EXTRACTION OF MILK FAT FROM GHEE RESIDUE USING LOW TOXICITY SOLVENTS

THESIS SUBMITTED TO THE

ICAR-NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL

(DEEMED UNIVERSITY)

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE AWARD OF THE DEGREE OF

MASTER OF TECHNOLOGY

IN

DAIRY TECHNOLOGY

BY

WANI AAKASH DADARAO

B. Tech (Dairy Technology)

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ICAR-NATIONAL DAIRY RESEARCH INSTITUTE

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KARNAL-132001 (HARYANA), INDIA

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Approved by:

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This is to certify that the thesis entitled, **"Extraction of milk fat from *ghee* residue using low toxicity solvents"** submitted by **Mr. WANI AAKASH DADARAO** towards the partial fulfilment of the award of the degree of **Master of Technology 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 supervision, and no part of the thesis has been submitted for any other degree or diploma.

Dated: 21th September, 2021

Dr. Writdhama Prasad
MAJOR ADVISOR & CHAIRMAN
(GUIDE)


DEDICATED TO MY
BELOVED FAMILY
AND
RESPECTED GUIDE

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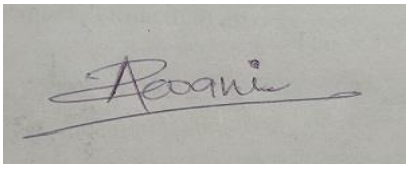

Date: - ***21th September, 2021*** ***WANI AAKASH DADARAO***

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List of Abbreviations

Abbreviated form	Full form
%	Percentage
°C	Degree centigrade
µg	Microgram
µl	Microlitre
A.R.	Analytical grade
CAGR	Cumulative annual growth rate
df	Degree of freedom
DM	Dry matter
et al.	et alii (and others)
F	Frequency
g	Gram
G’	Storage modulus
G’’	Loss modulus
hr	Hour
lt.	Liter
m	Meter
mg	Milligram
min.	Minute
mm	Millimeter
ml	Milliliter
mg / L	Milli gram per liter
mM	Milli molar
Max.	Maximum
N	Newton
P<0.05	Significant if probability is less than 0.05
P>0.05	Non-Significant if probability is more than 0.05
pH	Negative log of the hydrogen ion concentration
Rpm	Rotations per minute
s	Seconds
UV	Ultra-violate
viz.	Videlicet (namely)
Vol.	Volume
w/v	Weight by volume
w/w	Weight by weight
Wt.	Weight
kgbw	Kilogram of body weight
FSSR	Food Safety and Standard Regulation
US	United State
%OA	Percent Oleic acid
%LA	Percent Lactic acid
M	Molar
SD	Standard Deviation
mPa.s	Milli pascal second
Pa.s	Pascal second

GRAS	Generally Recognized as Safe
EA	Ethyl acetate
DMC	Dimethyl carbonate
2-MTHF	2-methyl tetrahydrofuran
J	Joules
FSSAI	Food Safety and Standards Authority of India
LD_{50}	Lethal dose 50
FTIR	Fourier transform infrared spectroscopy
ETP	Effluent treatment plant

“Extraction of milk fat from ghee residue using low toxicity solvents”

ABSTRACT

Ghee residue (GR) is a by-product obtained during ghee preparation. Amount of GR depends upon the method of ghee preparation, but its quantity is about one tenth of the amount of ghee produced. Fat content in the GR ranges from 32 to 70 % (weight basis). Considering the amount of fat in GR, different interventions for extraction of the milk fat from GR, viz., pressing, hydrothermal treatment and centrifugal separation has been explored. But these techniques are time consuming, labour intensive and generates huge amount of waste water. Solvent extraction is commonly used in oilseed industry to extract residual oil from the pressed oilseed cakes. It is reported to yield higher oil extraction efficiency as compared to mechanical extraction methods. The present investigation was aimed at using solvent extraction method to extract milk fat from GR using low toxicity solvents, viz., ethanol, 2-methyl tetrahydrofuran (MeTHF), ethyl acetate (EA) and dimethyl carbonate (DMC). Water and hexane were used as control solvent, and ghee was used for comparison with the solvent extracted milk fat. It was observed that milk fat extracted using ethanol, MeTHF and hexane was dark brown in color, which was entirely different from the control (ghee) sample. Milk fat could not be extracted from ghee residue using water, because of viscous paste formation that was difficult to filter. On the other hand, milk fat obtained using EA and DMC resembled more to the ghee sample and thus was selected for further studies. In the next part of the study, milk fat was extracted using EA and DMC at different temperatures. Increasing the extraction temperature increased the yield of milk fat extraction from GR. Highest milk fat extraction efficacy was obtained using EA at 60^{o}C extraction temperature. Free fatty acids (FFA) content increased and iodine value decreased with an increase in the extraction temperature. Ghee (control) sample had lower FFA content and higher iodine value as compared to the solvent extracted milk fat samples. The DMC extracted milk fat at 80oC had highest viscosity, followed by DMC (50^{o}C), EA (60^{o}C), ghee (control) and least in EA (50^{o}C) extracted sample. All the milk fat samples exhibited shear thinning behaviour, i.e., decreased viscosity with an increase in the applied shear rate. Frequency sweep analysis of extracted milk fat revealed that all the samples had higher G’ than G” value, which indicated about dominance of elastic behaviour. The FTIR spectra of ghee (control) were compared with solvent extracted milk fat samples. The EA (60^{o}C) extracted milk fat showed more similarity ($p>0.05$) to the FTIR spectra of control ghee sample, and thus was selected for further studies. The solvent extraction process for milk fat extraction from ghee residue was compared with conventional process for energy consumption, time duration and waste water generation. It was found that the solvent extraction process requires less amount of time, does not generate waste water and requires slightly higher energy. This indicates that the solvent extraction process using low toxicity solvents could be employed for milk fat extraction from GR as an environment friendly process then their conventional counterparts.

सार

घी अवशेष (जीआर) घी तैयार करने के दौरान प्राप्त एक उप-उत्पाद है। जीआर की मात्रा घी बनाने की विधि पर निर्भर करती है, लेकिन इसकी मात्रा उत्पादित घी की मात्रा का लगभग दसवां हिस्सा होती है। जीआर में वसा की मात्रा 32 से 70% (वजन के आधार पर) के बीच होती है। जीआर में वसा की मात्रा को ध्यान में रखते हुए, जीआर से दूध वसा निकालने के लिए विभिन्न हस्तक्षेपों, जैसे दबाने, हाइड्रोथर्मल उपचार और केन्द्रापसारक पृथक्करण का पता लगाया गया है। लेकिन ये तकनीकें समय लेने वाली, श्रमसाध्य हैं और भारी मात्रा में अपशिष्ट जल उत्पन्न करती हैं। सॉल्वेंट एक्सट्रैक्शन आमतौर पर तिलहन उद्योग में दबाए गए तिलहन केक से अवशिष्ट तेल निकालने के लिए उपयोग किया जाता है। यह यांत्रिक निष्कर्षण विधियों की तुलना में उच्च तेल निष्कर्षण दक्षता प्राप्त करने की सूचना है। वर्तमान जांच का उद्देश्य कम विषाक्तता वाले सॉल्वैंट्स, जैसे इथेनॉल, 2-मिथाइल टेट्राहाइड्रोफुरन (MeTHF), एथिल एसीटेट (EA) और डाइमिथाइल कार्बोनेट (DMC) का उपयोग करके GR से दूध वसा निकालने के लिए विलायक निष्कर्षण विधि का उपयोग करना था। पानी और हेक्सेन को नियंत्रण विलायक के रूप में इस्तेमाल किया गया था, और घी का इस्तेमाल विलायक निकाले गए दूध वसा के साथ तुलना करने के लिए किया गया था। यह देखा गया कि इथेनॉल, एमईटीएचएफ और हेक्सेन का उपयोग करके निकाला गया दूध वसा गहरे भूरे रंग का था, जो नियंत्रण (घी) के नमूने से बिल्कुल अलग था। पानी का उपयोग करके घी के अवशेषों से दूध की चर्बी नहीं निकाली जा सकती, क्योंकि चिपचिपा पेस्ट बनने के कारण जिसे छानना मुश्किल था। दूसरी ओर, ईए और डीएमसी का उपयोग करके प्राप्त दूध वसा घी के नमूने के समान था और इस प्रकार आगे के अध्ययन के लिए चुना गया था। अध्ययन के अगले भाग में, विभिन्न तापमानों पर ईए और डीएमसी का उपयोग करके दूध वसा निकाला गया। निष्कर्षण तापमान में वृद्धि से जीआर से दूध वसा निष्कर्षण की उपज में वृद्धि हुई। उच्चतम दूध वसा निष्कर्षण प्रभावकारिता 60^{o}C निष्कर्षण तापमान पर ईए का उपयोग करके प्राप्त की गई थी। निष्कर्षण तापमान में वृद्धि के साथ मुक्त फैटी एसिड (एफएफए) सामग्री में वृद्धि हुई और आयोडीन मूल्य में कमी आई। घी (नियंत्रण) के नमूने में विलायक से निकाले गए दूध वसा नमूनों की तुलना में कम एफएफए सामग्री और उच्च आयोडीन मूल्य था। 80^{o}C पर निकाले गए दूध वसा में सबसे अधिक चिपचिपापन था, इसके बाद DMC (50^{o}C), EA (60^{o}C), घी (नियंत्रण) और सबसे कम EA (50^{o}C) निकाले गए नमूने में था। सभी दूध वसा नमूनों ने अपरूपण थिनिंग व्यवहार प्रदर्शित किया, अर्थात लागू अपरूपण दर में वृद्धि के साथ श्यानता में कमी आई। निकाले गए दूध वसा के आवृत्ति स्वीप विश्लेषण से पता चला है कि सभी नमूनों में जी 'मूल्य से अधिक जी' था, जो लोचदार व्यवहार के प्रभुत्व के बारे में दर्शाता है। घी (नियंत्रण) के एफटीआईआर स्पेक्ट्रा की तुलना सॉल्वेंट एक्सट्रैक्टेड दूध वसा के नमूनों से की गई। ईए (60^{o}C) निकाले गए दूध वसा ने नियंत्रण घी के नमूने के FTIR स्पेक्ट्रा के लिए अधिक समानता ($p > 0.05$) दिखाई, और इस प्रकार आगे के अध्ययन के लिए चुना गया। घी के

अवशेषों से दूध वसा निष्कर्षण के लिए विलायक निष्कर्षण प्रक्रिया की तुलना ऊर्जा खपत, समय अवधि और अपशिष्ट जल उत्पादन के लिए पारंपरिक प्रक्रिया से की गई थी। यह पाया गया कि विलायक निष्कर्षण प्रक्रिया में कम समय लगता है, अपशिष्ट जल उत्पन्न नहीं होता है और थोड़ी अधिक ऊर्जा की आवश्यकता होती है। यह इंगित करता है कि कम विषाक्तता वाले सॉल्वैंट्स का उपयोग करने वाली विलायक निष्कर्षण प्रक्रिया को जीआर से दूध वसा निष्कर्षण के लिए पर्यावरण के अनुकूल प्रक्रिया के रूप में उनके पारंपरिक समकक्षों के रूप में नियोजित किया जा सकता है।

Chapter 1

Introduction

1. INTRODUCTION

A by-product may be defined as a product of commercial value obtained during the manufacture of the main product. Utilization of dairy by-products improves the plant's economy, makes valuable nutrients available for humans and decreases environmental pollution originating from dairy industries. Skim milk, buttermilk, ghee residue and whey are among the major by-products generated in a dairy plant, among which *ghee* residue has gained relatively less attention as compared to others. *Ghee* residue is a blackish brown residue obtained as a by-product during *ghee* preparation. It mainly consists of milk proteins, lactose and small quantity minerals.

Ghee is heat clarified milk fat with unique organoleptic attributes. About 30 - 35% of total milk produced in India is utilized for *ghee* preparation and the average yield of ghee residue is about a tenth of the *ghee* produced (Ramesh *et al*., 2018). Ghee is prepared using different methods, all of which involves a heating step in which milk fat is exposed to different time-temperature combination with small amount of serum solids. At the end of ghee preparation, the serum solid's part that coagulates during thermal processing appears as *ghee* residue. At industrial scale, it is mostly considered as waste because of lack of processing interventions owing to its intense flavour profile and dark brown colour. Ghee residue contains about 34% fat and most of the industries could not utilize this residue completely in the form of different confectionary products. This results into loss of milk fat in the form of ghee residue because of unavailability of proper processing intervention of the ghee residue to extract fat from it. Application of techniques such as pressing and centrifugal separation for extraction of ghee from the residue has an efficiency of about 45% and 70%, respectively. However, still the press cake contains 33-38 % ghee, which is a considerable amount and emphasizes on the need of alternative techniques for fat extraction form ghee residue.

Solvent extraction has been reported as an efficient method for recovering oil from materials with low oil content, including pre-pressed oil cakes (Xu and Godber, 2000). Solvent extraction is based on the solubility of compound of interest in a suitable solvent to extract it from the source material. Solvent extraction using various solvents is common and is more efficient than the methods replying mainly on mechanical means. This method is commonly used in various industries to extract residual oil from the pressed oilseed cake. Efeovbokhan *et al*. (2015) studied different solvents for extraction of oil from two types of *Moringa*

oleifera seeds (northern and southern). The author concluded that petroleum ether had highest yield of oil about 49.38% and 37.57%, followed by hexane with 44.94 and 34.71%, while isopropyl alcohol gave 36.39 and 28.43% yield for northern and southern samples, respectively. Use of petroleum-based solvents for oil extraction is still a common practice, but its higher toxicity is growing a concern for the food industries. Koteswararao *et al*. (2014) has reported about the adverse effects on respiratory and nervous systems on long-term exposures of petroleum solvent. Solvent treatment, such as hydrocarbon halogen, petroleum distillate and diethylene glycol, can cause renal tubular necrotic disease and other associated health problems. This has contributed to the quest for less toxic solvents as an alternative to conventionally used petroleum-based solvents. Using low toxicity solvents decreases the harmful health impacts that arises due to toxicity and pollution caused by the petroleum-based solvents. Biondo *et al.* (2015) reported higher efficiency of lipids extraction from canola and soyabean seeds using ethanol as compared to hexane. Gharby *et al*. (2020) examined suitability of 2-methyloxolane for lipid extraction from cactus seeds. The authors reported that 2-methyloxolane gave higher oil yield of (9.55 ± 0.12 g/100 g) than n-hexane (8.86 ± 0.25 g/100 g). Although these low toxicity solvents have been successively used for fat extraction from oilseeds, but their application in dairy industry to recover milk fat from ghee residue has never been studied. Considering this, it appears that there is an opportunity to use low toxicity solvents for extraction of milk fat from ghee residue using low toxicity solvents. Hence, the present investigation 'Extraction of milk fat from ghee residue using low-toxicity solvents' was undertaken with the following objectives:

1) **Optimization of extraction condition for milk fat extraction from ghee residues using low toxicity solvents**
2) **Comparison of extracted fat with ghee**

Chapter 2

Review of literature

2. REVIEW OF LITERATURE

India is a tropical country with wide variations in ambient temperature depending upon region and seasonal changes. Because of that, it is very difficult to maintain the cold chain for the perishable products such as milk and butter. Whenever surplus milk is available it is better approach to preserve it by converting into ghee, by following methods such as removal of cream and convert into butter. The butter fat is then clarified at higher temperature to prepare ghee.

2.1. *Ghee* residue

Dairy by-products have high nutritive value, and most of them are usually channelized to food industries for different product preparation (Tamine, 2009). Gandhi *et al*. (2013) stated that 30-35% of the overall milk produced in India (187.9 million tons in 2018-19) is converted into ghee. About 1.5 million tons of ghee and 45000 tons of ghee residue is produced in India (Gupta, 2017). Tamine (2009) reported *ghee* residue as a blackish brown residue, which is obtained as by-product during ghee preparation. It consists of mainly milk proteins and some quantity of lactose and minerals. The Ghee residue has been used in food industries for making sweets, bakery products and as a flavour enhancer. According to Verma and Narendra Raju (2008), ghee residue contains about 10, 11 and 132 time more carbonyls, FFA and lactone content as compared to ghee, respectively.

2.1.1 Chemical composition and nutritional properties

Ghee residue has been reported as a rich source of protein, energy and minerals, especially calcium and phosphorus. But mostly it is considered as a waste at the industry level and discarded as waste that increases load on the ETP plant. Verma and Narendra Raju (2008) reported the overall composition and yield of ghee residue produced from different sources (Table 2.1).

