Study on fatty acid composition of clarified butter prepared using different methods

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CARISSA NOEMIA DE SA

Seat Number: 22P0470004

ABC ID: 751314372328

PRN: 201902510

Under the Supervision of

DR. MEGHANATH PRABHU

School of Biological Sciences and Biotechnology

Goa University

GOA UNIVERSITY

Date: April 2024

Seal of the School

Examined by:

8/4/24

DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, "Study on fatty acid composition of clarified butter prepared using different methods" is based on the results of investigations carried out by me in the Biotechnology Discipline at the School of Biological Sciences and Biotechnology, Goa University under the Supervision of Dr. Meghanath Prabhu and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will be not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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Carissa Noemia De Sa
Signature and Name of Student
Seat no: 22P0470004

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Place: Goa University

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8/4/24
Dr. Meghanath Prabhu

Signature and Name of Supervising Teacher

Date: 8th April 2024

GOA UNIVERSITY ★ SBSB: Biotechnology ★ Taleigao Plateau, Goa 403206

School Stamp

Signature of Dean of the School
Date: 8th April 2024
Place: Goa University

Dean of School of Biological Sciences & Biotechnology
Goa University, Goa-403206

CONTENTS

PREFACE

The study of different types of clarified butter, also known as ghee, is of significant interest due to its prominent use in culinary traditions worldwide and its health benefits. In recent years, there has been a growing interest in understanding the nutritional composition of various types of clarified butter.

The fatty acid profile of clarified butter can vary depending on the source of milk, method of preparation, and regional differences in production. Different types of clarified butter may have varying levels of saturated and unsaturated fats, which can influence their health effects.

Clarified butter, being a concentrated source of fats, has implications for health due to its specific composition of fatty acids. Recent attention has focused on the types and proportions of these fatty acids – saturated, monounsaturated, and polyunsaturated as they play an important role in determining the nutritional value and health effects of clarified butter consumption.

This study aims to analyse and compare the fatty acid profile of different types of clarified butter. By examining the composition of ghee derived from different sources, this research seeks to provide valuable insights into their potential health implications and culinary uses. Understanding the variation in fatty acid profiles can guide consumers in making informed choices about their dietary fats.

The outcomes of this study could also contribute to a broader understanding of the health benefits and risks associated with the consumption of clarified butter. This knowledge can support ongoing discussions in nutrition and health, as well as offer guidance for culinary practices.

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TABLES

FIGURES

ABBREVIATIONS USED

Entity	Abbreviation
Sodium hydroxide	NaOH
Potassium iodide	KI
Sodium thiosulphate	$Na_2S_2O_3$
Potassium hydroxide	KOH
Thin Layer Chromatography	TLC

ABSTRACT

Clarified butter, commonly known as ghee, is a staple ingredient in many culinary traditions around the world. This study investigates the fatty acid profile of clarified butter prepared by different methods in comparison to branded ghee samples available in the market.

Thin layer chromatography (TLC) was employed to analyse and compare the fatty acid composition of each ghee type. The study involved dissolving each ghee sample in hexane to create a 1% (v/v) solution, which was then spotted onto TLC plates for analysis.

The results revealed significant variations in the fatty acid profiles among different types of ghee, reflecting their source and production methods. Different fatty acids like stearic acid, myristic acid, pentadecylic acid, oleic acid, linoleic acid, palmitoleic acid and arachidonic acid were found to be present in the clarified butter samples.

The findings highlight the importance of understanding the fatty acid composition of clarified butter, as it can impact nutritional properties and culinary applications. This comparative study provides valuable insights for consumers and producers regarding the selection and production of ghee with specific fatty acid profiles for health and culinary benefits.

CHAPTER 1: INTRODUCTION

1.1 Background

Clarified butter, commonly known as ghee, holds a significant place in culinary practices and dietary traditions. It is a form of butter where water and milk solids are removed, leaving behind pure butterfat. Despite its simplicity, the composition of clarified butter can vary significantly depending on factors such as the source of milk, the method of clarification, and regional preferences. One crucial aspect of understanding the nutritional and sensory attributes of clarified butter lies in analysing its fatty acid profile (Gupta *et al*., 2015).

