linum usitatissimum L.*) on blood lipid profiles in rats. *International Journal of Applied Science and Technology*, ***1(4),*** 84-89.

Kocchar, S. P. (1995). Oxidative Pathways to the Formation of Off-Flavours. *In: Food Taints and Off-Flavours*, (2nd ed., pp. 168–225). Blackie Academic and Professional, Glasgow, UK.

Kolanowski, F. S., Ewa, L. and Stanislaw Berger, W. (2001). Enrichment of spreadable fats with polyunsaturated fatty acids omega-3 using fish oil. *International Journal of Food Sciences and Nutrition*, ***52(6),*** 469-476.

Kolanowski, W., Jaworska, D. and Wei Xbrodt, J. (2007). Importance of instrumental and sensory analysis in the assessment of oxidative deterioration of omega-3 LC PUFA-rich foods. *Journal of the Science of Food and Agriculture*, ***87***, 181–191.

Kolanowski, W., Swiderski, F., Jaworska, D. and Berger, S. (2004). Stability, sensory quality, texture properties and nutritional value of fish oil-enriched spreadable fat. *Journal of the Science of Food and Agriculture*, ***84(15),*** 2135-2141.

Kris-Etherton, P. M., Harris, W. S. and Appel, L. J. (2002). Fish consumption, fish oil, omega-3 fatty acids and cardiovascular disease. *Circulation*, ***106*****(21),** 2747-2757.

Kristensen, M., Jensen, M. G., Aarestrup, J., Petersen, K. E., Søndergaard, L., Mikkelsen, M. S. and Astrup, A. (2012). Flaxseed dietary fibers lower cholesterol and increase fecal fat excretion, but magnitude of effect depends on food type. *Nutrition and Metabolism*, ***9*****(1),** 8.

Kristensen, M., Savorani, F., Christensen, S., Engelsen, S. B., Bügel, S., Toubro, S. and Astrup, A. (2013). Flaxseed dietary fibers suppress postprandial lipemia and appetite sensation in young men. *Nutrition, Metabolism and Cardiovascular Diseases*, ***23*****(2),** 136-143.

Kucukoner, E. and Haque, Z. U. (2003) Liyofilize edilmis protein kaynakli yag ikame maddelerinin Edam peynirinin tekstur ve olgunlasmasina etkisi. *Gida, **28,*** 227–233.

Kulkarni S. (1983). Studies on rheology of butter as influenced by processing conditions (*Doctoral dissertation*, NDRI, Karnal).

Kulkarni, S. and Ramamurthy, M. K. (1985). Studies on changes in rheological characteristics of butter stored at different temperature for different periods. *Indian Journal of Dairy Science.*

Kuruppu, D.P., Schmidt, K., Langerak, D. I., Van Duren, M. D. A. and Farkas, J. B. (1983). The effect of irradiation and fumigation on the antioxidative properties of some spices. IFFIT Report No: 37, 22.

Landis, B. (2006). Does The Addition of Flaxseed Oil To Homemade Peanut Butter Affect Taste and Texture?

Lee, P. E. and Choo, W. S. (2015). Characterization of flaxseed oil emulsions. *Journal of Food Science and Technology*, ***52*(7),** 4378-4386.

Let, M. B., Jacobsen, C. and Meyer, A. S. (2007). Lipid oxidation in milk, yoghurt, and salad dressing enriched with neat fish oil or pre-emulsified fish oil. *Journal Agricultural Food Chemistry,* ***55(19)****,* 7802–7809.

Levey, A. S., Eckardt, K.U. and Tsukamoto, Y. (2005). Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney International,* ***67(6)****,* 2089-2100.

Leyvaa, D. R., Zahradkab, P., Ramjiawanc, B., Guzmanc, R., Alianid, M. and Piercea, G. N. (2011). The effect of dietary flaxseed on improving symptoms of cardiovascular disease in patients with peripheral artery disease: Rationale and design of the FLAX-PAD randomized controlled trial. *Contemporary Clinical Trials,* ***32(5),*** 724–730.

Lim, C. W., Norziah, M. H. and Lu, H. F. S. (2010). Effect of flaxseed oil towards physico-chemical and sensory characteristics of reduced fat ice-cream and its stability in ice cream upon storage. *International Food Research Journal,* ***17****,* 393-403.