The authors reported that yield of ghee residue was highest in ghee prepared from sweet cream as the fat (7.7 kg/ 100kg), followed by sour cream (5.1 kg/100kg) and washed sweet cream (3.5kg/ 100 kg). Fat content was highest in washed sweet cream source and lowest yield in the makkhan as the fat source (33.4kg / 100kg). Janghu *et al.* (2014) reported that the yield of ghee obtained by direct cream method (DCM) and creamery butter method (CBM) was 444g and 760 g per kg of raw material, respectively and the ghee residue obtained was 131.6g and 49.6g per kg of raw material in DCM and CBM, respectively.

Table 2.1: Chemical composition of *ghee* residue produced from different sources

Source of Ghee residue	Chemical composition (%)					yield GR kg/100kg
	Moisture	Fat	Protein	Lactose	Ash	
Makkhan	13.4	33.4	32.8	15.4	5.2	1.6
Creamery butter (unsalted)	5.7	65.0	25.5	Trace	3.8	1.2
Sweet cream	4.1	63.2	18.0	12.3	2.4	7.7
Sour cream	8.0	38.8	41.6	7.3	4.3	5.1
Washed sweet cream	1.7	80.8	16.2	Trace	1.3	3.5

(Verma and Narendra Raju, 2008)

Prahlad (1954) reported that ghee residue was stable at ambient temperature for up to 20 days, with slight increase in rancidity. Hardening of the body and texture leads to difficulties during processing and they applied certain approaches to increase the stability of ghee residue handling. Methods given as follows-

Table 2.2: Effect of different treatments on *ghee* residue composition

Particulars	Treatment I		Treatment II		Treatment III		Treatment IV	Treatment V		Treatment VI
	Before	After	Before	After	Before	After	After	Before	After	After
Acidity	18.8	9.2	20.6	-	18.1	10.3	-	20.6	5.0	-
Moisture	13.3	49.7	13.8	65.0	15.3	61.5	70.7	13.8	49.0	68.0
Fat	52.2	26.7	49.8	18.5	46.8	18.2	15	49.8	22.0	15.4
Protein	19.7	17.6	19.9	10.8	19.9	16	10.1	19.9	23.6	12.0
Lactose	11.5	3.8	12.5	2.5	13.1	1.1	1.6	12.5	1.2	1.4
Ash	3.3	2.2	4.0	3.2	4.3	2.3	2.6	4.0	3.2	3.2

Reference - (Prahlad,1954)

Treatment I Loosely tying the residue in the form of bundle and cooking in boiling water for 30 min.

Treatment II Cooking the residue in boiling 1.0% sodium bicarbonate for 30 min.

Treatment III Washing the residue with 50% alcohol and then cooking in boiling water for 30 min.

Treatment IV Washing the residue with 50% alcohol followed by boiling in 1% sodium bicarbonate.

Treatment V Autoclaving the residue (15 PSI/10 min) obtained from III after incorporating 2% vinegar

Treatment VI Autoclaving the residue obtained from IV after incorporating 2% vinegar.

Above all the treatments were carried out for making ghee residue soft and smooth. The author found that there are several changes occurred in the composition of ghee residue after each treatment. Ghee residues absorb moisture and in case of treatment 2, 4 and 6 acidities decreased to nil. Fat and lactose content also reduced. Excessive fat removal from residues by washing with 50% alcohol followed by cooking in baking soda (treatment -4) was the best among all the treatments. Moisture content was lowered and texture improvement occurred as the ghee residue was autoclaved with 2% vinegar. The shelf life of all GR clarified at 120^{o}C was 3 months at ambient temperature and it further extended to more than 4 months by pressing it in cake form.

Table 2.3: Chemical composition of *ghee* residue

Proximate principles	Composition
Moisture (%)	12.10 $\pm$ 2.24
Dry matter (%)	87.90 $\pm$ 2.24
Crude protein (%)	19.86 $\pm$1.34
Crude fibre (%)	3.49 $\pm$1.74
Ether extract (%)	47.12 $\pm$ 3.62
Total ash (%)	3.90 $\pm$ 0.32
NFE (%)	25.63 $\pm$ 3.71
Gross energy (estimated) (kcal/kg)	6256.17 $\pm$ 266.36
Calcium (%)	0.62 $\pm$ 0.07
Phosphorus (%)	0.62 $\pm$ 0.04
Salt (%)	0.68 $\pm$ 0.09
Sand silica (%)	0.14 $\pm$ 0.03
AME (kcal/kg)	5160 $\pm$ 72.10
TME (kcal/kg)	5290 $\pm$ 76.06

(Ramesh *et al.* 2018)

Ghee residue is an energy rich and highly nutritious by-product of dairy industry. Ramesh *et al.* (2018) reported that the moisture, crude protein, crude fibre, ether extract, nitrogen free extract and total ash contents of ghee residue were 12.10, 19.86, 3.49, 47.12, 25.63 and 3.90 per cent, respectively. It is rich in calcium (0.62%) and phosphorus (0.62%). Total energy provided by 1kg ghee residue is about 6256.17 Kcal. (Ramesh *et al.* 2018). The author also analysed the fatty acid and amino acid composition of ghee residue. It was found that the palmitic acid was highest (38.88%) among saturated fatty acids and oleic acid accounted for the highest percentage (25.15%) among unsaturated fatty acids. Linoleic, linolenic, eicosapentaenoic and docosahexaenoic acid content in ghee residue were 2.02, 0.79, 0.36 and 0.25 per cent, respectively. Amino acid profile of ghee residue revealed that the lysine and methionine, content were 0.99 and 0.61%, respectively. Threonine and arginine levels were found to be at 1.44 and 0.76 per cent, respectively. Glutamic acid was recorded the highest percentage (5.26), while cystine was in level (0.35%) among the amino acids in ghee residue.

2.1.2 Fat recovery methods

Discarding ghee residue containing high fat content increases the load on ETP plant and decreased the recovery of the dairy industry. To extract this fat from ghee residues, some work has been carried out. Since 1970 Viswanathan *et al*. (1973) studied two methods, namely centrifugal and direct pressure, for recovery of ghee from ghee residue.

Table 2.4: Methods of fat recovery from ghee residue

Sr.no	Method	Scale of experiment (gm)	Ghee yield (%)	Extraction efficiency (%)	Ghee in press cake (%)
1.	Centrifugal method	1500	24.9	46	-
2.	Pressure method				
a)	Hydraulic press	750	46.7	71	35
b)	Hand screw press	2500	48.2	74	33 - 38
		5000	45.0	70	
		7000	40.0	61	

Reference – Viswanathan *et al*. (1973)

The author reported that transferring the occluded ghee to water by heating the ghee residue in water and centrifuging the water-fat phase (centrifugal method) gave 25 % ghee yield (46 % efficiency). By pressure methods (hand screw-press and hydraulic press), the corresponding ghee yields were between 44.2 to 46.7% with the extraction efficiency of 61 to 74%, respectively. Thus, the centrifugal method appeared to be less efficient and hand screw-press method simple, efficient, practical and economical for adoption in ghee preparing dairy plants. However, the press cake which still contains 33-38 % ghee and 31 % protein.

Reddy *et al.* (1978) studied extraction of fat from ghee residue using hot water and brine solution. The authors reported that the average recovery of ghee from ghee residue treated with water was 48.51 %, whereas brine treatment had 53.46 % fat recovery. Another author did work on improved ghee yield from ghee residue, which involves multiple separation of cream and its effect on ghee recovery was determined. Usage of high fat cream produced by multiple ghee extraction reduced the fat loss in ghee residue. Single separated cream (60% fat) on second and third separation resulted in 64.60% and 77.12% fat with corresponding ghee recoveries of 94.74%, 95.64% and 95.80%, respectively. Further, negative correlation was observed between SNF of cream and recovery of ghee and a positive correlation between SNF of cream and fat loss in ghee residue (Pal and Rajorhia,1975).

2.1.3 Utilization in food Industry

Ghee residues are having various nutritional and beneficial properties. It contains excessive amount of fat, protein and minerals such as calcium and phosphorus. Also, it is a rich source of flavouring compounds such as carbonyls, lactones and FFAs. Analysis of ghee residue showed that palmitic acid (saturated fatty acid) and oleic acid (unsaturated fatty acid) present in high amount. Amino acid profile revealed that ghee residue contained high level of glutamic acid compare with the other. In addition, ghee residue is also a rich source of phospholipids. Because of these attributes, it has been used in various products preparation as flavouring agent.

Ranjan *et al.* (2020) studied the “nutritive value of ghee residue incorporated bakery product” with an objective to utilize ghee residue for the preparation of bakery products. Cake and muffins were prepared by addition of ghee residue in different proportions. The authors reported for both cake and muffins, 60% refined flour and 40% ghee residue incorporation yielded best product with regard to colour, body and texture and flavour attributes. In case of cake, content of calcium (71.35/100g), protein (23.48g/100g) and fat

(89.96g/100g) increased as compared to control. And in case of muffins, calcium (68.01mg/100g), protein (21.48g/100g) and fat (90.72g/100g) increased as compared to control. Cost of the products on the basis of raw ingredients per 100g was found to be Rs 29.00 for cake, Rs 31.00 for muffins.

Janghu *et al.* (2014) utilized ghee residue in confectionaries preparation *viz*., candy, chocolate and burfi. Candy, chocolate and burfi had sensory scores of 7.92, 7.77 and 7.12 points for overall acceptability on 9-point hedonic scale. During the storage study in polyethylene bags and glass containers, its significant increases in free fatty acid from 1.400 to 1.531% and 1.410 to 1.558%, respectively in candy, 0.939 to 1.043%, and 0.930 to 1.108%, respectively in chocolate and 1.128 to 1.249% and 1.128 to 1.278%, respectively in burfi, was observed till 30 days of storage. Verma & De (1978) reported the process for the preparation of ghee residues burfi using SMP, khoa, chocolate and sugar.

Dua *et al.* (2018) utilized ghee residue in burfi preparation by optimizing the level for ghee residue, sugar and khoa levels in the formulation of burfi. Highest sensory parameters were obtained at 40% ghee residue, 60% khoa and 30% sugar level. Further, different levels of corn flour viz. 3%, 6% and 9% were incorporated (by replacing the respective proportions of khoa) in the *ghee* residue *burfi*. The study concluded that ghee residue burfi had higher nutritional value (23.68% protein, 27.96% fat, lactose 18.79%, calcium 0.56% and phosphorus 0.50%) and could be a potential source for overcoming protein-energy malnutrition. Further, enrichment with 6% corn flour improved the textural score and overall acceptability of the product (Dua *et al.* 2018). Prasad (2012) utilized ghee residue for chocolate burfi preparation. The author prepared burfi by ghee residue addition at 5%, 10%, 15% and 20% level and reported that increasing the ghee residue level resulted into significant increase among the samples for energy, fat, protein, carbohydrate, moisture content along with sensory attributes. Geeta *et al.* (2010) prepared ghee residue based 'sweet cubes' comprising of ghee residue, khoa and different binding agents.

Ananthakumar *et al.* (2016) prepared candy using by-products from dairy (ghee residue) and juice industry (orange-peel). The authors reported that aqueous extract of orange peel powder increased the anti-oxidant activity in the product and the sample was stable up to 30 days at refrigerated temperatures. Similarly, Wadhwa (1997) formulated a candy using 1kg ghee residue, 500 – 625 gm sugar and 125 – 250 gm of dry coconut powder. Application of 50% sugar syrup instead of direct sugar addition improved the sensory acceptance of the product (Wadhwa, 1997). Panvelwala *et al.* (2016) prepared nutritional 'Choco-fudge' using ghee residue. The formulation comprised of 20gm ghee residue, 5g chocolate powder, 5g

orange zest (a candy), 15ml milk, ¼ teaspoon rose water and 1g almond. The developed product had sensory scores of 16.21 for flavour, 8 for texture and 19.92 for overall acceptability.

Table 2.5: Application of ghee residue in food industry

Product	Particulars	Reference
Bakery products (cakes and muffins)	Refined flour: GR in 60:40 had overall best quality in both products.	Ranjan *et al.* (2020)
Confectionaries (chocolate, burfi and candy)	Sensory score was found in between 7 to 8 measured on 9-point hedonic scale.	Janghu *et al.* (2014)
Chocolate burfi	Significant difference obtained in energy, fat, protein, carbohydrate, moisture with ghee residue addition.	Prasad *et al.* (2012)
Candy	1kg GR, 500 – 625gm sugar (50% syrup) and 125 – 250gm of dry coconut powder.	Wadhwa (1997)
'Choco-fudge'	Improvement in texture and flavour. Got 19.92 out of 25 points for overall acceptability.	Panvelwala *et al.* (2016)
Orange peel candy	Increase in antioxidant activity and sensory properties compare to control sample.	Ananthakumar *et al.* (2016)
Confectionary (corn flour burfi)	Sensory optimized formulation of 40% ghee residue, 60% khoa and 30g sugar. Enrichment with 6% corn flour improved the textural value and overall acceptability of the product.	Dua *et al.* (2018)
Ghee residue sweet cubes	Good in all sensory parameters. 6.64 out of 9 score for overall acceptability of the product,	Geeta *et al.* (2010)

2.1.4 Antioxidant and flavouring property of ghee residues

Ghee residue has been reported to be a good source of antioxidant compounds such as phospholipids and phenolics. Many studies have been reported for better recovery of these compounds from residues and application in different product to increase their shelf life. Lal *et al.* (1984) studied the process standardization for phospholipids transfer from ghee residue to ghee in order to improve its keeping quality. The authors reported that phospholipids could be extracted however; it was not convenient when compared with heat processing method. In heat-processing method, heating of ghee-residue with ghee in the ratio of 1: 4 at 130°C yielded optimum transfer of phospholipids from ghee-residue to ghee.

Murthy *et al*. (1968) reported that phospholipids possess antioxidant activity which help to improve quality of preservation. Addition of 1–5% at different levels ghee residue to ghee made from cow and buffalo milk fat at different levels containing about 6.012% of phospholipids in the final product. The antioxidant effects of ghee residue have been studied by Santha and Narayanan (1978) based on the clarification temperature of butter-fat and reported that antioxidant property of ghee residue has decreased with a rise in clarification temperature. The ghee preparation process also influenced antioxidant properties of the ghee residue. The antioxidant activity in ghee residue produced by various preparation methods decreased in the following sequence: creamery-butter ghee residue > desi butter ghee residue > cream ghee residue > ghee residue (Santha and Narayanan, 1978).

Khanam and Prasuna (2017) used various polar and non-polar solvents for extraction of phenolic compounds using Soxhlet and liquid-liquid extraction method. Although, both the methods used for phenolic extraction from ghee residue were found beneficial but, maximum phenolic compounds were obtained by Dichloromethane using Soxhlet method. The author also reported that liquid-liquid extraction method by using Ethyl Acetate was an easier and better method for extraction of phenolic compounds compared to the Soxhlet extraction method (Khanam and Prasuna (2017).

Ghee residue possess large number of flavouring components such as carbonyls, lactones and free fatty acids. Galhotra and Wadhwa (1993) reported that FFAs, lactones and carbonyls as the major flavouring components in ghee residue. Also, Galhotra and Wadhwa, (1991b) reported that C_{12}, C_{14} and C_{18} delta-lactones were the major lactones in ghee residue at mean level of 237.32, 2859.42 and 533.62 µg/g of ghee residue, respectively. Galhotra and Wadhwa, (1991a) reported that ghee residue contained carbonyls at level of 43.65 µmol/g ghee residue and FFAs content of about 627.48 µmol/g ghee residue. Wadhwa and Bindal (1995)

reported that ghee residue instead of synthetic flavours, can be utilized for flavouring bland products such as butter oil and vanaspati oil.

2.2 Solvent extraction: -

Solvent extraction is basically a chemical method to extract oil from vegetables, oilseeds and nuts by using suitable solvents. Extraction is a process in which one or more components are separated selectively from a liquid or solid mixture, the feed (Phase 1), by means of a liquid immiscible solvent (Phase 2). The transmission of components from feed to solvent is regulated in the subsequent step by their solubility behaviour. The extraction stage result in two stages, one enriched (Extract Phase) and the other depleted (Raffinate Phase), respectively in the components to be segregated. After this, a new separation stage (e.g., distillation) is eventually necessary in order to recycle the solvent (Zhang *et al*. 2018).