The composition of clarified butter is primarily determined by the types and proportions of fatty acids present in the fat. Fatty acids are organic compounds that vary in their saturation levels and chain lengths, which influence the texture, flavour, and nutritional properties of the fat (Kaliyaperumal *et al*., 2017).

It is complex lipids of glycerides, sterols, sterol esters, free fatty acids, phospholipids, free fatty acids, hydrocarbons, carotenoids and carbonyls. It also contains small amount of iron and calcium as well as moisture. Clarified butter is majorly composed of glycerides which comprises 98% of total material in clarified butter and remaining 2% consists of sterols, mainly cholesterol which occurs to the extent of around 0.5% (Tengku-Rozaina *et al*., 2017).

The main fatty acids present in clarified butter include saturated fatty acids (SFAs) which make up a significant portion of clarified butter and contribute to its solid state at room temperature. The high concentration of SFAs may have implications for heart health. Monounsaturated fatty acids (MFAs), such as oleic acid, are moderately present in clarified butter, which are considered beneficial for heart health and have other benefits. Clarified butter contains lower

level of polyunsaturated fatty acids (PUFAs), including essential fatty acids like omega-3 and omega-6, which play a significant role in overall health (Talpur *et al*., 2008).

Clarified butter is commonly made at home in India. Usually there are four methods of preparation: Desi Method, Creamery Butter Method, Direct Cream Method and Pre-stratification Method. The desi method is mostly used (Rahman *et al*., 2008).

The quality of the prepared clarified butter depends on the quality of milk, cream, curd, butter, preparation method, temperature, storage conditions and type of animal feed. These factors help in assessing the physicochemical properties of clarified butter. Clarified butter quality can be determined by its peroxide value, flavour and acidity. The quality of clarified butter on storage is checked by finding out its acid and peroxide value (Das et al., 2017).

Clarification temperature controls the flavour of ghee. When ghee is prepared at a temperature of 120℃ or above, it has an intense flavour and is known as cooked or burnt ghee whereas if it has been prepared at 110℃, it has a mild flavour. The flavour of ghee is affected by the acidity of cream or butter (Lamsal *et al*., 2020).

Fatty acids are essential components of dietary fats, playing critical roles in human health and culinary properties. Various factors influence the fatty acid profile of clarified butter, such as the type of milk source, animal husbandry practices, processing methods, and storage conditions (Kumar & Rajhoria., 2019).

By examining the fatty acid composition of clarified butter, we can gain insights into its nutritional value, flavour characteristics, and potential health implications. Understanding the

fatty acid profile of clarified butter is essential for various reasons. Firstly, it provides information regarding the nutritional composition, including the types and proportions of saturated, monounsaturated, and polyunsaturated fatty acids present. Secondly, the fatty acid profile influences the flavour, aroma, and texture of clarified butter, thereby impacting consumer preferences (Anandan *et al.*, 2014).

The general chemical composition of clarified butter is as follows:

Table 1: General chemical composition of clarified butter

Component	**Content**
Milk fat	99-99.5%
Moisture	Less than 0.5%
Non-saponifiable matter	0.5-1%
Solids not fat (charred casein, salts, etc.)	Traces
Free fatty acids	Maximum 2.5%

This study aims to investigate and compare the fatty acid profiles of different types of clarified butter prepared and derived from various sources and processed using different methods. By analysing these profiles, we can identify variations in fatty acid composition and their implications in cooking and nutritional benefits. Through analysis and comparison, the aim is to provide valuable insights into the diversity of clarified butter products available in the market and their potential impacts on consumer preferences and dietary choices. This research holds significance for consumers, food manufacturers, and health professionals seeking to make informed decisions regarding fat consumption and dietary practices.

1.2 Aim and Objectives

Aim: To study the fatty acid composition of clarified butter prepared using different methods.

Objectives:

i. Preparation of clarified butter by different methods.

ii. Assessment and comparison of fatty acid profile of different types of clarified butter.