Lin, X., Wang, Q., Li, W. and Wright, A. J. (2014). Emulsification of algal oil with soy lecithin improved DHA bio accessibility but did not change overall in vitro digestibility. *Food and Function*, ***5(11),*** 2913-2921.

Lőrinczy, D., Regdon Jr, G., Keller, B., Szakály, S. and Schäffer, B. (2007). Development of a DSC method for determination of certain technological parameters of margarine and mixed-fat spread. *Journal of Thermal Analysis and Calorimetry*, ***88*(2),** 351-354.

Makhlouf, J., Arul, J., Boudreau, A., Verret, P. and Sahasrabudhe, M. R. (1987). Fractionnement de la matière grasse laitière par cristallisation simple et son utilisation dans la fabrication de beurres mous. *Canadian Institute of Food Science and Technology Journal*, ***20*(4),** 236-245.

Mantzioris, E., Cleland, L. G., Gibson, R. A., Neumann, M. A., Demasi, M. and James, M. J. (2000). Biochemical effects of a diet containing foods enriched with n− 3 fatty acids. *The American Journal of Clinical Nutrition*, ***72*****(1),** 42-48.

Mani, I., Biswas, M. and Kumar, S. N. (2011). An open-label study on the effect of flax seed oil supplementation in the management of diabetes mellitus. *Journal of Dietary Supplements*, ***8(6),*** 77-95.

Markiewicz-Kęszycka, M., Czyżak-Runowska, G., Lipińska, P. and Wójtowski, J. (2013). Fatty acid profile of milk-a review. *Bulletin of the Veterinary Institute in Pulawy*, ***57*****(2),** 135-139.

Martinchik, A. N., Baturin, A. K., Zubtsov, V. V. and Molofeev, V. (2012). Nutritional value and functional properties of flaxseed. *Voprosy Pitaniia*, ***81*****(3),** 4-10.

McClements, D. J. (2004). Protein-stabilized emulsions. *Current Opinion in Colloid and Interface Science*, ***9*****(5),** 305-313.

McGillivray, W. A. (1972). Softer butter from fractionated fat or by modified processing. *New Zealand Journal of Dairy Science and Technology,* ***7,*** 111-112.

McKenna, B. M. and Kilcast, D. (2003). Using emulsifiers to improve food texture. *In: Texture in Food-Semi-Solid Foods,* (Vol. 1, pp. 216–250). Woodhead Publishing, England.

Mohamed, O. E., Satter, L. D., Grummer, R. R. and Ehle F.R. (1988). Influence of dietary cottonseed and soybean on milk production and composition. *Journal of Dairy Science,* ***71,*** 2677-2688.

Mondello, L., Casilli, A., Tranchida, P. Q., Costa, R., Chiofalo, B., Dugo, P. and Dugo, G. (2004). Evaluation of fast gas chromatography and gas chromatography–mass spectrometry in the analysis of lipids. *Journal of Chromatography Analysis*, ***1035*****(2)**, 237-247.

Moraes, É. A., Dantas, M. I. de, S., Morais, D., de, C., Silva, C. O., da, Castro, F. A. F. de, Martino, H. S. D. and Ribeiro, S. M. R. (2010). *Sensory evaluation and nutritional value of cakes prepared with whole flaxseed flour. Ciência e Tecnologia de Alimentos,* ***30(4),*** *974–979.*

Mori, T. A., Burke V., Puddey, I. B., Watts, G. F. and O'Neal, D. N. (2000). Purified eicosapentaenoic and docosahexaenoicacids have differential effects on serum lipids and lipoproteins LDL particle size glucose, and insulin in mildly hyperlipidemic men. *American Journal of Clinical Nutrition*, ***71,*** 1085–94.

Mridula, D., Kaur, D., Nagra, S., Barnwal, P., Gurumayum S. and Singh, K. (2012). Effect of dietary flaxseed supplementation on egg production and quality in laying hens. *Indian Journal of Poultry Science,* ***47,*** 40-47.

Mridula, D., Singh, K. K. and Barnwal, P. (2013). Development of omega-3 rich energy bar with flaxseed. *Journal of Food Science and Technology*, ***50*****(5),** 950-957.