2.2.1 Fat extraction Process

Two methods are commonly used at the commercial scale for extraction of edible oil from oil seeds *viz*., mechanical expression and solvent extraction. For seeds with high oil content (sunflower seeds, groundnuts, palm kernels or rapeseed) both the steps are usually involved, whereas materials of lower oil content, such as soybeans, can be directly used for solvent-extraction (Ward, 1984).

2.2.1.1 Mechanical expression

Mechanical expression is used for oil extraction from oilseeds and juice extraction from fruits. In this process, size reduction is carried out to increase yield of the product. Components are extracted from plant parts either for direct use or for use in subsequent processing such as refining. Expression is achieved either in two stages (size reduction to produce pulp or slurry, followed by separation in a press) or in a single stage, in which both the cell rupture and expression of oil is achieved in the same step. Often in mechanical expression, hydraulic press is usually employed due to their lower initial and maintenance costs. Savoire *et al*. (2013) used different mechanical press for oil extraction from oleaginous seeds. The authors studied the expression with expeller, expander and twin-screw extruder. Singh *et al*. (1987) stated that although mechanical expression method is simple but is inefficient. Thus, this method is relatively less used for oil extraction.

2.2.1.2 Solvent extraction

Solvent extraction refers to preferential dissolution of oil by mixing oilseeds with a solvent (Dunford, 2013). The type of solvent being used depends upon cost, safety and solubility of oil. Oil solubility in the solvent increases with an increase in the extraction temperature. Viscosity has an inverse relation with diffusivity as the extraction temperature increases. If the extraction temperature is high then the energy required for solvent recovery is low. Solvent extraction is currently the most efficient way of extracting soybean oil, which is commonly used in the laboratory scale (Oliveira *et al.* 2012). The author reported that the average residual oil, left in the soybean meal after extraction, is only 1.2% in the solvent extraction method, compared to the 6.3% residual oil in screw-pressed and 7.2% residual oil in extruding expelling methods (Oliveira, 2012). Xu and Godber, (2000) reported that low oil material or pre- pressed oil cake when applied with solvent extraction gave high oil yield and efficiency of extraction.

Garba *et al.* (2017) is studied the extraction of rice bran oil from its seeds using solvent extraction method with hexane as solvent and reported an extraction yield of 92%. Arisanu (2013) studied extraction of oleiferous seed oil by solvent extraction and reported that oil extraction efficiency of about 99–99.5%. Oliveira *et al.* (2012) reported that ethanol to rice bran ratio of 2.5:1 and 4.5:1 at temperature range of 60 to 90 ^{o}C yielded about 42.7-99.9% oil extraction efficiency. Hu *et al.* (1996) reported that increasing the extraction temperature from 40^{o}C to 60^{o}C and solvent (hexane and isopropanol) to bran ratios (w/w) of 2:1 and 3:1 increased the Rice bran oil yield. Author added that oil extraction at 60^{o}C for 10 min with 3:1 (hexane solvent to bran ratio) yielded about 3.6% more oil, while extraction with isopropanol produces 6.4% more oil than at 40^{o}C. Efeovbokhan *et al.* (2015) conducted study on alternative solvents for *Moringa oleifera* seeds extraction. The authors concluded that petroleum ether had the highest yield of 49.38 and 37.57%, followed by hexane with 44.94 and 34.71% while isopropyl alcohol gave 36.39 and 28.43%, respectively.

Table 2.6: Application of solvent extraction in food industry

Application	Results	Reference
Oil extraction from oleiferous seed	nearly 99– 99.5% oil yield obtained.	Arisanu (2013)
Rice bran oil extraction	42.7 – 99.9% yield obtained when ethanol: rice bran ration taken 2.5:1and 4.5:1 with 60 - 90 ^{o}C temp. range for solvent extraction.	Oliveira *et al.* (2012)
Rice bran oil extraction	At 60^{o}C for 10 min with 3:1 (solvent to bran ratio) there was 3.6% increase in yield with hexane and 6.4% with isopropanol at 40 ^{o}C.	Hu *et al.* (1996)
Moringa oleifera seed oil extraction	Oil yield – Petroleum ether> Hexane > Iso-propyl alcohol. % Oil yield increased with time reaching (opt 8 – 10 hr).	Efeovbokhan *et al.* (2015)

2.2.2 Application in food industry

In order to recover valuable soluble components from raw materials, extraction is done by firstly dissolving them into a liquid solvent so that the target compound can be separated and recovered later from solvent by using different desolvation techniques. There as two types of extraction processes –

a) Solid/ liquid extraction – when raw material in solid form from which we have to recover the compound of interest.
b) Liquid / liquid extraction – when target compound to be recovered is from liquid raw material such as slurry, juice etc.

Commonly solvents used for extraction in food industries are water, organic solvents such as ethyl acetate, hexane, alcohols.

Applications of solvent extraction in food industry: –

i. Sugar extraction from sugar-beets or sugar-cane.
ii. Oil extraction from oilseeds and virgin pomace.

iii. Extraction of coffee extract from coffee beans.

iv. Caffeine extraction form coffee beans.

v. Extraction of various components such as proteins, pectin, vitamins, pigments, essential oils, flavouring/aroma compounds from many different materials.

2.2.2.1 Oilseed industry

Solvent extraction is mostly used in the oil industry for recovery of residual oil from the oil seeds after pressing. Solvent extraction is known for its high yielding oil output, ease and swiftness to carry out; relatively cost effective, high overhead cost effectiveness. Use of this method requires a complete refining process to ensure traces of the solvents are removed completely.

Li *et al.* (2014) evaluated alternative solvents for oil extraction from rapeseeds. This study was done to evaluate the comparative performance of five different solvents (ethanol, isopropanol and terpenes) with n-hexane. Hansen solubility methodology has been used to theoretically study the interaction between rapeseed oil and solvents. The author reported that n-hexane which is mostly used solvent for oil extraction can be replaced by p-cymene because of its high lipid yield and selectivity. It extracted more polar lipids with lower tocopherol and tocotrienol content in extract (Li *et al.* 2014). Eganathan *et al*. (2005) during a study on oil analysis in seeds of *Salicornia brachiate* found that extraction with hexane yielded maximum oil (22.4%) from seeds followed by petroleum ether which yielded 10.5% oil from 100 gm seed.

Table 2.7: Total oil extraction using petroleum ether and hexane

Sr.no.	Seed quantity (gm)	Oil extraction (%)	
		Petroleum ether	Hexane
1.	100	8.2 $\pm$ 0.25	18.1 $\pm$ 0.25
2.	100	9.1 $\pm$ 0.12	21.6 $\pm$ 0.31
3.	100	10.5 $\pm$ 0.14	22.4 $\pm$ 0.28

(Eganathan *et al*. 2005)

Garba *et al.* (2017) extracted rice bran oil from its seeds using solvent extraction method with hexane as a solvent. There are several techniques used for the extraction of the RBO, but solvent extraction using hexane is the most popular used conventional method for commercial extraction. The author reported 92% oil yield was obtained from solvent extraction using hexane of ohmic heating-stabilized rice bran.

Table 2.8: Application of solvent extraction in oilseed industry

Application	Results	Reference
Rapeseed oil extraction	p-cymene (green solvent) can be replaced n-hexane because of high yield and selectivity. It also extracts more polar lipids with lower tocopherol and tocotrienol content in final extract.	Li *et al.* (2014)
Oil extraction from seeds of *Salicornia brachiate.*	High yield 22.4% with hexane. Oil obtained with high ester of about 538.32 mg/g and saponification value of 547.52 mg/g.	Eganathan *et al*. (2005)
Rice bran oil extraction	Product with 92% yield obtained.	Garba *et al.* (2017)
Soyabean oil	Increase in yield as compare to traditional pressing methods.	Galloway (1976)

2.2.2.2 Flavour extraction

Flavour perception is the sensory impression of food or any other chemical substance, determined by senses of taste and smell (Reineccius, 2005). Flavours are a mixture of volatile aroma compounds which are classified to natural, natural identical, and artificial flavourings (Erten & Cadwallader, 2017). These flavour-active components are widely used in beverage industry with the largest market in North America, followed by Asia-pacific and Europe. These markets are highly mature and emerging in Latin America and Eastern countries (marketsandsearch.com). Growth rate of about 5% is projected for food flavour market since 2015 and continuous growth is expected till 2020 (marketsandsearch.com).

Isolation of volatile aroma components from plants and fruits with vegetable oils extraction from pre-processed seeds has been widely done with the supercritical (CO_2) extraction (Bejarano and Del valle, 2017). Recovery of volatile flavour-active aroma

compounds which are key components of processed liquid food streams is of utmost concern to food industry, as these compounds contribute to the quality of the final product. Among the available techniques for flavour recovery in food industry, distillation, pervaporation, supercritical fluid extraction, and adsorption showed potential for selective recovery of the flavour components from liquid food streams (Saffarionpour and Ottens, 2018). These techniques can be combined in different stages of the process or applied as an alternative to the conventional techniques for aroma recovery. Recent research is concerned with extraction of catechins and caffeine from green tea, using different co-solvents (i.e., ethyl lactate, ethyl acetate, and ethanol) and supercritical CO_2 (SC-CO_2) (Bermejo *et al*. 2016).

Peev *et al.* (2011) studied the solvent extraction of rosmarinic acid from lemon balm and concentration of extracts by nanofiltration. Ethanol-water mixture (50:50 and 80:20 (v/v)) for rosmarinic acid extraction was used to extract rosmarinic acid from dry lemon aerial parts. After extraction about 94% recovery of rosmarinic acid was obtained. Another work done by Miller *et al*. (1995) on isolation of semi-volatile flavour from the cinnamons using solvent assisted supercritical fluid extraction. Ethyl acetate used as extraction solvent. After extraction about 21 compounds identified (Miller *et al.* 1995).

Table 2.9: Application of solvent extraction in flavour industry

Application	Result	References
Solvent extraction of rosmarinic acid from lemon balm	Ethanol-water mixture (50:50 and 80:20 (v/v)) used for rosmarinic acid extraction. About 94% recovery of rosmarinic acid was obtained.	Peev *et al.* (2011)
Isolation of semi-volatile flavour from cinnamons using solvent assisted supercritical fluid extraction	Ethyl acetate used as solvent and 21 components are identified from 24 samples of 24 samples of cinnamon and cassia.	Miller *et al.* (1995)
Semi-volatile flavour and fragrance extraction from cinnamon.	Pentane used as an extraction solvent and 1.5 hr of extraction time results in clean extracts.	Jayatilaka *et al.* (1995).

Simultaneous micro-distillation / solvent extraction is another efficient for semi-volatile flavour and fragrance extraction from cinnamon. Pentane was used as an extraction solvent and 1.5 hr of extraction time results in clean extracts requiring no further sample preparation prior to gas chromatographic analysis (Jayatilaka *et al*. 1995).

2.2.2.3 Spices industry

Spices industry also uses solvent extraction method for recovery of the essential oil from spices and herbs. Spices are mostly known for its specific flavour and taste which is due to the essential oil content in it. Essential oils can be extracted by using solvents such as petroleum ether, methanol, hexane etc. and is often used on fragile material such as jasmine, tuberose etc. which would not be able to handle the heat of steam distillation. Solvent extraction helps in better extraction of target compounds.

Roohinejad *et al*. (2017) conducted extraction methods of essential oils from herbs and spices. The author studied both non-conventional and conventional extraction methods.

Table 2.10: Application of solvent extraction in spices industry

Study	Results	Reference
Extraction methods of essential oils from herbs and spices.	Extraction methods significantly influenced the essential oil composition.	Roohinejad *et al*. (2017)
Antioxidant Potential of Garlic (Allium sativum) Extracts.	Methanolic extract with max. total phenolics and flavonoids obtained. Also, highest DPPH, b-carotene and linoleic acid potential also observed in it.	Awan *et al.* 2017
Extraction of phenolics and triterpenoid antioxidants from herbs such as *Origanum majorana L.*	Ethanolic extract showed stronger antioxidant activities than SFE extract. In SFE pressure and temp. showed positive impact on extraction yield.	Vagi *et al.* (2005)

Extraction of phenolics and triterpenoid antioxidants from herbs such as *Origanum majorana L.* was carried out by Vagi *et al.* (2005). Ethanolic extract showed stronger antioxidant activities than SFE extract. Awan *et al*. (2017) investigated the antioxidant potential of garlic (*Allium sativum*) extracts through different extraction modes. Garlic extract obtained using different solvents such as methanol, ethyl acetate and hexane at different time intervals of 35, 50 and 65 min. Methanolic extracts obtained at 50 min extraction time showed maximum total phenolics as 60.38±0.23 mg GAE/100g and flavonoids as 58.45±1.24 mg/100g. Similarly, the highest DPPH activity (61.59±1.58%) and β-carotene and linoleic acid potential (64.96±1.72%) were also observed for methanolic extract (Awan *et al*. 2017).

2.2.3 Solvents for fat extraction

Solvents are the heart of the solvent extraction method which is mostly used at industrial scale for commercial oil production. They act by dissolving component into them and separate it out from the mixture. Commercial solvent extraction does not include any pre-pressing operation due to the relative disadvantages of low oil content and slower oil recoveries.

2.2.3.1 Hexane

Hexane, a petroleum-derived solvent has been mostly used as solvent for the oil extraction of oilseeds because of its low boiling point (boiling point 63 to 69 ^{o}C), good stability, minimum corrosiveness, low greasy residual effect, and acceptable aroma and flavour productivity for the milled products (Becker, 1978). Lusas *et al*., (1991) reported that application of hexane in oil extraction may adversely affects CNS of workers working with the solvent. Released vapours when mixed with air at 25 ^{o}C may leads to the spontaneous ignition. Narain and Singh, (1988) reported that application of Hexane at small scale extraction make this process expensive because of its huge operational losses. Research, however, is being carried out to determine the solvents that are easily available and less harmful than hexane. Johnson and Lusas, (1983) reported that the ideal solvent must be easily removed from the meal and oil, non-flammable, stable, non-reactive with oil; meal or equipment, pure, only slightly soluble in water and readily available at low prices.

Hu *et al.* (1996) conducted a comparative study on isopropanol and hexane. In this study, extraction of vitamin E and oryzanols from stabilized rice bran was carried out. Effect of temperature, time and solvent: rice bran ratio was studied. Result revealed that solvent: rice bran ratio and extraction temperature positively influence yield of vit-E, oryzanol and crude oil

for both the solvents. For hexane increase in ratio from 2:1 to 3:1 extracted 10.8% more crude oil (Hu *et al.* 1996).

2.2.3.2 Petroleum ether

It is another type of solvent that is used for extraction of fat from the samples such as oilseeds, nuts etc. it is a group of various volatile liquid hydrocarbon mixture such as naphtha, petroleum naphtha etc. These are highly flammable and irritant in nature because of this more care is to be taken during its use. It is mostly used as a non-polar solvent during the fat extraction from different material. Dari (2009) reported that petroleum ether has a boiling point of 75 ^{o}C and melting point of -73 ^{o}C. Also, it has molar mass of between 87-90 g/mol. Seth *et al*., (2007) reported that petroleum ether, being a petroleum product, faces occasional scarcity and fluctuation in price depending upon supply and demand of gasoline.

2.2.3.3 Isopropyl alcohol (IPA)

It is a type of alcohol which dries quickly and is non-toxic in nature. Zhang *et al.* (2002) reported that IPA is one of the favourable alternatives to hexane for oil extraction. When compared with ethanol and hexane, IPA is less toxic, low flammable and legal in use without any restriction. The IPA is a volatile, colourless liquid with sharp musty odour. It has a boiling point of 82.3^{o}C with 53^{o}F of flash point helps in better extraction and solvent-product mixture separation. It has melting point of -89^{o}C and density of about 0.786 g/cm3. All these parameters help in better isolation properties and final product quality.

A study conducted by Zhang *et al.* (2002) on the isopropyl alcohol extraction of oil from cottonseed collects. In this study, first the authors flacked the cottonseed meats and cooked it using Hivex expander before processing. Hexane and IPA at different concentrations (88, 93, 95 and 97%) were used as solvent to study the effect of collects versus flake. Hexane had better oil carrying capacity and fast extraction as compared to aqueous IPA extraction. Residual oil content, when cottonseed collects extracted with 88, 93 and 97% IPA was, 2.4, 1.9 and 1.5% respectively while for hexane it was 1.2%. Capellini *et al*. (2017) studied on rice bran oil extraction using alcoholic solvents under the objective of assess the feasibility of replacing hexane with safer solvents such as ethanol and isopropanol in rice bran oil extraction. Results showed that aqueous nature of alcoholic solvents negatively affect their oil extraction capacity, however use of absolute solvents gave approximate 80% oil yield in single stage batch extraction at 80^{o}C. On other hand, absolute isopropanol extract had higher tocopherol content

of 98.1 mg/kg as compared to other solvents. The authors concluded that short chain alcohols are better alternatives to hexane for solvent extraction of oilseeds. Gandhi *et al.* (2003) obtained the highest degree of purity in soya bean oil for extraction with IPA and reported that IPA extraction process is equally effective when compared with hexane.