1.2 Hypothesis/Research question

The fatty acid profile of different types of clarified butter varies significantly based on the source of the milk (cow, buffalo, goat, or sheep) and the methods of production (traditional versus industrial processing). By examining the composition of various fatty acids present in clarified butter, valuable insights can be offered into the potential health effects of clarified butter composition and guide both producers and consumers in making healthy choices regarding its use in the diet. This study hypothesises that the traditional method of ghee preparation is responsible for the fatty acid content that are beneficial for health. The study of the fatty acids present in clarified butter aims to provide a wide understanding of the different types of fatty acids found in clarified butter and how they contribute to its overall nutritional profile.

1.3 Scope

The present study will help in learning various methods of clarified butter preparation, identification of various fatty acids present in each clarified butter sample, including saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids.

It will also focus on comparison of the fatty acid profiles across different types of clarified butter based on milk source, production method, and regional differences.

It will aid in evaluation of how variations in fatty acid profiles may impact the nutritional properties and health benefits and risks associated with consuming different types of clarified butter.

Producers can gain guidance to optimise their clarified butter products for both flavour and nutritional value.

Information for consumers to make dietary choices regarding the use of different types of clarified butter in their diet.

Further research can be carried out for refining production methods to enhance the nutritional profile of clarified butter.

CHAPTER 2: LITERATURE REVIEW

Dairy activities and business have traditionally been rooted to India's rural economy. India is the leading producer and consumer of dairy products. As per the report of India Dairy Products Market Forecast & Opportunities, 2017, the market for dairy products has opened a wide window in food processing sector, simultaneously if provided with proper stable and sanitised conditions to achieve the international standards. The Indian market has grown rapidly over the last few decades and predicted to be growing at a faster rate as compared to the global dairy market. Amongst all dairy products, clarified butter (ghee) is most valuable. People eating trans fatty acids plus clarified butter or those consuming clarified butter as total visible fat had a significantly higher prevalence of coronary artery disease in both rural and urban men as well as women (Singh *et al*., 2016).

To reduce the risk of cardiovascular disease, the American Heart Association and American College of Cardiology recommended that saturated fat intake be limited to 5% to 6% of total daily calorie intake. The scientific basis for reducing dietary saturated fatty acids has been focused on the effects of rising low-density lipoprotein cholesterol along with a decrease in non-low-density lipoprotein cholesterol, in contributing to atherosclerosis (Asif *et al.*, 2022).

Clarified butter is a culturally significant and nutritionally valued commodity cherished for centuries. Originating in ancient India, clarified butter holds a great place in Ayurveda, the traditional Indian system of medicine, and culinary practices across diverse cultures. Renowned for its distinct flavour, longer shelf life, and potential health benefits, clarified butter has transcended its traditional roots to gain global popularity (Patil *et al*., 2011).

Traditionally, clarified butter is obtained from milk by fermenting it to produce curd which is then churned to obtain butter and buttermilk followed by simmering butter to obtain ghee. The method of processing and storage leads to different fatty acids distribution and is responsible for the taste of the clarified butter (Lamsal *et al.*, 2020).

Its preparation involves simmering butter to separate the milk solids and water, leaving behind a golden, aromatic, and pure fat. The removal of milk solids makes ghee lactose-free, rendering it suitable for individuals with lactose intolerance (Gupta *et al*., 2021).

Beyond its culinary use, clarified butter holds a place in ancient healing practices for its perceived medicinal properties. Its therapeutic value suggests that it supports digestion, enhances immunity, and promotes overall well-being (Nanda *et al*., 2019).

The fatty acid components of clarified butter were determined from their corresponding fatty acid methyl esters (FAMEs) followed by gas chromatography. Clarified butter is composed of mixed glycerides, fatty acids, phospholipids, sterols, fat soluble vitamins, hydrocarbons, trace amounts of casein, calcium, iron, etc (Asif *et al*, 2022).

Modern scientific research has also spotlighted the nutritional composition of clarified butter, revealing it to be rich in fat-soluble vitamins such as A, D, E, and K, along with conjugated linoleic acid (CLA) and butyric acid, which may impart potential health benefits (Kumar *et al*., 2020).