Nabar, A. B., Srinivasan, M. R. and Iya, K. K. (1969). Studies on the rheological properties of butter made from buffalo cream. *Indian Journal of Dairy Science*.

Nadeem, M., Abdullah, M., Hussain, I., Inayat, S., Javid, A. and Zahoor, Y. (2013). Antioxidant potential of Moringa oleifera leaf extract for the stabilisation of butter at refrigeration temperature. *Czech Journal of Food Sciences*, ***31*****(4),** 332-339.

Najgebauer-Lejko, D., Grega, T., Sady, M. and Domagała, J. (2009). The quality and storage stability of butter made from sour cream with addition of dried sage and rosemary. *Biotechnology in Animal Husbandry*, ***25***(5-6-2), 753-761.

National kidney Foundation, (2002). K/DOQI Clinical practice guidelines for chronic kidney disease evaluation, classification and stratification. *American Journal of Kidney Diseases*, ***39(2)*** (suppl 1): S1-S266.

Nazni, P., Amrithaveni, M. and Poongodi, V. T. (2006). Impact of flaxseed based therapeutic food on selected type II diabetic patients. *Indian Journal of Nutrition and Dietetics*, ***43,*** 141-145.

Nelson, K. A. S. and Martini, S. (2009). Increasing omega fatty acid content in cow's milk through diet manipulation: Effect on milk flavor. *Journal of Dairy Science*, ***92(4),*** 1378-1386.

Oomah, B. D. and Mazza, G. (1998). Flaxseed products for disease prevention. *In: Functional Foods: Biochemical and Processing Aspects,* (pp. 91–138). Mazza, G., Ed., Technomic Publishing Co, Inc., Lancaster, PA.

Ozcan, M. and Ayar, A. (2003). Effect of propolis extracts on butter stability. *Journal of Food Quality*, 65-73.

Pathare, P. B., Opara, U. L. and Al-Said, F. A. (2013). Colour measurement and analysis in fresh and processed foods, A Review. *Food Technology and Biotechnology, **6,*** 36-60.

Pekka, P., Bo, N. and World Health Organization (2011). Global Atlas on Cardiovascular Disease Prevention and Control (PDF). World Health Organization in collaboration with the World Heart Federation and the World Stroke Organization. pp. 3–18.

Popa, V. M., Gruia, A., Raba, D. N., Dumbrava, D., Moldovan, C., Bordean, D. and Mateescu, C. (2012). Fatty acids composition and oil characteristics of linseed (*Linum Usitatissimum L.)* from Romania. *Journal of Agro Alimentary Processes and Technologies*, ***18*****(2),** 136-140.

Potter, N.W. and Hotchkis, J. (1995). Fat, Oils, and Related Products. *In: Food Science,* (pp. 359-380). AVI Publishing Co. Inc., New York.

Potter, N.W. and Hotchkis, J. (1998). *Food Science,* AVI Publishing Co. Inc., New York.

Prentice, J. H. (1972). Rheology and texture of dairy products. *Journal of Texture Studies*, ***3****,* 415–458.

Rachana, C. R., Nath, B. S., Reshma, M. V. and Armughan, C. (2013). Variation in grainy texture of commercial ghee in relation to laboratory ghee and its blends. *International Journal of Dairy Technology, **66(1),*** 90-97.

Rajiv, J., Indrani, D., Prabhasankar, P. and Rao, G. V. (2012). Rheology, fatty acid profile and storage characteristics of cookies as influenced by flaxseed (*Linum usitatissimum*). *Journal of Food Science and Technology*, ***49*****(5),** 587-593.

Ramcharitar, A., Badrie, N., Mattfeldt-Beman, M., Matsuo, H. and Ridley, C. (2005). Consumer acceptability of muffins with flaxseed (*Linum usitatissimum*). *Journal of Food Science, **70*****(7),** 504-507.

Rousseau, D., Zilnik, L., Khan, R. and Hodge, S. (2003). Dispersed phase destabilization in table spreads. *Journal of the American Oil Chemists' Society, **80*****(10),** 957-961.