Table 2.11: Different solvents used in fat extraction

Solvent	n-Hexane	Isopropyl alcohol	Petroleum ether
Molecular formula	C_6H_{14}	C_3H_8O	$C_4H_{10}O$
Boiling point (°C)	69	82.3	34.6
Molecular weight (g·mol−1)	86.18	60.1	74.12
Density (25 °C, g·cm−3)	0.675	0.785	0.7134
Viscosity (25 °C)	0.31 Cp	2.038 mPa s	0.2448 Cp
Flash point (°C)	-23	12	-45

2.2.4 Environmental and health impacts of low toxicity solvents: -

Solvents are important for in many research and industrial processes and consumer product formulations. Applications of poisonous and harmful chemical solvents in industry increase concern about health of operators and impact on environmental pollution. Jutz *et al.* (2011) stated that each year, more than twenty million tons of waste residues from organic solvents are emitted into the atmosphere, causing unnecessary waste of solvents and polluting the environment. Dharmarajan (2019) stated that, of all analytical techniques, extraction is a huge solvent-consuming process that adversely affects the environment. Use of petroleum-based solvents for oil extraction from oilseeds is still a common practice, despite the potential hazard and toxic water pollution. Increasing awareness about toxicity of petroleum-based solvents, immense need for sustainable development schemes and strategies are required to address the environmental impact without compromising the yield. In the course of developing green extraction techniques, automation, alternative solvents, and selective extractions are the growing trend.

Agata (2017) stated that long-term exposure of living organisms to solvent has harmful impact on respiratory and nervous system. Moreover, application of hazardous solvents is dangerous to organs, e.g., carbon tetrachloride and chloroform are hepatotoxic

(Agata, 2017). Glycol ethers and chlorinated solvents may lead to kidney failure (Sanni and Mutta Reddy, 2014). Lauwerys *et al.* (1985) stated that after short term exposure halogenated hydrocarbons and diethyl glycol causes renal tubular necrosis and other health issues. According to a WHO report, about one-fourth of diseases arises due to the long-term exposure of environmental pollutants. Decreasing the use of their highly toxic and non-eco-friendly solvents with green and low toxicity solvents makes the processing easier and eco-friendly with lower impact on the human health. It has been reported that solvents like diethyl ether, tetrahydrofuran (THF), 1,2- dimethoxy ethane (DME), and 1,4-dioxane have been widely used in extraction for a long time because of their high solubility for a wide range of organic compounds (sigma-aldrich.com/solvents). Different bio-based and low toxic solvents are recommended by researchers for use in extraction process and studies also carried out by them. Use of these low toxicity solvents may leads to lower pollution, minimum or no health impact and better extraction efficiency also.

2.2.5. Low toxicity solvents

Low toxicity solvents are the solvents which are does not affect the health of the human being. These are eco-friendly in nature reduce pollution as compare with the other synthetic and highly toxic solvents.

2.2.5.1. Application in oil extraction

During solvent extraction separation of solvent and desired compound is done by evaporation of volatile solvent because of which air pollution occurs. These vapours may lead to irritation or ignition under suitable conditions. Sometimes due to high toxicity, it directly affects the central nervous system of the workers.

2-Methyl tetrahydrofuran is a green alternate to tetrahydrofuran and dichloromethane as it synthesized from waste such as sugarcane bagasse and corncobs of natural sources. It is a polar aprotic and produces lower peroxides as compare to tetrahydrofuran. In a study of 2-methyloxolane as alternative solvent for lipid extraction and its effect on the cactus (Opuntia Ficus-indica L.) seed oil fractions was conducted by Gharby et al. (2020). The author found that oil extraction with 2-MeO was higher (9.55 ± 0.12 g/100 g) than the oil extracted with n-hexane (8.86 ± 0.25 g/100 g). The chemical and physical parameters quality indices acidity, peroxide value of 2-methyloxolane extracted oil was found to be much higher than that of oil extracted using n-hexane. Sterol content in the oil extracted with n-hexane (102.1 $\underline{+}$7.54 mg/100gm) was higher than the oil obtained with 2- MeO (111.5$\underline{+}$ 2.5 mg/100gm). However, fatty acid and tocopherol content was unchanged by the extraction

solvent. Cherukuri *et al*., (1999) suggested a liquid-liquid extraction process using lower aliphatic alcohols (C1 to C6, preferably methanol, ethanol, or isopropanol). The process involves mixing rice bran oil and alcohol, separating the alcohol layer and subsequently distilling this layer in order to recover rice bran oil. Lee *et al*., 2013 reported that dimethyl carbonate (DMC) is good extraction solvent and has ability to extract triglyceride from microalgae biomass.

Tundo *et al*. (2008) reported that dimethyl carbonate produced using clean process, has no toxicity and possess biodegradability, which make it a preferable green solvent. According to Plonis and Trujillo-Quijano (1995), the deacidification of palm oil by liquid-liquid extraction may produce an olein (the fraction of the palm oil enriched in unsaturated fatty acids) with a carotene content of 750-1000 mg/kg. The proposed solvents are short chain alcohols or ketones, preferred ethanol, containing 1 to 25 % (in volume) of water and about 1% of citric acid. It was also reported that the process may generate a deacidified oil with an enhanced flavour and aroma, and containing high levels of carotene and reduced amounts of diacylglycerols and free fatty acids (Plonis and Trujillo-Quijano,1995). Saxena *et al.* (2011) conducted a comparative study on the extraction of cottonseed oil using n-hexane and ethanol. Based on the results, the solvent to solid ratio of 7:1 was found sufficient for both solvents to extract the maximum amount of oil and only marginal increment of oil amount was obtained by increasing the ratio to 8:1. Chioma *et al.* (2020) also conducted same study on comparative oil analysis extracted using n-Hexane and ethanol in various raw materials, such as soybean, cocoon and palm kernel seeds and concluded that no shift in values of free fatty acid detected after soybean oil extraction but for cocoa and palm kernel oil extracted with ethanol, it was much higher. Also added, ethanol extracted oil reported higher saponification and acid values than the n-hexane extracted oil.

Table 2.12: Application of low toxicity solvents in oil extraction

Application	Results	References
2-methyloxalane in Cactus seed oil extraction	Oil yield, acidity, and peroxide value was higher than n-hexane extracted oil. Sterol content higher in n-hexane extracted oil. No change in FFAs and tocopherol content.	Gharby *et al.* (2020)
Rice bran oil extraction using IPA and ethanol.	Aqueous nature negatively affects oil extraction yield. Aqueous ethanol yielded rice bran oil with 1.53% γ-oryzanol and 769 mg/kg tocotrienols.	Capellini *et al.* (2017)
Liquid-liquid extraction process using lower aliphatic alcohols for rice bran oil.	Enriched oil with solvent high purity was obtained.	Cherukuri *et al.* (1999)
Deacidification of palm oil by liquid-liquid extraction.	Olein (the fraction of the palm oil enriched in unsaturated fatty acids) with high carotene content produce after extraction. Enhancement in flavour and aroma, and containing high levels of carotene and reduced amounts of diacylglycerols and free fatty acids in extracted product.	Plonis and Trujillo-Quijano (1995)
Comparative oil extraction analysis on the use of ethanol and n-hexane in various raw materials, such as soybean,	No shift in values of free fatty acid detected after soybean oil extraction but for cocoa and palm kernel oil extracted with ethanol, it was much higher. Ethanol extracted oil reported higher saponification and acid values than the n-hexane extracted oil.	Chioma *et al.* (2020)

cocoon and palm kernel seeds.		
Comparative study of the extraction of cottonseed oils using N-hexane and ethanol solvents.	Solvent-to-solid ratio of 7:1 was sufficient for all solvents to extract the oil. Maximum production of cottonseed oil for n-hexane and ethanol amounts to 68.50 wt% and 65.07 wt %.	Saxena *et al*. (2011)

Chapter 3

Materials and methods

3. MATERIALS AND METHODS

3.1 Materials

1. **Ghee residue**

   Ghee residue was obtained from Experimental Dairy, ICAR-National Dairy Research Institute, Karnal.

3.2 Chemicals –

All the chemicals used for the preparation of different reagents and for carrying out chemical analysis were of Analytical Grade (AR) and were procured from standard companies. The reagents used for analyse were freshly prepared adopting standard procedures.

Ethanol, 2- Methyl tetrahydrofuran, Ethyl acetate, Dimethyl carbonate, Water, Ethyl alcohol (95%), Phenolphthalein indicator, Sodium hydroxide / Pot. Hydroxide (0.1N and 0.5N), Starch indicator, Sodium thiosulphate, Glacial Acetic acid, Ammonium hydroxide, Diethyl ether, Petroleum ether, Conc. Sulphuric acid (AR), Cyclohexane, Wij's reagent, Hexane and Sodium methoxide ($NaOCH_3$).

3.2.1 Reagents

1) Ethyl alcohol (95%) – 99.9% absolute ethanol was diluted to get 95% ethanol using distilled water.

2) Ethyl alcohol (neutralized) – 95% (v/v) ethanol was neutralized to pH 7.0 with 0.1N NaOH solution using phenolphthalein indicator.

3) Phenolphthalein indicator (1%) – 1.0g of phenolphthalein powder was dissolved in approx. 100mL of 95% (v/v) ethanol.

4) Potassium iodide solution: 10%, free from iodine and iodates

5) Hydrochloric acid (6.0 M)

6) 0.1 N Sodium thiosulfate solution: Dissolved approximately 24.8 gm of sodium thiosulphate crystals in previously boiled and cooled distilled water and made the volume to 1000 ml. Stored the solution in a cool place in a dark-coloured stock bottle. After staring the solution for about two weeks, filter if necessary and standardize as follows:

5 gm of finely ground potassium dichromate which has been previously dried to a constant weight at 105 ± 2°C in to a clean 1000ml volumetric flask was weighed. Dissolved in water make up to the mark; solution was shaked thoroughly and kept in dark place. 25 ml of this solution was pipette into a clean glass stoppered 250ml conical flask. 5 ml of concentrated hydrochloric acid and 15 ml of 10% potassium iodide solution was added. Allowed to stand in dark for 5 minutes and titrated the mixture with the solution of sodium thiosulfate using starch solution as an indicator towards the end. The end point was taken when blue colour changed to green. Calculated the normality (N) of the sodium thiosulfate as follows:

Calculate the normality of the sodium thiosulfate solution as follows:

$$N = \frac{(25 \times W)}{(49.03 \times V)}$$

Where:

W= weight in grams of potassium dichromate,

V= volume in ml of sodium thiosulfate solution required for the titration

7) 1% starch indicator solution: Make a paste of 1 gm soluble starch in 30 ml of water. Transfer the paste to 80 ml of boiling water and heat until a clear solution is formed. Cool the contents and store in a tight stoppered bottle. It can be stored for 2-3 weeks at 4'C.

8) Starch solution: Mix 5 g of soluble starch and 10 mg of mercuric iodide in 30 ml water. Add this mixture to 1000 ml of boiling waler and continue to boil for 3 minutes.

9) Wij's reagent - Dissolve 10 ml of iodine monochloride in about 1800 ml of glacial acetic acid and shake vigorously. Pipette 5 ml of this, add 10 ml of potassium iodide solution and titrate with 0.1 N sodium thiosulphate solution, using starch as indicator. Adjust the volume of the solution till it is approximately 0.2N.

3.3 Apparatus and glassware:

All volumetric flasks, pipettes and burettes were class "A". Burette (50 ml), funnels (small and large), Mojonnier tubes, measuring cylinder (10, 50, 100, 250, 500 and 1000 ml), volumetric flasks (5, 10, 50, 100, 250, 500 and 1000 ml) procured from Borosil India Ltd., Mumbai, India and Whatman filter papers (Whatman no. 1) from Whatman International Ltd., Kent, UK.

3.4 Equipment's and Instruments:

The following equipments and instruments were used for extraction of milk fat from ghee residue.

1. **Weighing balance** - Sartorius India Pvt. Ltd. Mumbai India
2. **Magnetic stirrer** - SPINOT MC 02, Tarsons Products Pvt. Ltd., Kolkata, India
3. **High speed refrigerated centrifuge** - KUBOTA-6500, Kubota Corporation, Tokyo, Japan
4. **Laboratory shaker -** Spinix, Vortex shaker, Tarsons Products Pvt. Ltd., Kolkata, India
5. **Auto pipettes -** (10 – 100µl and 100-1000µl) Tarsons Products Pvt. Ltd., Kolkata, India
6. **Desiccator**- Tarsons products Pvt. Ltd., Kolkata, India
7. **Digital temperature controller oven**- Narang Scientific Works Pvt. Limited, New Delhi, India
8. **Distillation unit**- Laboratory Glass Co. LABCO, Ambala, India
9. **Electric heater**- Vikrant, Jain Enterprises, Indi
10. **Electronic balance**: Precisa XB 220A, Switzerland
11. **Hot air oven** - Tempo Instruments and Equipments (I) P. Ltd., Mumbai, India
12. **Water bath with thermostat**- The laboratory Glassware Co., Ambala Cantt, India.
13. **FTIR (Fourier transform infra-red spectroscopy) –** IR Affinity-1 206-73500-38 SHIMADZU cooperation, Kyoto, Japan
14. **Rheometer -** MCR 52, Anton Paar, Germany
15. **Thermometer**: The thermometers were evaluated under the specification of ISI (IS: 1223 -1970)
16. **Hunter Color lab** - Hunter Lab Colour Flex® (MiniScan XE plus, Hunter Associates Laboratory Inc. Reston, Virginia, U.S.A.)
17. **Refrigerator** – DW 40L508, Haier Medical and Laboratory Products Co. Ltd. Qingdao, China

3.5 Methods –

3.5.1 Solvent selection –

As per discussion different solvents were selected for the extraction of milk fat from ghee residues. Three criteria used for the selection of these solvents

1) Low toxicity
2) Boiling point lower than 100^{o}C.
3) Lower residual in extracted product with high extraction yield.

According to first two criteria solvents are selected are provided in the following Table no. 3.1

Table no. 3.1 Selection of solvents

Sr.no	Solvents	Boiling point (^{o}C)	Toxicity (LD_{50})
1	Ethanol	78	7.06gm/kgbw
2	2- Methyl tetrahydrofuran	80.2	5.75gm/kgbw
3	Ethyl acetate	77.1	5.62gm/kgbw
4	Dimethyl carbonate	90.5	13.8 gm/Kgbw
5	Water	100	-

3.5.2 Screening of solvents for extraction efficiency and low residual solvent

3.5.2.1 Extraction of milk fat from ghee residues

For the extraction of milk fat from ghee residue, firstly the ghee residues were collected from the experimental dairy plant of National Dairy Research Institute. Ghee residues was crushed/ milled using the piston-mortal. This ghee residues were then put into glass beakers of 500 ml capacity. Then selected solvent was added in to ghee residue beaker at 5:1 ration (5 is solvent and 1 is ghee residue). Solvent and ghee residue mixture were properly mixed. The mixture then heated at 50^{o}C for 30 min of time with stirring at 100 Rpm using magnetic stirrer and another trial conducted with same sample - solvent at 10^{o}C below the boiling point of each solvent. Then the mixture was filtered through whatman paper no.1 in to the previously weighed conical flasks added with glass beads to stop boiling or spillage of mixture during boiling. The filtrate was heated to evaporate the solvent from it and after heating the remaining fat again heated to 110 + 2^{o}C for maximum or fully removal of solvent from the extracted milk fat. The extracted sample was desiccated, cooled and weighed to determine the extraction yield.