Studies have also compared the fatty acid profiles of commercially available clarified butter products with homemade varieties. Upon analysis of fatty acid composition of different clarified butter brands, variability has been found, with some commercial brands exhibiting higher levels of saturated fatty acids compared to homemade clarified butter (Tian *et al*., 2023).

According to literature, ghee is a lipophilic product with 99-99.5% lipids out of which 46-47.8% is saturated fat, 36% monounsaturated fat and 18% polyunsaturated (Sharma *et al*., 2010).

Ghee is also considered a good source of lipophilic vitamins, especially vitamin A and E, and conjugated linoleic acid – CLA (Upadhyay *et al*., 2017).

In a study carried out on clarified butter prepared from cow milk and buffalo milk, the clarified butter samples showed no oxidation after production since the peroxide value was 0 meq/kg. Clarified butter from cow milk showed higher acid value than that prepared from buffalo milk. This indicated that clarified butter from cow milk likely undergoes higher oxidation and rancidity throughout time. The saponification value of the samples did not show any difference which implies that the molecular weight of the fatty acids present in both the samples was similar and corresponded to long-chain fatty acids. According to the iodine value, clarified butter from cow milk contained a higher amount of unsaturated fatty acids than clarified butter from buffalo milk (Liu *et al*., 2018).

Palmitic – C16:0 (24-28%), stearic – C18:0 (9-14%) and myristic – C14:0 (8-10%) acids were the three main saturated fatty acids present in cow and buffalo clarified butter (Antony *et al*., 2018).

As clarified butter is a dairy product obtained from ruminants, the trans fatty acids (TFAs) contained in it are a consequence of the hydrogenation of unsaturated fatty acids produced by rumen bacteria, thus they are ruminant TFA (rTFA). According to some epidemiological studies, rTFA have exhibited no negative effect on coronary heart disease risk factors (Farlay *et al*., 2017).

Cow and buffalo clarified butter samples displayed a concentration of vaccenic acid (C18:1 t-11) above 1.7% and up to 1% of conjugated linoleic acid – CLA (C18:2 c-9, t-11), which were the most abundant rTFA (Gavin *et al*., 2016).

The sensory profile of buffalo and cow clarified butter was characterized by a predominantly lactic, cooked and fatty odour. The taste was mainly fatty, lactic, sweet and cooked. The texture was found to be fatty, lumpy and greasy, and the appearance was described by no uniform

colour and the presence of particles, exhibiting higher content in cow than in buffalo clarified butter (Gavin *et al*., 2016).

A study suggested that due to palm oil adulteration in clarified butter, the concentration of short chain fatty acids gets decreased and long chain saturated and unsaturated fatty acids increase. In addition, change was also observed in the ratio of the total of saturated fatty acids to unsaturated fatty acids (Hazra *et al*., 2020).

Traditional ghee from Tibet when collected and analysed for their systemic characteristic indices, including physicochemical parameters, minerals, fatty acid composition, and thermal behavior showed that the samples contained large amount of fat (71.68% - 93.3%) and a small quantity of protein (0.51% - 1.81%). The acid and peroxide values varied from 0.02 to 1.30 mg/g and 0.07 to 5.93 meq/kg, respectively (Jing *et al*., 2019).

During heating various changes occur in fats because at high temperature both thermolytic and oxidative reactions occur at the same time. Saturated as well as unsaturated fatty acids undergo chemical decomposition when exposed to heat and oxygen resulting in the loss of valuable nutrients like essential fatty acids (especially polyunsaturated fatty acids), which are destroyed due to oxidation reactions on the unsaturated bonds (Sen *et al*., 2017).

As a human food, clarified butter has been accepted universally as superior fat over other fats, primarily due to its characteristic short chain fatty acids content, which are responsible for its better digestibility. Clarified butter is also an important carrier of fat soluble vitamins (A, D, E, K) and essential fatty acids (linoleic and arachidonic acid), besides its rich and pleasant sensory properties (Kumar *et al*., 2018).

In a research study carried out on seasonal variation in physico-chemical composition of clarified butter processing marketable attributes, 36 samples of ghee were collected over a year and examined for various properties and it was found that clarified butter in winter contained more C12, C14 and total saturated fatty acids and less C14:1, C16:1, C18:1 and total unsaturated fatty acids than in summer or monsoon (CH Joshi, 2019).