Santos, A. P., Rogero, M. M. and Bastos, D. H. (2010). Edible plants, their secondary metabolites and antiobesogenic potential. *Recent Patents on Food, Nutrition, and Agriculture*, ***2,*** 195–212.

Sarnak, M. J., Levey, A. S., Schoolwerth, A. C., Coresh, J., Culleton, B., Hamm, L. L., and Parfrey, P. (2003). Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. *Circulation*, ***108*****(17),** 2154-2169.

Schaap, J. E., Hagedoorn, H. G., and Rutten, G. A. M. (1981). Effect of storage time, temperature and working on the firmness and spreadability of butter. *Zuivelzicht (Netherlands).*

Schmidt, S. J. and Fontana Jr, A. J. (2008). Water activity values of select food ingredients and products. In: *Water Acitivity in Foods: Fundamentals and Applications,* (*pp.* 407-420). *GV Barbosa-Cánovas, AJ Fontana Jr., SJ Schmidt, TP Labuza (Eds.), Blackwell Publishing and the Institute of Food Technologists, Ames, Iowa, USA.*

Serna-Saldivar, S. O., Zorrilla, R., De La Parra, C., Stagnitti, G. and Abril, R. (2006). Effect of DHA containing oils and powders on baking performance and quality of white pan bread. *Plant Foods Human Nutrition*, ***61(3),*** 121–129.

Shiota, M., Konishi, H. and Tatsumi, K. (1999). Oxidative stability of fish oil blended with butter. *Journal of Dairy Science*, ***82*****(9),** 1877-1881.

Shukla, A., Bhaskar, A. R., Rizvi, S. S. H. and Mulvaney, S. J. (1994). Physicochemical and rheological properties of butter made from super critically fractionated milk fat. *Journal of Dairy Science*, ***77*****(1),** 45-54.

Silva-Kazama, D. C. D., Santos, G. T. D., Pintro, P. T. M., Visentainer, J. V., Kazama, R., Petit, H. V. and Marchi, F. E. D. (2010). Effect of storage on fatty acid profile of butter from cows fed whole or ground flaxseed with or without monensin. *Revista Brasileira de Zootecnia*, ***39*****(10),** 2297-2303.

Simopoulos, A. P. (2010). Genetic variants in the metabolism of omega-6 and omega-3 fatty acids: their role in the determination of nutritional requirements and chronic disease risk. *Experimental Biology and Medicine*, ***235*****(7),** 785-795.

Simopoulos, A. P., Leaf, A. and Salem, N. (1999). Essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. *Annals of Nutrition and Metabolism*, ***43,*** 127–131.

Singh, K. K., Mridula, D., Rehal, J. and Barnwal, P. (2011a). Flaxseed: a potential source of food, feed and fiber. *Critical Reviews in Food Science and Nutrition*, ***51*****(3),** 210-222.

Singh, R. B., Moshiri, M., De Meester, F., Juneja, L., Muthusamy, V. and Manoharan, S. (2011b). The evolution of low ω-6/ ω-3 ratio dietary pattern and risk of cardiovascular diseases and diabetes. *Journal of Alternative Medicine Research,* ***3,*** 45-70.

Starling, S. (2008). Omega-3 in infant formula world first. Available from www.nutraingredients.com/Industry/Omega-3-in-infant-formulaworld-first. Accessed January 20, 2019.

Stillwell, W. and Wassall, S. R. (2003). Docosahexaenoic acid: membrane properties of a unique fatty acid. *Chemistry and Physics of Lipids*, ***126*****(1),** 1-27.

Tambade P. B. (2018). Microencapsulation of flaxseed oil for fortification of milk with omega- 3 fatty acids, *M. Tech thesis,* NDRI, SRS, Bangalore.

Tomaszewska-Gras, J. (2012). Detection of butter adulteration with water using differential scanning calorimetry. *Journal of Thermal Analysis and Calorimetry*, ***108*****(2),** 433-438.

Tomaszewska-Gras, J. (2016). Rapid quantitative determination of butter adulteration with palm oil using the DSC technique. *Food Control,* ***60,*** 629-635.

Trautwein, E. A. (2001). n-3 Fatty acids-physiological and technical aspects for their use in food. *European Journal of Lipid Science and Technology,* ***103****,* 45–51.