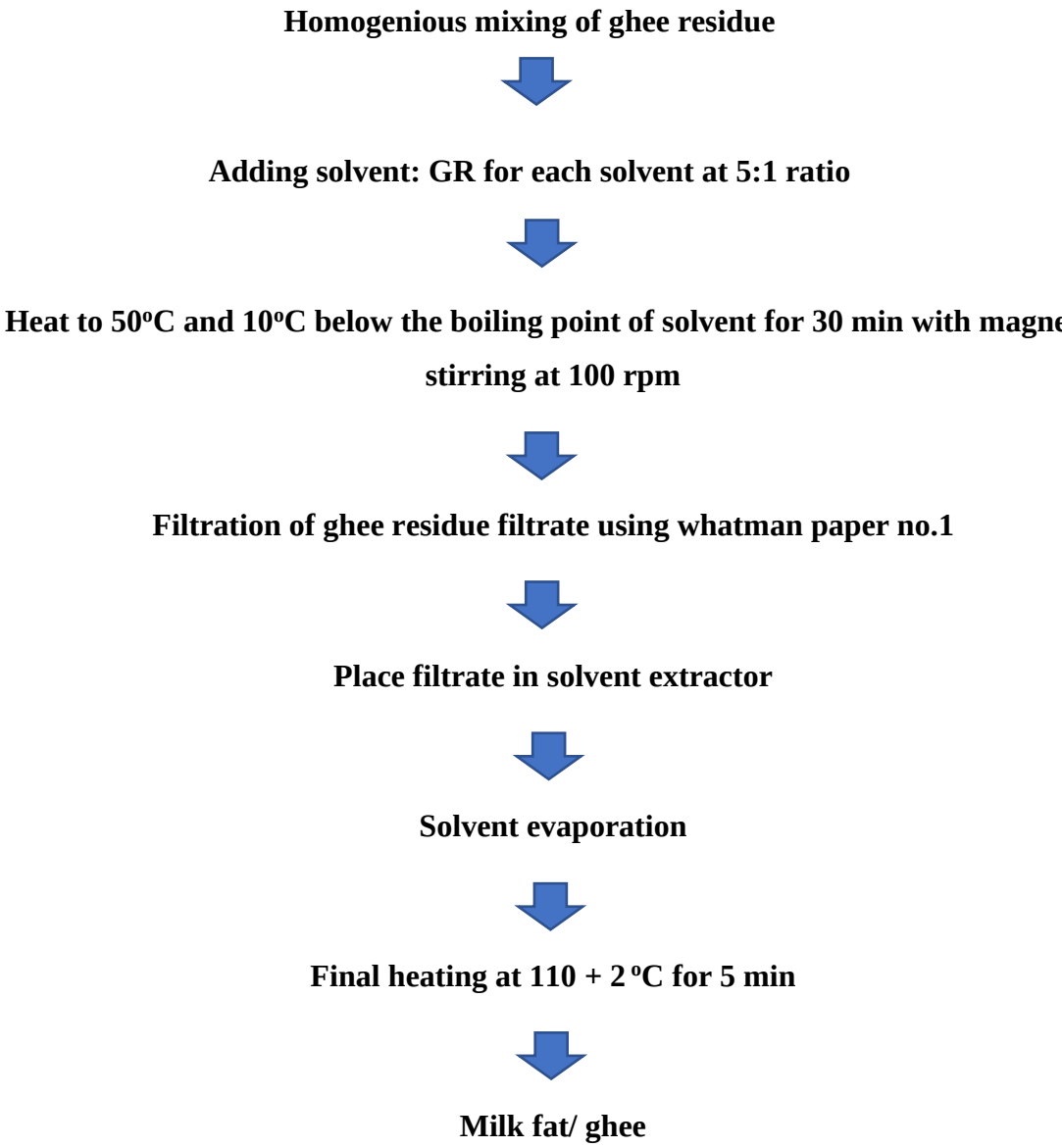


Fig no. 3.1 Flow chart for optimization of solvent extraction of ghee residue using low toxicity solvents

- Solvent yielding lowest residual in milk fat and highest fat recovery from ghee residue will be selected for further study.

3.6 Chemical Analysis ghee residue –

The chemical analysis of ghee residue like fat content were determined by the methods given in BIS Handbook (SP 18: Part XI, 1981). The details of the procedure for these constituents are as follows:

3.6.1 Fat content of ghee residue –

Fat content of ghee residue was determined by Mojonnier fat extraction method as per AOAC, 2000 official method. In brief, about 10 g of sample (liquid milk) was transferred to extraction tube. After 1.25 ml of ammonia sp. gr. 0.91 (or an equivalent volume of a more concentrated ammonia solution may be used) was mixed and shake thoroughly. 10 ml ethyl alcohol was added and mix again. Then 25 ml of diethyl ether (peroxide free) was added stoppered and shake vigorously for about a minute. Then again 25 ml petroleum ether (boiling range 40 – 60°C) was mixed and shake again vigorously for about half a minute. The mixture was put for stand until the upper ethereal layer has separated completely and is clear. (Alternatively use low r.p.m. Mojonnier centrifuge). If there is a tendency to form emulsion, a little alcohol may be added to help separation of the layers. The clear ethereal layer was taken out into a suitable vessel (flask, glass bowl, aluminium dish, etc.). Delivery end of the extraction tube was washed with a little ether and added the washings to the flask. Extraction of the liquid remaining in the extraction tube was repeated twice using 15 ml of each solvent every time. The ethereal extract was added to the same container and evaporated off completely. The flask was dried in an air oven at 102 ± 2°C for two hours, cooled in a desiccator and weighed. The flask again heated in the oven for 30 min. Cooled in a desiccator and weighed. The process of heating and cooling and weighing was repeated until the difference between two successive weights does not exceed 1 mg. The fat washed out from the flask with petroleum ether carefully leaving any insoluble residue in the flask. Dried the flask in the oven and reweighed. The difference in weights represents the weight of fat extracted from the milk. Correct weight of extracted fat was counted by blank determination on reagents used. If reagent blank is more than 0.5 mg purify or replace reagents. Difference between duplicate determinations obtained simultaneously by the same analyst should not be more than 0.03 g fat /100g product.

Calculation: -

$$\text{Fat \% (w/w)} = \frac{We}{Ws} \times 100$$

Where,

We = weight of extracted fat

Ws = weight of ghee residue sample

3.7 Determination of Physico-chemical characteristics of solvent extracted ghee -

The Physico-chemical characteristics of ghee such as % Fat extraction efficiency, Free fatty acids (%OA), Iodine value (IV) and Instrumental color values of solvent extracted ghee samples were determined by the different methods. The details of the procedures for these are as follows: -

3.7.1 Fat extraction efficiency –

For all the solvent extracted milk fat from ghee residues, fat extraction efficiencies were calculated using given equation –

$$\% \text{ Fat extraction efficiency} = \frac{We}{Ws} \times 100$$

Where;

We = gm fat extracted from given amount of ghee residue

Ws = Initial gm fat present in ghee residue sample

3.7.2 Free fatty acid in ghee –

Free fatty acid (FFA) levels, expressed as % oleic acid, of ghee samples were determined by the method IS 3508 – 1966 (Reaffirmed 1997), which is described below -

Ten grams of ghee sample was accurately weighed in a 250 ml conical flask. 50 ml of neutralized alcohol (at 70°C) was added into the flask containing ghee. The contents were brought to boil on a boiling water bath. The solution, while hot, was titrated against 0.1N sodium hydroxide solution, shaking vigorously during titration. The end point of the titration was perceived when the addition of a single drop produced a slight but definite colour change (pink color) for at least 15 s. The FFA levels were expressed as per percent oleic acid.

Calculation -

$$\text{FFA (\%oleic acid)} = 2.82 \times \frac{V}{W}$$

Where;

V- volume (ml) of NaOH required for titration of sample

W- weight of the sample

3.7.3 Iodine value (Wij's method) –

Molten ghee was taken and filtered it through Whatman No 1 or No 4-filter paper to remove any solid debris and small amounts of water. To ensure the complete removal of moisture from the sample, used anhydrous sodium sulfate (Na_2S0_4). This can be done by placing small amount of anhydrous sodium sulfate in the filter paper and slowly pouring the molten fat through this. Accurately 0.4 to 0.45 g of the clear ghee was weighed in a clean dried Erlenmeyer flask. Then 20 ml cyclohexane (Cyclohexane serves to solubilize the fat) was added and swirled to dissolve the sample. Added by means of a burette exactly 25 ml of the Wij's reagent. Closed the flask with its stopper, mixed carefully and leave it standing for 1 hour in the dark. At the end of the hold, 20 ml potassium iodide solution was added. Then immediately approximately 150 ml of distilled water was added, swirled lightly to mix, and then promptly titrated with vigorous shaking I stirring, with 0.1 N sodium thiosulphate solution using 50 ml burette. Continued titration until the yellow-brown colour almost disappears, then 1-2 ml starch indicator was added and continued to titrate until the blue colour just disappeared. Recorded the volume of sodium thiosulfate used in the titration. Carried out a blank test, using the same quantities of the reagents. Calculated the iodine value by means of the following formula:

Calculation-

$$\text{Iodine value (IV)} = 12.69 \text{ x } \frac{(a - b)\text{x N}}{W}$$

Where,

a – volume in ml of 0.1 N sodium thiosulfate used in the blank test

b – volume in ml of 0.1 N sodium thiosulphate used in the titration with the ghee sample.

N – normality of sodium thiosulphate

W – weight of ghee sample taken for the analysis.

(Ref: - SP: 18 Part (XI) – 1981)

3.7.4 Color

The colour of solvent extracted milk fat was measured using Colorflex calorimeter supplied by hunter lab (Hunter Associates Laboratory, Inc., Reston, VA, USA) along with the software (version 4.10) and the results were expressed in terms of CIELAB system (plate-8). Before the test, the instrument was calibrated with standard black and white tiles as specified by the

manufacturer. The light source was dual beam xenon flash lamp. Data was received through the software in terms of L* (lightness), ranging from 0 (black) to 100 (white), a* (redness), ranging from -60 (red) to +60 (green) and b* (yellowness), ranging from -60 (yellow) to +60 (blue) values. For this, the heated and cooled solvent extracted milk fat was filled into the sample container attached with the Colorflex instrument. During transfer care was taken to avoid solidified fat. Three readings were taken for each sample.

3.8 Rheological analysis

3.8.1 Flow behaviour

The rotational Rheometer (MCR 52, Anton Parr, Austria) fitted with probe CP-75 is used to conduct rheological analysis. Flow behaviour test was performed at temperature of 30ºC by adjusting the gap of 0.149 mm between the probe and platform. The ghee sample (1.0 ml) was immediately put between the plates and the measurement conducted. To investigate changes in shear stress s and dynamic shear viscosity, the shear rate was varied from 0.01 to 100 s^{-1}. During the shearing, a total of 25 data points were generated in a ramp-wise decrease in time length. Every measurement was carried out three times.

3.8.2 Frequency sweep

The rotational Rheometer (MCR 52, Anton Parr, Austria) fitted with probe PP-50 is used to conduct rheological analysis. Amplitude sweep test was performed at temperature of 18ºC by adjusting the gap of 0.5 mm and LVE (linear viscoelastic region) range as % strain obtained by this test is used in frequency sweep and temperature sweep test. The frequency sweep test was performed at 20°C with frequency varied from 0.01 to 100 Hz. The values of G' (storage modulus) and G'' (loss modulus) was obtained over frequency range of 0.01 to 100 Hz.

Fig no 3.2 Rheometer

3.9 FTIR (Fourier transform infrared spectroscopy) –

This method used to determine adulteration or characterization of the ghee. The procedure of this method is as follows- Just before the analysis the ghee samples were melted and stored in the glass container and a small amount was then put onto a sample holder. FTIR spectra were scanned using a FTIR spectrometer Bruker with a resolution of 4 cm−1, in the 400–4000 cm−1 region. The samples were placed in contact with attenuated total reflectance (ATR) element (ZnSe crystal) at controlled ambient temperature (20^0C). The instrument was calibrated before the analysis of the ghee samples. The small amount of ghee from each sample was taken in a sequence and was analysed at the rate of 60 scans/minute. Between the analysis of every ghee sample, the sample holder of the instrument was cleaned with methanol using tissue paper three to four times in order to avoid the contamination of one sample by another. The spectrogram obtained after the analysis was saved and studied for the determination of the functional group.

3.10 Statistical analysis

Data obtained from various activities was analysed statistically to provide meaningful interpretations. Data was analysed through Two-way ANOVA using IBM SPSS 23.0 software.

Chapter 4

Results and discussions

4. RESULTS AND DISCUSSION

The main aim of this work was to evaluate the ability of different low-toxic solvents to extract the residual milk fat from *ghee* residue. This chapter deals with the various results that were obtained during the present investigation. The study was carried out in two phases. In first phase, different low toxicity solvents were screened for their suitability to extract milk fat from ghee residue. In the second phase, milk fat extraction from ghee residue was carried out different extraction temperature so as to optimize the extraction temperature.

4.1 Selection of solvents

4.1.1 Low toxicity solvents

Solvent is considered as the heart of the solvent extraction process. Selection of solvent is an important activity as the economy of the plant and extracted product's quality depends upon the solvent. In the present investigation, solvents were short-listed based upon their lower toxicity and boiling point in the range of 50-90^{o}C. Maximum boiling point of 90^{o}C was considered primarily because of easy evaporation and solvent removal from the extracted fat. Using a solvent with higher boiling point would have used higher amount of energy during solvent evaporation and might have led to residual solvent in the extracted fat, both of which is not desired by the dairy industry. After a thorough review of the available solvents, a total of four solvents were short-listed (Table 4.1). In addition to this, water and hexane were also used as control solvents to compare the fat extraction efficacy with low toxicity solvents.

Table 4.1 Low toxicity solvents used in the present investigation

Sr. no.	Solvents	Toxicity level	Boiling point (^{o}C)
1	Ethyl acetate (EA)	GRAS	77.1
2	Dimethyl carbonate (DMC)	LD_{50} – 13.8 g/kg	90.5
3	2-MTHF	LD_{50} - 5.75gm/kg	80.2
4	Ethanol	GRAS	78

4.1.2 Screening of solvents

Screening of solvent for the extraction of milk fat from *ghee* residue was carried out to select the suitable solvents with respect to the extracted fat quality. All the previously short-listed solvents were subjected to an extraction process in which the thoroughly grounded *ghee* residue was mixed with different solvents at 50^{o}C in a 5:1 ratio (solvent: ghee residue) and

mixed at 100 rpm for 30 minutes throughout this activity. Following this, the contents were filtered through Whatman filter paper no.1. Solvent in the filtrate was evaporated by heating the contents in a water bath, and the resulting fat was then heated to 110 ± 2 ^{o}C for 5 - 10 minutes to evaporate the residual solvent. The results obtained after every step are presented in picturised form (Fig 4.1) and in Table no. 4.2.

When water was used as a solvent for fat extraction (to mimic the industrial practice), a thin layer of fat appeared at the top and remaining bottom (aqueous) portion turned into a hazy solution having paste like consistency. This could be attributed to the presence of hydrophilic compounds that remained in ghee residue after the ghee boiling step. The characteristics of solvent + ghee residue mixture changed when other solvents were used for fat extraction in place of water, which could be attributed to the difference in their polarity. Polarity of the solvents is in the order of: water > ethanol > DMC > EA > MeTHF > Hexane. Solvent having polarity close to water (ethanol) had solvent + ghee residue mixture characteristics similar to that of water (i.e., hazy with paste like constituency) and could not be filtered. Al-Hamamre *et al*. (2012) reported that polar solvents such as alcohols are known to extract higher amounts proteins, carbohydrates, Maillard reaction products, phosphatides and other compounds, which causes haziness in filtrate. Solvent + milk fat filtrate was obtained from all other solvents. Upon solvent evaporation from the solvent – fat filtrate, milk fat of different color characteristic was obtained. The milk fat extracted from EA and DMC was yellow coloured while the milk fat extracted using MeTHF and hexane was dark brown in color. During the solvent mixing step with *ghee* residue, solvent would have penetrated inside the ghee residue particles, and solubilized and extracted the compounds (including fat) having polarity similar to them. Extraction of compounds resulted into decrease in the size of ghee residue particles and enabled them to pass through the filtration process and appear in the filtrate along with fat. During the solvent evaporation step, the ghee residue particles got concentrated along with the fat and imparted a dark brown color.