A study suggested that because of adulteration of ghee with palm oil, the concentration of short chain fatty acids decreases while that of long chain saturated and unsaturated fatty acids increases (Hazra *et al*., 2020).

Western countries have displayed an increase in ghee intake due to globalisation as well as replacement of margarine consumption as it contains high content of industrial trans fatty acids (iTFA). According to current scientific evidence, iTFA shows a higher negative impact on cardiovascular disease, diabetes and depression than saturated fatty acids (Ford *et al.*, 2016).

A study was conducted to determine the physico-chemical qualities of ghee samples sold in Meerut City. The samples of ghee were also tested to check whether they meet the specifications or not. The samples were collected from three locations of local market. The moisture or volatile content of sample 1 & 2 were (0.24 and 0.27%) within the range, while, it exceeded for sample 3 (0.40%). Free Fatty Acid value of sample 1 & 3 were (1.26 and 1.33%) confirm the standard range, whereas it was more than standard specification (1.6%) for sample 2 (Kumar *et al*., 2016).

Due to the increasing consumption of ghee in the Western countries, a complete characterisation of buffalo and cow ghee was performed. It was found that ghee is a lipophilic dairy product

with 98.9% lipids, 0.3% water and less than 0.9% nonfat solids. Fatty acids being the major lipid fraction and representing 85.1% and 83.65% for buffalo and cow ghee, respectively. More than 52% of the fatty acids were saturated, and palmitic (24-28.8%), stearic (9.4-14%) and myristic (8.5-10%) acids were predominant. Monounsaturated fatty acids were approximately 23.8% and the major component was oleic acid. Polyunsaturated fatty acid content was 2.45% (buffalo) and 4% (cow). The vaccenic acid (2.18%) and the conjugated linoleic acid (CLA cis-9, trans-11) with a concentration of 0.77% in buffalo and 1% in cow ghee, were the main ruminant trans fatty acids (Pena-Serna & Betancur, 2020).

CHAPTER 3: METHODOLOGY

3.1 Preparation of clarified butter

3.1.1 Traditional Method

Goa Dairy milk (7 litres) was boiled and cooled to room temperature. 2 tablespoons of curd was added as inoculum followed by overnight incubation at room temperature for 16 hours. This led to the formation of curd from the milk. Chilled water was added to the curd and it was then churned using a wooden hand churner (Raavi) which resulted in the separation of curd into butter and buttermilk. The butter was collected and washed. It was then heated in a container on the stove till it melted and froth formation in the form of bubble was observed along with characteristic odour of clarified butter. It was then filtered and stored (Jing *et al*., 2019).

3.1.2 Direct Cream Method

Goa Dairy milk (1 litre) was boiled daily for 7 days and the cream was collected while the milk was hot and stored in a container. At the end of the 7th day, curd was added as inoculum to the cream and was left at room temperature overnight for 16 hours. The cream was transferred to a mixer grinder along with chilled water and churned till separation of butter and buttermilk was observed. The butter was washed and heated till it melted giving rise to bubbles which indicated the formation of clarified butter followed by filtration and storage (Serna *et al*., 2019).

3.1.3 Cold Cream Method

Goa Dairy milk (1 litre) was boiled daily for 7 days, cooled and stored in the refrigerator. The cold milk cream was collected and accumulated in a container. On the 7th day, the cream was inoculated with curd and left overnight at room temperature. After 16 hours, the cream was churned in the mixer grinder with chilled water which facilitated the formation of butter and buttermilk. The butter was collected, washed and heated till it melted and formed clarified butter which was indicated by the formation of bubbles. This was followed by filtration and storage (Vasudeva *et al*., 2019).

3.1.4. Creamery Butter Method

Amul butter was taken in a container and heated on medium flame till the butter melted and turned frothy. The flame was reduced and simmered till the froth evaporated. It was stirred occasionally as the butter started bubbling. The colour changed from yellow to golden brown indicating the formation of clarified butter. It was then strained and stored in a container (Serna *et al*., 2019).