Truan, J. S., Chen, J. M. and Thompson, L. U. (2010). Flaxseed oil reduces the growth of human breast tumors (MCF7) at high levels of circulating estrogen. *Molecular Nutrition and Food Research*, ***54(10),*** 1414-1421.

Ullah, R., Nadeem, M. and Imran, M. (2017). Omega-3 fatty acids and oxidative stability of ice cream supplemented with olein fraction of chia (*Salvia hispanica L.*) oil. *Lipids in Health and Disease*, ***16*****(1),** 34.

Umesha, S. S., Manohar, R. S., Indiramma, A. R., Akshitha, S. and Naidu, K. A. (2015). Enrichment of biscuits with microencapsulated omega-3 fatty acid (Alpha-linolenic acid) rich Garden cress (*Lepidium sativum*) seed oil: Physical, sensory and storage quality characteristics of biscuits. *LWT-Food Science and Technology*, ***62(1),*** 654-661.

USDA Nutrient Data Laboratory. Available at: http://www.nalusda.gov/fnic/foodcomp./ Accessed October 3, 2018.

Veena, N., Nath, B. S., Srinivas, B. and Balasubramanyam, B. V. (2017). Quality attributes of dahi prepared from milk fortified with omega-3 fatty acids, phytosterols and polydetxrose. *Journal of Food Science and Technology*, ***54*(7),** 1765-1775.

Venegas-Calerón, M., Sayanova, O. and Napier, J. A. (2010). An alternative to fish oils: metabolic engineering of oil-seed crops to produce omega-3 long chain polyunsaturated fatty acids. *Progress in Lipid Research*, ***49*(2),** 108-119.

Vijaimohan, K., Jainu, M., Sabitha, K. E., Subramaniyam, S., Anandhan, C. and Devi, C. S. (2006). Beneficial effects of alpha linolenic acid rich flaxseed oil on growth performance and hepatic cholesterol metabolism in high fat diet fed rats. *Life Sciences*, ***79*(5),** 448-454.

Vuksan, V., Whitham, D., Sievenpiper, J. L., Jenkins, A. L., Rogovik, A. L., Bazinet, R. P. and Hanna, A. (2007). Supplementation of conventional therapy with the novel grain Salba (*Salvia hispanica L.*) improves major and emerging cardiovascular risk factors in type 2 diabetes: results of a randomized controlled trial. *Diabetes Care*, ***30*(11),** 2804-2810.

Wainwright, P. E., Bulman-Fleming, M. B., Levesque, S., Mustsaers, L. and Mutcheon, D. (1998). A saturated fat diet during development alters dendritic growth in mouse brain. *Nutrition Neuroscience*, ***1,*** 49–58.

Ward, G. R., Huang, Y. S., Bobik, E., Xing, H. C., Mutsaers, L., Auestad, N. and Wainwright, P. (1998). Long-chain polyunsaturated fatty acid levels in formulae influence deposition of docosahexaenoic acid and arachidonic acid in brain and red blood cells of artificially reared neonatal rats. *The Journal of Nutrition*, ***128*(12),** 2473-2487.

Wasnik, P. G., Menon, R. R., Surendra Nath, B., Balasubramanyam., B. V., Manjunatha, M. and Sivaram, M. (2017). Application of particle analysis and colour parameters for detection of adulteration of cow ghee with vanaspati derived from image analysis. *Indian Journal of Dairy Science*, ***70(2),*** 200-208.

Wilbey, R. A. (1994). Production of butter and dairy based spreads. In: *Advances in milk processing*, (Vol. 1, pp. 107-158). Robinson, R. K., Ed., Chapman and Hall, New York.

Woods, V. B. and Fearon, A. M. (2009). Dietary sources of unsaturated fatty acids for animals and their transfer into meat, milk and eggs: A review. *Livestock Science*, ***126(1-3),*** 1-20.

Yep, Y. L., Li, D., Mann, N. J., Bode, O. and Sinclair, A. J. (2002). Bread enriched with microencapsulated tuna oil increases plasma docosahexaenoic acid and total omega-3 fatty acids in humans. *Asia Pacific Journal of Clinical Nutrition*, ***11*****(4),** 285-291.