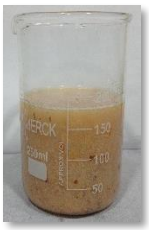
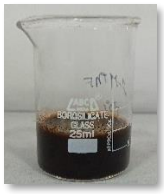
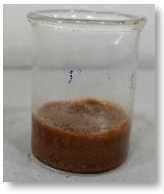
A	**Solvent + Ghee residue**					
						
	Water	**Ethanol**	**Dimethyl carbonate**	**Ethyl acetate**	**2 – MTHF**	**Hexane**
B	**Solvent and ghee residue mixed images**					
						
	Water	**Ethanol**	**Dimethyl carbonate**	**Ethyl acetate**	**2-MTHF**	**Hexane**
C	**Filtered solvent images**					
						
	Water	**Ethanol**	**Dimethyl carbonate**	**Ethyl acetate**	**2 - MTHF**	**Hexane**
D	**Images of solvent extracted fat**					
						
	Water	**Ethanol**	**Dimethyl carbonate**	**Ethyl acetate**	**2-MTHF**	**Hexane**

Fig. 4.1 Images of milk fat extraction process using different solvents at different stages

Difference in the color parameters of extracted milk fat could be attributed to the difference in the polarity and solubilizing characteristics of these solvents. It was also interesting to observe that even though potable water is used at dairies for milk fat extraction from ghee residue; however, in our study, no such fat extraction could take place using water. This could be due to three possible reasons, viz., (a) the amount of water used at commercial scale is too high (about 4 – 6 times more) than the amount of water used in our study. Large

amount of water results into higher dilution of serum part and this made the fat easy to come out from ghee residue particles and float at the top of serum. (b) Temperature difference between the two processes. At the industrial scale boiling water is used for fat extraction from ghee residue while in our study the extraction was performed at 50^{o}C. Higher temperature results into decreasing the viscosity of serum and enables easy migration of fat to the top. (c) Difference in the fat extraction method in the two processes. At the industrial scale, whole of the contents (i.e., ghee residue + water mixture) are transferred to cold store (maintained at 4-8 ^{o}C) where the fat solidifies and then the solidified fat is removed manually from the bottom serum which is in liquid state. On the other hand, in our study, the contents were filtered through a filter paper and no such heating – cooling process was done.

It could possibly be assumed that the solvent extracted milk fat will be mixed with the main ghee lot at the industrial scale. The milk fat extracted using EA and DMC appeared similar to *ghee*, then the sample obtained hexane and MeTHF. Considering this, addition of hexane or MeTHF extracted fat could possibly alter the ghee characteristics to large extent as compared to addition of EA or DMC extracted fat. Hence, EA and DMC were selected for further studies.

Table no.4.2 Visual observation of milk fat extraction process using different solvents

Extraction stages	Solvent					
	Water	**Ethanol**	**Dimethyl carbonate**	**Ethyl acetate**	**2-MTHF**	**Hexane**
After mixing	No color change		Light yellow	Dark yellow	Brownish with haziness	
After heating & mixing	Paste formation	Slight cream color	Light yellow	Dark yellow	Brownish with haziness	
After filtration	No filtrate	Hazy colourless filtrate	Dark yellow filtrate	Dark yellow filtrate	Hazy dark brown filtrate	Hazy blackish yellow filtrate
After solvent evaporation	No fat obtained	Dark residue at bottom	Slight brown fat	Dark yellow fat	Dark black fat obtained	Dark black fat obtained
After final heating	-	Burnt appearance	Ghee like appearance		Burnt appearance	

4.2 Optimization of solvent extraction temperature

In this activity, the previously milk fat was extracted from ghee residue using the selected solvents, *viz*., EA and DMC, at two different temperatures, *viz*., 50^{o}C and 10^{o}C below the boiling point of the respective solvent (EA=70^{o}C and DMC=90^{o}C). The fat obtained using the previously provided protocol at different extraction temperatures was studied for various attributes and are discussed in the following section.

4.2.1 Milk fat extraction efficiency

Extraction efficiency of a solvent is an important parameter to evaluate the recovery of product and economy of the solvent extraction plant. It is represented as % of the total fat extracted from the source material. In this activity, milk fat was extracted from *ghee* residue at two different temperatures, viz., 50 ^{o}C and 10 ^{o}C below the boiling point of the solvent (60 ^{o}C for ethyl acetate and 80 ^{o}C for di-methyl carbonate). The results obtained are presented in Table 4.3 and Fig 4.2.

Table 4.3 Fat extraction efficiency of solvents at different extraction temperatures

Sr. no	Solvents	% Fat in ghee residue	Temperature	
			50 ^{o}C	60 ^{o}C/ 80 ^{o}C
1	Ethyl acetate	39.3 ± 0.69	48.70 $\pm$ 0.70^{aA}	60.40 $\pm$ 1.90^{bA}
2	Dimethyl carbonate	39.3 ± 0.69	45.62 $\pm$ 0.62^{aB}	47.60 $\pm$ 0.69^{aB}

Values are mean ± SE (n=3);
Means with different superscript (a, b) differ significantly ($p<0.05$) within the same row for the same attribute
Means with different superscript (A, B) differ significantly ($p<0.05$) within the same column for the same attribute

It was observed that EA had significantly higher ($p<0.05$) fat extraction efficacy as compared to DMC at both the studied extraction temperature. Further, for the same solvent, it was found that increasing the extraction temperature resulted into significant ($p<0.05$) increase in fat extraction efficacy from 48.70 to 60.40 % for EA, while no such significant effect ($p>0.05$) was obtained in case of DMC and the values were 45.62 and 47.60 % at 50 and 80^{o}C extraction temperature, respectively. Increase in fat extraction efficacy with increasing extraction temperature could be attributed to decrease in the viscosity of the solvent and fat, which enables easy penetration of solvent into the ghee residue particles and migration of fat

outside the particles along with the solvent. In addition to this, fat solubility also increases in the solvent with an increase in the temperature. Further, Giergielewicz-Mozajska *et al*. (2001) reported that increased heat energy because of higher temperature results into breakdown of solute-matrix linkages and promotes solute diffusion from the matrix. Upon comparison of the solvent extraction process with the conventional methods (centrifugal and pressing), it was observed that EA extracted sample at 60 ^{o}C reported higher fat extraction efficiency than centrifugal method and similar to pressing method. Viswanathan *et al.* (1973) reported that centrifugal fat recovery method, hydraulic press and hand screw press had extraction efficiency about 46%, 71% and 61 %, respectively.

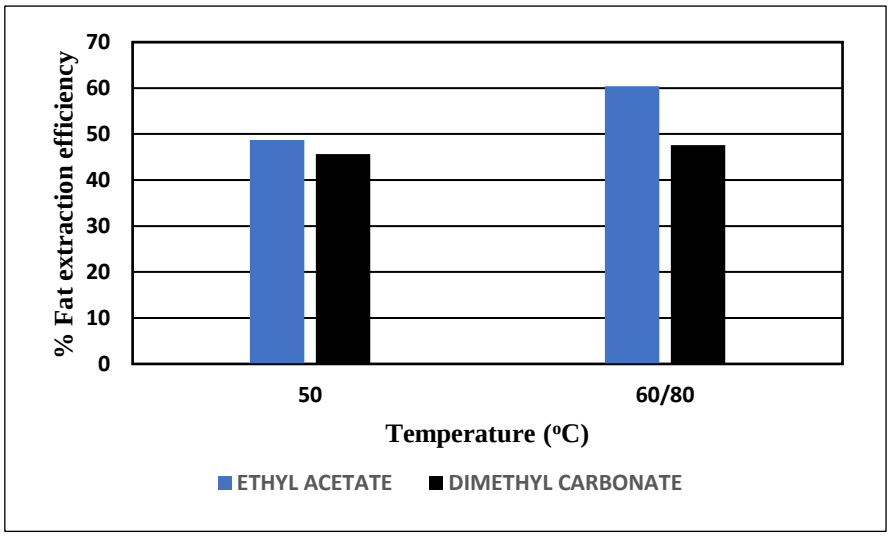


Fig.: 4.2 Fat extraction efficiency of solvents at different extraction temperatures

4.2.2 Free fatty acids content in solvent extracted milk fat

The free fatty acid (FFA) content is an indicator of the number of fatty acids present in free form in the fat sample. The value is a measure of fatty acids which have been liberated by hydrolysis from the glycerides due to the action of moisture, temperature and/or lipolytic enzyme (lipase). It has importance related to the stability and flavour changes in the milk fat. The FFA content (% oleic acid) in the solvent extracted milk fat is provided in the Table 4.4 and Fig 4.3.

Table 4.4 Free fatty acids content in extracted milk fat at different extraction temperature

Solvents	Free fatty acids (% oleic acid)	
	50°C	**60°C/80°C**
Ethyl acetate	0.50 ± 0.02^{aA}	0.53 ± 0.03^{aA}
Dimethyl carbonate	0.51 ± 0.04^{aA}	0.59 ± 0.02^{aA}
Control (*ghee*)	0.34 ± 0.01	

Values are mean ± SE (n=3);
Means with different superscript (a, b) differ significantly ($p<0.05$) within the same row for the same attribute
Means with different superscript (A, B) differ significantly ($p<0.05$) within the same column for the same attribute

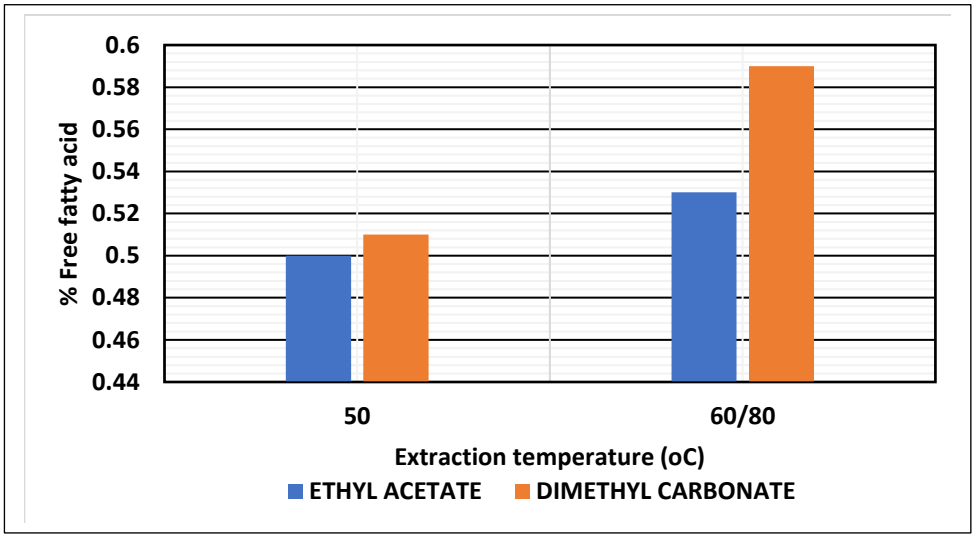


Fig. 4.3: Free fatty acid content in solvent extracted milk fat at different extraction temperature

It was observed that both the solvents at both the studied extraction temperatures had no significant difference ($p>0.05$) in their FFA content. The FFA content in EA extracted milk fat at 60°C was slightly higher (0.53 ± 0.03) than at 50°C extraction temperature (0.50 ± 0.02). Similarly, DMC extracted milk fat had higher FFA values (0.59 ± 0.02) at 80°C than at 50°C (0.51 ± 0.04) extraction temperature. Among the two solvents, highest FFA content was observed in DMC extracted milk fat than EA, which could be attributed to the lesser polarity of EA that led to lower extraction of FFA in the extract. Efthymiopoulos *et al*. (2018) reported that FFA content in solvent extracted oil increased with an increase in the polarity of

the solvent. When compared with the control (*ghee*) sample, it was found that solvent extracted milk fat had higher FFA concentration than the control ghee sample. This could be due to the additional heat treatment provided to the solvent extracted milk fat during the final heating step (110^{o}C for 5-10 minutes) to evaporate the residual solvent. Dawodu *et al.* (2017) reported that an increase in the extraction temperature resulted into an increase in lipolytic activity and led to an increase in the acid value of vegetable oil.

4.2.3 Iodine value

Iodine value indicates about the presence of unsaturation in the lipid samples. A higher iodine value reflects higher amount unsaturated bonds present in the sample. The results of iodine values of extracted milk fat and control sample is provided in the Table no. 4.5 and Figure no. 4.4

Table 4.5 Iodine value of extracted milk fat at different extraction temperature

Solvents	Temperature	
	50^{o}C	**60/80^{o}C**
Ethyl acetate	28.16 ± 0.44^{aA}	27.01 ± 0.58^{aA}
Dimethyl carbonate	30.36 ± 0.65^{aB}	28.77 ± 0.42^{bA}
Control	31.36 ± 0.51	

Values are mean ± SE (n=3);
Means with different superscript (a, b) differ significantly ($p<0.05$) within the same row for the same attribute
Means with different superscript (A, B) differ significantly ($p<0.05$) within the same column for the same attribute

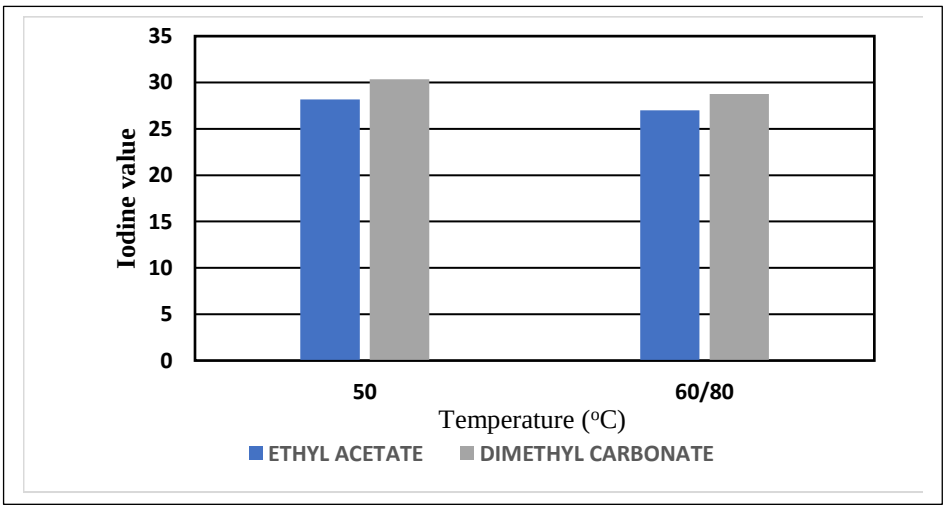


Fig. 4.4: Iodine value of extracted milk fat at different extraction temperature

When compared among EA and DMC at different extraction temperatures, it was found that both the solvents showed significant difference ($p<0.05$) in iodine value at 50 ^oC of extraction temperature whereas no such significant difference ($p>0.05$) was obtained at elevated (60 or 80 ^oC) extraction temperature. At 50^oC extraction temperature, DMC had higher iodine value (30.36) than EA (28.16). Compared with control (*ghee*) sample, it was found that the solvent extracted milk fat had lower iodine value. This indicated that the experimental samples contain lower amount of unsaturated fatty acids. Lower iodine value in the experimental samples could be attributed to the additional heating provided to these samples during the solvent evaporation step. Dawodu *et al*. (2015) reported that iodine value in vegetable oils (olive oil, palm kernel oil, peanut oil, sunflower oil and soybean oil) decreased with an increase in the heating temperature. Ngassapa *et al*. (2012) reported that lowering of iodine value upon heating oils could be due to the thermal destruction of π-bonds and increase in the amount of saturated bonds in the fatty acids.

4.2.4 Instrumental color attributes of solvent extracted milk fat

The solvent extracted milk fat from ethyl acetate and dimethyl carbonate were tested for instrumental color values. The color attributes L* (lightness (100: white, 0: black), a* (+: red, -: green) and b* (+: yellow, -: blue) values of the samples are provided in Table 4.6 and Figure 4.5.

Table 4.6 Instrumental colour values of extracted milk fat at different extraction temperature

Solvent	L* value		a* value		b* value	
	50 ^oC	**60/80 ^oC**	**50 ^oC**	**60/80 ^oC**	**50 ^oC**	**60/80 ^oC**
EA	5.39 ± 0.12^{aA}	5.98 ± 0.18^{aA}	3.97 ± 0.9^{aA}	4.8 ± 0.52^{aA}	6.62 ± 0.86^{aA}	5.13 ± 0.63^{aA}
DMC	6.38 ± 0.02^{aB}	6.68 ± 0.4^{aA}	3.39 ± 0.36^{aA}	2.35 ± 0.29^{bB}	5.08 ± 0.77^{aA}	7 ± 0.32^{bB}
Control (ghee)	6.18 ± 0.24		5.1 ± 0.44		5.49 ± 0.37	

Values are mean ± SE (n=3);
Means with different superscript (a, b) differ significantly ($p<0.05$) within the same row for the same attribute
Means with different superscript (A, B) differ significantly ($p<0.05$) within the same column for the same attribute

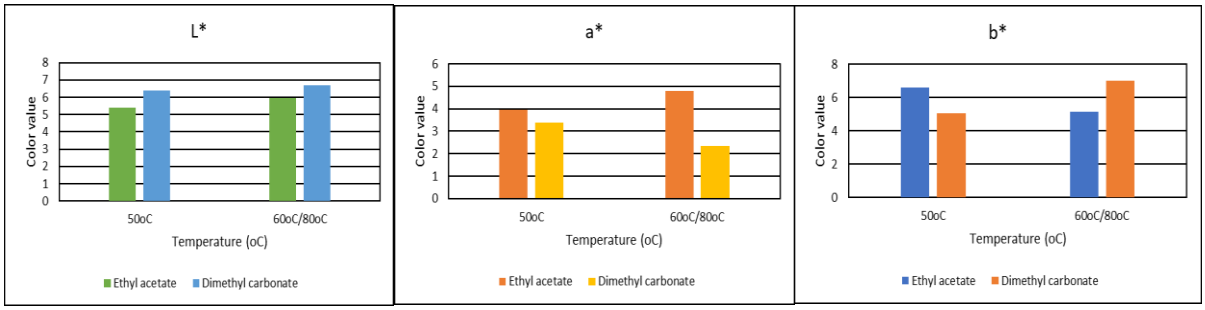


Figure 4.5 Instrumental colour values of extracted milk fat at different extraction temperature

When compared for the same solvent at different extraction temperatures, it was observed that both the solvent extracted milk fat samples showed no significant difference ($p>0.05$) in L* value with an increase in the extraction temperature. The EA extracted milk fat sample had higher L* value (5.98±0.18) at 60^{o}C extraction temperature than at 50 ^{o}C extraction temperature (5.39±0.12). Similarly, the DMC extracted milk fat had higher L* value (6.68±0.40) at 80^{o}C extraction temperature as compared to the sample extracted at 50^{o}C (6.38±0.02). Among the two solvents at both the extraction temperatures, it was found that EA extracted milk fat had lowest L* value (5.39±0.12) and DMC extracted milk fat at 80^{o}C had highest value (6.68±0.40) among all the samples. In case of a* value, it was found that the solvent extracted milk fat from *ghee* residue using EA at 50˚C did not differ significantly ($p>0.05$) from the milk fat sample extracted at 60˚C, whereas the value was significantly different ($p<0.05$) for DMC at different extraction temperature. Among the two solvents at different extraction temperature, it was observed that the two solvent extracted milk fat samples had no significant difference ($p>0.05$) at 50^{o}C extraction temperature, while significant difference ($p<0.05$) was obtained at higher extraction temperature. It was observed that the b* value of solvent extracted milk fat sample using EA at 50˚C extraction temperature did not differ significantly ($p>0.05$) from the sample extracted 60˚C. On the other hand, b* value of sample extracted using DMC differed significantly ($p<0.05$) with an increase in the extraction temperature. The EA extracted milk fat sample had higher a* value (6.62±0.86) at 50 ^{o}C extraction temperature than at 60 ^{o}C extraction temperature (5.13±0.63). On the other hand, DMC extracted milk fat sample had higher b* value at higher extraction temperature.

When compared with the control *ghee* sample, it was observed that the L* values of EA extracted sample at both the extraction temperature was lower than the control *ghee* sample, but it was lower than the DMC extracted samples. The a* value of control *ghee* sample was

higher than all the solvent extracted milk fat samples. On the other hand, b* value of EA extracted sample at 50 ^{o}C extraction temperature was higher (6.62±0.86) than control ghee (5.49 ± 0.37) sample but at 60 ^{o}C it was lower (5.13±0.63) than control ghee sample.

4.2.5 Rheological analysis of solvent extracted milk fat

Flow behaviour of solvent extracted milk fat

Rheological analysis of the solvent extracted milk fat was performed to evaluate the flow behaviour properties of the sample. Relationship between the shear rate and apparent viscosity of the samples are presented in Fig. 4.6 and 4.7. It was observed that viscosity of solvent extracted milk fat samples as well as control ghee sample decreased with an increase in the shear rate. This indicates about the shear thinning or pseudo-plastic deformation of milk fat samples. Highest viscosity was observed in case of DMC 80 extracted milk fat sample and the least from EA 50 extracted sample. Viscosity of DMC 80 extracted milk fat decreased from 0.333 Pas to 0.06463 Pas, DMC 50 extracted sample from 0.297Pas to 0.06456 Pas, EA 60 extracted sample from 0.2486 Pas to 0.06653 Pas, control sample (*ghee*) from 0.2263 Pas to 0.058967 Pas and EA 50 extracted sample from 0.2015 Pas to 0.674 Pas. Differences in the viscosity of samples could be due to the differences in their fatty acid composition. Similar observations have been reported by Harry-O'kuru and Carriere (2002) for milkweed oil and Kim *et al*. (2010) for rapeseed oil.

Frequency sweep of extracted milk fat samples

The rheological analysis of solvent extracted milk fat (extracted using EA and DMC at 50^{o}C and 10^{o}C below the boiling point) was performed to check the viscoelastic properties of the extracted milk fat. The rotational Rheometer (MCR 52, Anton Parr, Austria) fitted with probe PP-50 is used to conduct rheological tests. Amplitude sweep test was performed at temperature of 18^{o}C by adjusting the gap of 0.5 mm and LVE (linear viscoelastic region) range as % strain obtained by this test is used in frequency sweep test. LVE range was obtained by amplitude sweep test was 0.01 (as % strain) and that was used for frequency sweep test. The frequency sweep test was performed at 18°C with constant 0.01% strain and frequency varied from 0.01 to 100 Hz. The values of G' (storage modulus) and G'' (loss modulus) was obtained over frequency range of 0.01 to 100 Hz and the results are presented in Fig. 4.8. It was observed that the storage modulus values of all the solvent extracted samples increased with an increase in the angular frequency. Higher values of storage modulus were observed in case of DMC80 followed by EA50, DMC50, control and lower in

EA60 sample. Storage modulus indicates about the elastic component of the viscoelastic behaviour, which resembles the sample's solid-state behaviour. Loss modulus represents the viscous component of viscoelastic behaviour, which may be referred of as the sample's liquid-state behaviour. In our study, loss modulus (G") values were lower than the storage modulus (G') values, which indicated that elastic characteristics dominated over the viscous characteristics in the linear viscoelastic range. Similar results are reported for by Duhan *et al.* (2018) for Indian cow milk ghee samples.

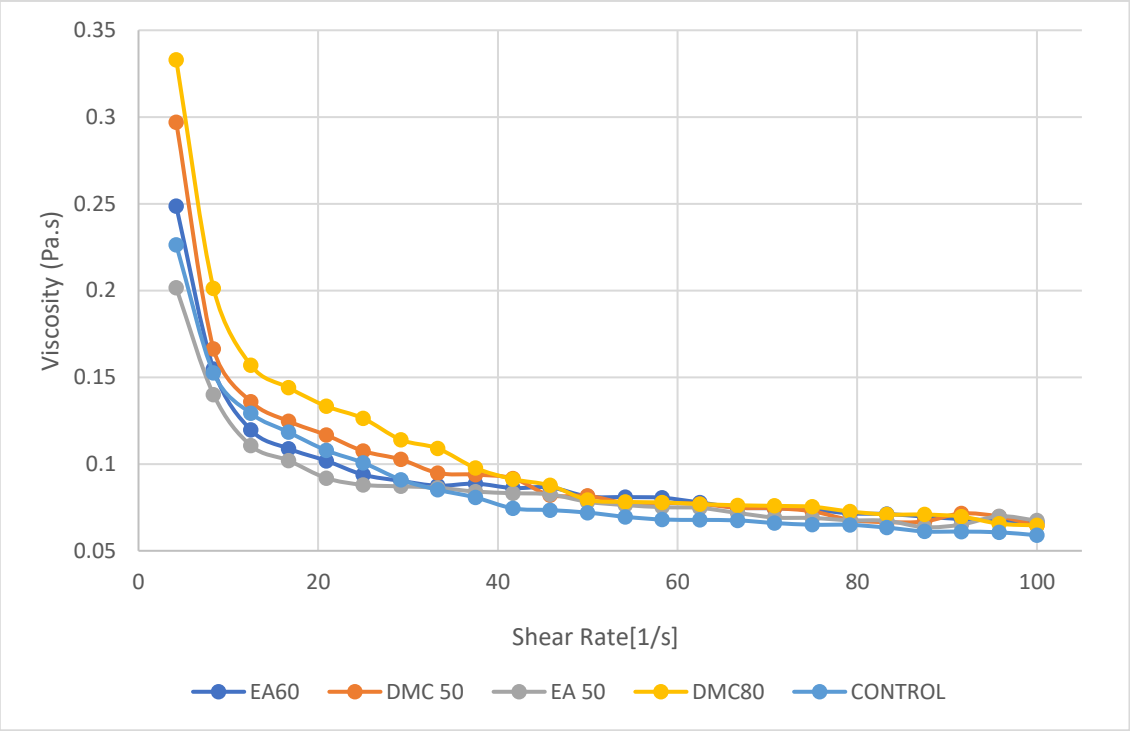


Figure 4.6 Flow behaviour of extracted milk fat at different extraction temperature

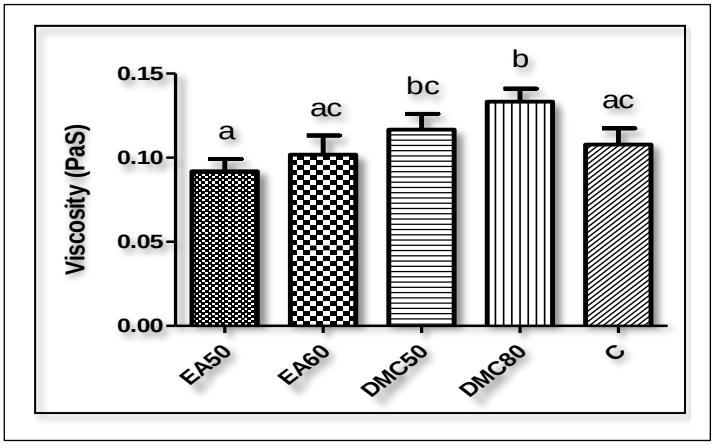


Figure 4.7 Viscosity of solvent extracted milk fat samples at 25(1/s) shear rate

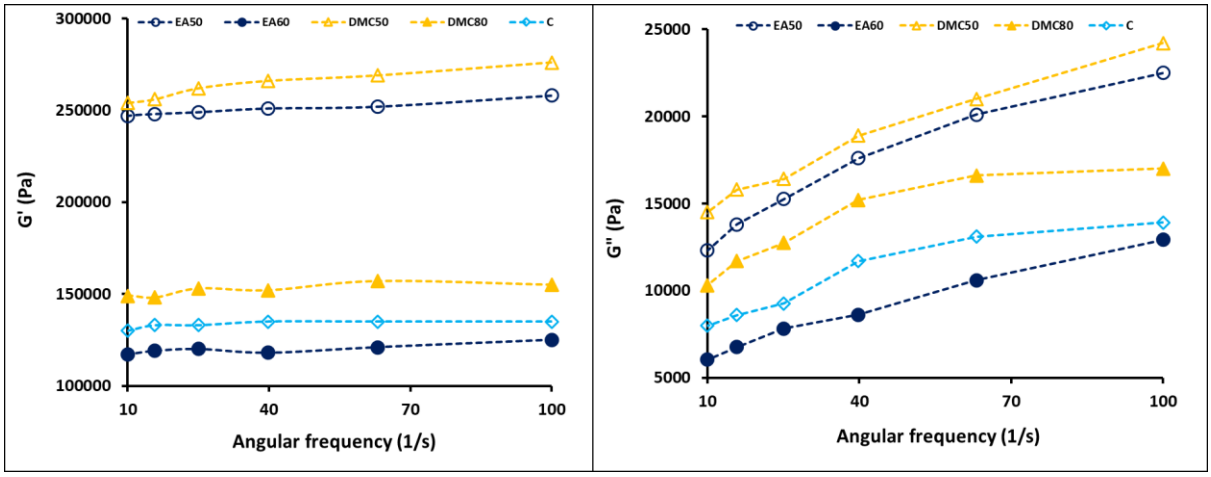


Figure 4.8 Frequency sweep of solvent extracted milk fat at different extraction temperature

4.2.6 Selection of solvent using Fourier transform infrared (FTIR) spectroscopy

When ghee residues are applied with solvent extraction to extract the residual milk fat, there lies a probability that the solvent extracted - heat treated samples might contain some traces of the solvent. Although, the solvents used in the present investigation does not possess high toxicity but their level should be as low as minimum as possible. Among the different tests performed to check the quality and purity of ghee samples, FTIR spectroscopy is an emerging method and increasingly being used for quality inspection of different products. FTIR spectra of different samples were obtained using FTIR spectrometer in the 400–4000 cm^{-1} frequency region with a resolution of 4 cm^{-1}. The sample was placed in contact with attenuated total reflectance (ATR) element (ZnSe crystal) at controlled ambient temperature (20-22 ^{o}C). Before analysis ATR diamond was washed using 70% 2-propanol and undergone for background scanning. Results were recorded in the form of peaks obtained at different wave numbers along with their absorption value. The recorded absorption waves and values then compared with the literature reported values using Perkin Elmer software. For identity acceptance about 4 peaks should be matching absorption bands. The spectra and results obtained are listed in Table 4.7 and Fig. 4.9.

The FTIR spectra (4000 - 400 cm^{-1}) of oils comprises of four different regions, viz., hydroxyl region (4000 – 3100 cm^{-1}), C-H stretching vibration (3100 – 2800 cm^{-1}), C=O stretching vibration (1600 – 1390 cm^{-1}) and the fingerprint region (1390 – 700 cm^{-1}) comprising of C-H, C-O, C-C and C=C stretching vibration (Alexa *et al*., 2009). The FTIR spectra of control ghee samples and solvent extracted ghee samples were examined for the absorption

intensities and peaks position in the spectra. Visual inspection of spectra and data analysis for the position of distinct absorption peaks in the spectra indicated that the total number of peaks and their positions were almost similar in some regions but showed discernible changes in some regions.

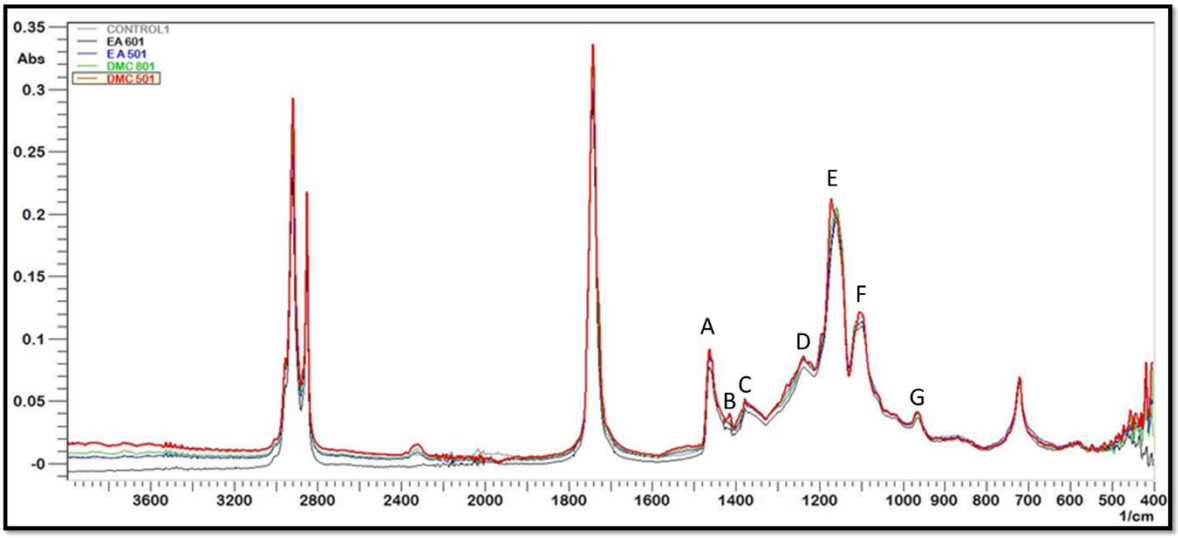


Figure 4.9 FTIR absorbance spectra of solvent extracted milk fat at different extraction temperature

Different peaks in the FTIR spectra are denoted by A, B, C, D, E, F and G. The wave numbers of standard peaks (Table no. 4.8) of control sample were compared with solvent extracted samples to identify the presence of residual solvent in them. The absorbance peaks (wave number) of the solvent extracted milk fat samples were compared with the control (ghee) samples and the results are presented in Table no. 4.7. The finger printing area (1390-700 cm^{-1}) in the MIR spectrum of an edible oil or fat contains vibrations of C–H, C=O, C–O, C–C, C–C, C=C, etc. in stretching and/or bending mode. It was found that none of the solvent extracted milk fat sample had absorbance peak of the solvent as no new absorbance peak emerged in their FTIR spectra. This indicated that solvent was completely evaporated from the extracted milk fat. Further, it was observed that the absorbance spectra of EA 60 extracted sample were found to be closer to the control sample.