APPENDIX

Sensory Evaluation Performa

Name: ______________________ **Date:**

Sample: Butter fortified with omega-3 fatty acid

Kindly evaluate the given samples for various attributes on the nine-point Hedonic scale given below:

Like extremely	9	Dislike slightly	4
Like very much	8	Dislike moderately	3
Like moderately	7	Dislike very much	2
Like slightly	6	Dislike extremely	1
Neither like nor dislike	5		

Attributes / Sample Code	Colour & appearance	Body & texture	Spreadability	Flavour	Overall acceptability

Remarks, if any

Signature

Pandule Vishal Shrirang

National Dairy Research Institute, (Ind.)

To carve out a niche for myself in dairy industry by applying my knowledge and working in challenging and competitive environment, thereby contributing to the growth of self and that of organization.

vishalspandule@gmail.com | +918805942111 | Pusad, Maharashtra | DOB: 1 Jul 1995

EDUCATION

M.Tech
National Dairy Research Institute

08/2017 – 06/2019 *Karnataka, India*

OGPA 8.26

- Dairy Technology
- Thesis Title: Development and quality evaluation of omega-3 fatty acid fortified butter

B.Tech
College of Dairy Technology, Warud

08/2013 – 06/2017 *Pusad, Maharashtra*

OGPA 8.12

- Dairy Technology

High School & Intermediate
Maharashtra State Board

2011 – 2013 *Pusad, Maharashtra*

Courses

- Koshatwar Daulatkhan Vidyalaya,Pusad, 93.07% - High School
- Phulsing Naik Mahavidyalaya, Pusad, 75.00 %, Intermediate

WORK EXPERIENCE

In plant training
ICAR-National Dairy Research Institute

02/2017 – 06/2017 *Bangalore, India*

Southern Regional Station

Tasks/Achievements

- Market Milk Section, QC, Utilities and Maintenance
- Plant capacity of 1000 LPD. - Experience processing of liquid milk and packaging. - Experience production of Kunda, Paneer, Cheddar Cheese, Chhana Podo, Processed cheese

Vaishno Devi Food Products Pvt. Ltd., Babhalgaon
Internship *Osmanabad, Maharashtra*

01/2016 – 02/2016

Tasks/Achievements

- Plant capacity of 3,00,000 LPD
- Underwent training in the areas of RMRD section, Powder section, Butter section, Ghee section, Quality assurance section
- Experience processing of liquid milk and packaging

Six Month Hands-On Training
Student Training Dairy Plant

07/2015 – 12/2015 *Pusad, Maharashtra*

- College of Dairy Technology, Warud (Pusad)

SKILLS & COMPETENCES

Communication Skills	●●●●○
Computer Application	●●●○○
Quality Assurance	●●●●○
Research & Development	●●●●○
Team Work	●●●●●

FELLOWSHIPS & AWARDS

Secured 7th rank in ICAR-PG entrance (2017)

- Organized by Indian Council of Agricultural Research

Institutional Scholarship (08/2017 – Present)

- ICAR-National Dairy Research Institute fellowship for post graduate students

Rajarshi Chhatrapati Shahu Maharaj Scholarship (2011-2013)

- Government of Maharashtra for 11th and 12th standards students

CERTIFICATIONS

Maharashtra State Certificate in Information Technology (2011)
Maharashtra State Board of Technical Education, Grade 95%

Participated and presented poster in national conference on “Innovative Approach for Enhancing Dairy Farmers Income” (02/19)
Organised by Indian Dairy Association (East zone)

Participated and presented paper in international conference on “Emerging Scenario in Agribusiness” (03/19)
Organised by Indian Institute of Plantation Management, Bengaluru

REFERENCES

Dr. Monika Sharma (2017 – Present)
Scientist, Dairy Technology Division, ICAR-NDRI
Email: sharma.monikaft@gmail.com

Dr. Bikash Chandra Ghosh (2017 – Present)
Principal Scientist, Dairy Technology Division, ICAR-NDRI
Email: ghosgoga@hotmail.com

LANGUAGES

Marathi
Native

Hindi
Expert level

English
Upper-intermediate

INTERESTS

Travelling | Music | Singing | Sketching