Table 4.7 FTIR absorbance spectra of extracted milk fat at different extraction temperature

Peak no.	Wave number	Control	EA50	EA60	DMC50	DMC80
A	1466	1464.45 (0.07925)	1463.14 (0.0841)	1465 (0.07699)	1463.14 (0.091)	1465.11 (0.08643)
B	1418	1417.02 (0.03172)	1413.98* (0.033)	1415.04 (0.02949)	1417.91 (0.040)	1415.95 (0.03496)
C	1377	1379.47 (0.04611)	1380.55 (0.050)	1379.84 (0.044)	1380.55 (0.0522)	1380.55 (0.0502)
D	1236	1237.18 (0.07863)	1237 (0.083)	1237.16 (0.0775)	1240.93* (0.08567)	1238.96 (0.0845)
E	1161	1160.11 (0.1955)	1162.27 (0.1963)	1161.06 (0.195)	1174.07* (0.211)	1162.27 (0.2039)
F	1083	1084.47 (0.1130)	1088.31* (0.1145)	1085.85 (0.1125)	1090.31* (0.121)	1089.34* (0.1182)
G	966	966.439 (0.04173)	965.61 (0.040)	966.71 (0.03747)	967.585 (0.04159)	965.618 (0.03877)

Values are mean (n=9); * indicates significant difference ($p<0.05$) from the control (ghee) sample

The peaks at A, B, C, D, E, F and G positions (wave number) were significantly not different ($p>0.05$) from that of the control *ghee* sample. On the other hand, all other solvent extracted samples had at least one of the absorbance peak positions (wave number) significantly different ($p<0.05$) from that of the control (*ghee*) sample. Changes in the absorbance peak at 1466-1463 cm^{-1} region was related to H-C-H bending of CH_2 and CH_3, 1417-1413 cm^{-1} region was related to C-H bending (rocking) of cis C=C-H, 1381-1379 cm^{-1} region was related to H-C-H symmetric bending of CH2, 1240-1237 cm^{-1} region was related to C-O stretching in O-C (=O)-CH_2 of ester, 1164-1160 cm^{-1} region was related to C-O stretching in HC-O-(C=O) of ester, 1101-1096 cm^{-1} region was related to C-C links of hydrocarbon chain

and 968-965 cm^{-1} region was related to C-H bending of trans double bond (Antony *et al.*, 2017).

Table 4.8 Description of peaks obtained in the FTIR spectra

Peak	Wave numbers region (cm^{-1})	Functional group & vibration modes
A	1466	H–C–H bending of CH_2 and CH_3 (scissoring)
B	1418	C–H bending (rocking) of cis C=C–H
C	1377	H–C–H symmetric bending of CH_2
D	1236	C–O stretching in O–C(=O)–CH2 of ester
E	1161	C–O stretching in HC–O–(C=O) of ester
F	1083	–C–H– bending
G	966	C–H bending of trans double bond

(Anthony *et al*., 2017)

The observed differences in the position of absorption peak could be attributed to the difference in the composition of fatty acids present in these samples, i.e., variation in the number of carbon atoms in the chain length, number of double or triple bonds (level of unsaturation), position and arrangement of these bonds (Lerma-Garcia *et al*., 2010). Further, it could be seen that all the samples exhibited small absorbance peaks in the 2400–2000cm^{-1} region, which indicates about the presence residues of thermally denatured protein in these samples. Shi *et al.* (2019) attributed these peaks to a combination of C–N, N–H, C–O, C=O and C–H bonds present in the digested wheat proteins. Presence of protein in ghee samples could be attributed to two reasons, viz., (a) emergence of hydrophobic compounds (including milk proteins) upon heat treatment (b) small size of protein aggregate that tend to pass the filtration process during ghee clarification. Development of hydrophobic nature in milk proteins upon heat treatment is related to the denaturation and exposure of sulphahydral groups from whey proteins, which are reported to possess hydrophobic nature (Zhu & Damodaran, 1994).

From the FTIR spectra, it was observed that none of the solvent extracted milk fat samples had solvent residues and the samples obtained using EA at 60 ^{o}C extraction temperature was similar ($p>0.05$) to that of the control (*ghee*) sample, hence it was selected for further studies.

4.3 Comparison of conventional and solvent extraction method for milk fat extraction from *ghee* residue

In this activity, we are compared the conventional fat extraction method from ghee residue with that of the previously selected solvent extraction method (Fig.4.10) for energy consumption, time requirement and waste water generation.

At the industrial scale, a hydrothermal method (Fig.4.11) is used for extraction of residual milk fat from the *ghee* residue. In this method, first the *ghee* residue is mixed with hot boiling water in about 1:10 ratio (*ghee* residue: hot water) in milk cans. The contents are then left undisturbed for about 3 – 4 hrs (preferably overnight). After this, the cans containing ghee residue are transferred to cold store till the temperature decreases to about 4-6 ^{o}C. During this, a fat layer solidifies at the top of the contents. This solidified milk fat layer is collected carefully from the top and the remaining waster is discarded and sent to effluent treatment. There are some drawbacks of this method such as uneconomical, labour intensive and required lots of water as well as generate waste water. Solvent extraction method does not generate waste water and provide higher extraction efficiency.

Method for milk fat extraction from ghee residue using low toxicity solvent

Homogeneous mixing of Ghee residue (GR)

↓

Adding Ethyl acetate: GR for each solvent at 5:1

↓

Heating at 60 ^{o}C and Continuous mixing (100 rpm) for 30 minutes

↓

Filtration of ghee residue filtrate

↓

Place filtrate in solvent extractor for solvent evaporation

↓

Solvent evaporation

↓

Milk fat/ ghee

Figure no. 4.10 Process flow chart for fat extraction from ghee residue (solvent extraction method)

a) Energy consumption calculation for solvent extraction method -

Assumption: Initial temperature of solvent & ghee residue is 30 ^{o}C

Data – ghee residue (Cp) – 2.218 KJ/Kg ^{o}C, Ethyl acetate (Cp) – 1.904 KJ/Kg ^{o}C, Qty of ghee residues (M) – 100 gm, Qty of ethyl acetate (m) – 500ml, latent heat of evaporation of ethyl acetate (L) – 3.67 x 10^5 J/Kg

1) Energy consumed to sensible heating of solvent from ambient temperature to 60^{o}C –

$Q = m \times Cp \times (t_2 - t_1)$

$= 0.451 \times 1.904 \times (60 - 30) = 25761.10$ Joules

2) Energy consumed to sensible heating of ghee residues

$Q = m \times Cp \times (t_2 - t_1)$

$= 0.1 \times 2.218 \times (60 - 30) = 6654$ Joules

3) Energy consumed by magnetic stirrer –

Measured on Wattmeter – 0.07 KWh = 70 Wh = 252000 Joules

4) Energy consumed for sensible heating of solvent before evaporation

$Q = m \times Cp \times (t_2 - t_1)$

$= 0.440 \times 1.904 \times (77.1 - 45) = 26892.096$ Joules

5) Energy consumed for evaporation heating of solvent –

$q = mL = 0.430 \times 3.67 \times 10^5 = 157810$ Joules

After the energy consumption and time calculation, it was found that total energy required to extract milk fat from 100gm of ghee residue using 500ml of solvents was 469117.2 joules and 102.5 minutes. In addition, no waste water was generated during the solvent extraction process.

b) Energy consumption calculation for hydrothermal treatment -

Data – Solid fat (Cp) – 2.12 KJ/Kg. oC, water (Cp) – 4.18 KJ/Kg. oC, Qty of ghee residues (M) – 100 gm, Qty of water(m) – 1000 gm, latent heat of fusion of water (L_f) & Fat - 334KJ/Kg & 120 KJ/Kg respectively.

1) Energy consumed for sensible heating of water for boiling –

$Q = m * Cp * (t_2 - t_1)$

$= 1 * 4.18 * (100 - 29) = 296780$ Joules

2) Energy consumed to sensible cooling of water –

$Q = m * Cp * (t_2 - t_1) = 0.966 \times 4.18 \times (4 - 40) = 145363.68$ Joules

3) Energy consumed for sensible cooling of fat –

$Q = m * Cp * (t_2 – t_1) = 0.017 \times 1.928 \times (4 – 40) = 1179.9$ Joules

4) Energy consumed for solidification of fat –

$q = mL = 0.017 \times 120 = 2040$ Joule

5) Energy consumed for sensible cooling of ghee residues –

$Q = m * Cp * (t_2 – t_1) = 0.100 \times 2.218 \times (4 – 40) = 7984.8$ Joules

6) Waste water generation – 800 to 900 gm per 100gm ghee residue processing

Extraction method using hydrothermal treatment (Industrial method)

Ghee residue (GR) mixing

↓

Filling in cans

↓

Addition of boiling water (1:10) & mixing the content

↓

Leaving undisturbed for 3 – 4 hrs

↓

Cool the mixture of fat and water (4°C – 8°C)

↓

Collection of top layers of solidified fat

↓

Recovered milk fat / Ghee

Figure no. 4.11 – Process chart for fat extraction from ghee residue by hydrothermal method

It was found that energy consumption was 453348.38 joules and 215 minutes was taken to complete the extraction from 100gm ghee residue using 1000 ml boiled water. Further, waste water of about 800-900 g was generated during the conventional milk fat extraction process.

Upon comparison of the two methods for energy consumption, time requirement and waste water generation (Table 4.9), it was found that the solvent extraction method require slightly more amount of energy (4,69,117.2 Joules) as compared to the conventional method (4,53,348.38 Joules). This is primarily because of the latent heat of solvent evaporation to remove the solvent from the extracted milk fat. Besides of this, the solvent extraction method

requires about half of the time consumed by conventional method and does not generate waste water.

Table 4.9 Comparison of energy requirement between solvent extraction and industrial fat recovery method

Stages	Solvent extraction method		Industrial fat recovery method	
	Energy consumed (Joules)	**Time taken (minutes)**	**Energy consumed (Joules)**	**Time taken (minutes)**
1	25761.1	20	296780	30
2	6654		226121.28	185
3	252000	30	1341.89	
4	26892.1	16.5	2040	
5	157810	36	12420.8	
Total	**4,69,117.2**	**102.5**	**4,53,348.38**	**215**

Chapter 5

Summary and conclusion

5. SUMMARY AND CONCLUSION

Ghee is a heat clarified milk fat with unique organoleptic attributes. About 30 - 35% of total milk produced in India is utilized for *ghee* preparation and the average yield of ghee residue is about a tenth of the *ghee* produced. *Ghee* residue (GR) is a blackish brown residue obtained as a by-product during *ghee* preparation. It mainly consists of milk proteins, lactose and small quantity minerals. Fat content in the GR ranges from 32 to 70 % (weight basis). Considering the amount of fat in GR, different interventions for extraction of milk fat from GR with extraction efficiencies, viz., pressing (Hydraulic – 71% & hand screw pressed – 61%), hydrothermal treatment and centrifugal separation (46%) has been explored. But these techniques are time consuming, labour intensive and generates huge amount of waste water. A strategy to overcome this could be by using solvent extraction method to recover residual milk fat from ghee residues. The present investigation was carried out for extraction of milk fat from GR using low toxicity solvents. The study was carried out in two phases. In first phase, low toxicity solvents were screened for their suitability to extract milk fat from GR. In the subsequent stage, the extraction temperature was studied of the screened solvents to extract maximum amount of fat. The major finding of present study was summarized as follows:

- Ethanol, 2- Methyl tetrahydrofuran (MeTHF), dimethyl carbonate (DMC) and ethyl acetate (EA) solvents were studied on the basis of parameters low toxicity and boiling point (< 100 ^{o}C). Water & hexane were considered as control solvents.
- The solvents were screened for suitability to extract milk fat from GR. It was observed that milk fat could not be extracted using water as a solvent, while the fat obtained using ethanol had dark residues at bottom; hexane and MeTHF extracted fat had dark blackish fat with burnt like appearance. The fat obtained using DMC and EA had ghee like appearance. Because of which DMC and EA solvents were selected for further studies.
- The fat extraction efficiency of ethyl acetate extracted milk fat sample at 50^{o}C (EA50) and 60^{o}C (EA60) and dimethyl carbonate extracted milk fat sample at 50^{o}C (DMC50) and 80^{o}C (DMC80) were found to be, 48.70 ± 0.70, 60.40 ± 1.90, 45.62 ± 0.62 and 47.60 ± 0.69, respectively. The free fatty acid (%OA) in ethyl acetate extracted milk fat sample at 50^{o}C and 60^{o}C, dimethyl carbonate extracted milk fat sample at 50^{o}C and 80^{o}C and control ghee were found to be 0.50 ± 0.02, 0.53 ± 0.03, 0.51 ± 0.04,

0.59 ± 0.02 and 0.34 ± 0.01, respectively and iodine value in this solvent extracted milk fat samples and control ghee were found to 28.16 ± 0.44, 27.01 ± 0.58, 30.36 ± 0.65, 28.77 ± 0.42 and 31.36 ± 0.51, respectively. Among all solvent extracted milk fat samples, percentage fat extraction efficiency of EA 60 was found to be higher as compare to others. Also, free fatty acids content increased and iodine value decreased with an increase in extraction temperature in all solvent extracted milk fat samples.

- Flow behaviour analysis of solvent extracted milk fat and control ghee sample revealed that all the samples showed shear thinning behaviour with increase in the shear rate. Among all the samples, DMC80 extracted milk fat sample had highest viscosity, followed by DMC50, EA60, control (*ghee*) and lowest was in case of EA50 extracted milk fat sample. Viscosity of EA50, EA60, DMC50, DMC80 and control ghee sample was found to be 0.093867±0.014 Pa.s, 0.1075±0.013 Pa.s, 0.088±0.008 Pa.s, 0.126±0.01 Pa.s and 0.1±0.014 Pa.s, respectively at 30^0C at 25 (1/s) shear rate.
- During the frequency sweep analysis, it was found that both storage and loss modulus values of all the samples increased with an increase in the angular frequency. Further, storage modulus (G') values were higher than the loss modulus (G") values for all the samples, which indicated that elastic behaviour dominated viscous behaviour in the linear viscoelastic range, and it remained phase stable throughout the LVR range.
- In the FTIR analysis, it was found that almost all of the variation in peak value and absorbance were observed in the range 1500 cm^{-1} to 900 cm^{-1} wave number region. The peaks were identified by notation A (1466 cm^{-1}), B (1418 cm^{-1}), C (1377 cm^{-1}), D (1236 cm^{-1}), E (1161 cm^{-1}), F (1083 cm^{-1}) and G (966 cm^{-1}). Upon examination of the FTIR spectra of the experimental sample, it was found that no absorbance peak of the respective solvent was observed. This indicated that the solvent was completely evaporated from the milk fat samples. Further, all except EA60 sample exhibited no significant difference ($p>0.05$) from that of the control ghee sample. This indicated that EA60 sample was most similar to the control ghee sample and thus was selected for further studies.
- The solvent extraction method for milk fat extraction from ghee residue was compared with the conventional (hydrothermal) treatment for energy requirement, waste water generation and time requirement. It was found that solvent extraction method using ethyl acetate at 60^oC of extraction temperature required 4,69,117.2 joules and 102.5 minutes time to process 100 grams of ghee residues whereas

industrial fat recovery method required 4,53,348.38 joules and 215 minutes to complete the fat extraction from 100 gm ghee residue using 1000 ml boiled water. Further, waste water of about 800-900 g was generated during the conventional milk fat extraction process.

Chapter 6

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