3.2 Analysis of organoleptic characters of clarified butter: Color, texture, taste, appearance and odor

The prepared clarified butter samples as well as some branded market samples which were labelled as AML Ghee, GVRDN Ghee, BRTNA Ghee and A2 Ghee were analysed for their organoleptic characteristics such as color, texture, taste, appearance and odor. For analysing the color, the clarified butter samples were taken in a transparent jar and their colors were observed and compared. The clarified butter samples were touched to determine their texture whether smooth, rough, grainy etc. A small portion of the different samples of clarified butter were tasted to determine their taste. The samples were melted and poured into a transparent bottle. They were then placed in proper light condition. The clarity was recorded depending on whether they were clear, hazy or cloudy. Odor was determined by melting the clarified butter samples and their characteristic odor was recorded (Rathi *et al.*, 2018).

3.3 Analytical techniques for the assessment of physico-chemical properties of clarified butter

3.3.1 Estimation of pH of clarified butter samples

The pH of the clarified butter samples was measured using a pH meter (pH 700 pH/mV/℃/℉ meter). Each sample was diluted with distilled water in a ratio of 1:10 and the pH was measured using a pH probe (Nekera *et al*., 2023).

3.3.2 Estimation of Specific Gravity of clarified butter samples

1 gram of the clarified butter samples was melted and poured into a measuring cylinder and the volume in mL was checked if it corresponds to ≤0.9 ml (Nekera *et al*., 2023).

3.3.3 Estimation of Acid Value of clarified butter samples

20 g of the clarified butter sample was weighed in a 250 mL conical flask and 50 mL of freshly neutralized hot ethyl alcohol was added followed by 1 ml of phenolphthalein indicator solution. The mixture was boiled for 5 minutes and titrated while hot against standard alkali solution (0.1 N sodium hydroxide) while shaking vigorously during the titration. The end point was a pink colour which did not persist for more than 15 seconds (Jariwala, K.N., 2014).

$$\text{Acid Value} = \frac{56.1 \text{ V x N}}{\text{W}}$$

Where,

V = Volume in mL of standard sodium hydroxide used

N = Normality of the sodium hydroxide solution

W = Weight in grams of the sample

3.3.4 Estimation of Peroxide Value of clarified butter samples

5 g (+ 50 mg) clarified butter sample was weighed into a 250 ml conical flask. 30 ml acetic acid chloroform solvent mixture was added and swirled to dissolve. 0.5 ml saturated potassium iodide solution was then added with a pipette and the flask was allowed to stand for 1 minute in dark with occasional shaking, followed by addition of 30 ml of water. The liberated iodine was slowly titrated with 0.1 N sodium thiosulphate solution, with vigorous shaking until the yellow colour almost went. 0.5 ml starch solution was added as indicator and titration was continued, shaking vigorously to release all iodine from chloroform layer until blue colour disappeared (Jariwala, K.N., 2014).

Peroxide Value = $\frac{(S-B) \times N \times 1000}{W}$

Where,

S = Volume in mL of sodium thiosulphate required for the sample

B = Volume in mL of sodium thiosulphate required for the blank

N = Normality of the sodium thiosulphate used

W = Weight in grams of the clarified butter sample used

3.3.5 Estimation of Saponification Value of clarified butter samples

0.2 g of clarified butter sample was taken in a 250 ml Erlenmeyer flask. 10 ml of 0.5 N ethanolic potassium hydroxide solution was added and mixed. The flask was heated at 80℃ in a water bath for 30 minutes. It was then cooled to room temperature and 2-3 drops of phenolphthalein indicator was added. It was then titrated with 0.5 N HCl standard solution until a faint pink colour was obtained. The number of milligrams of potassium hydroxide required to neutralise the fatty acids resulting from the complete hydrolysis of 1 g of the sample was calculated as follows: (Jariwala, K.N., 2014).

Saponification Value (mg/g) = $\frac{56.1 (B-S) N}{W}$

Where,

B = Volume in mL of standard hydrochloric acid required for the blank

S = Volume in mL of standard hydrochloric acid required for the sample

N = Normality of the standard hydrochloric acid

W = Weight in grams of the clarified butter sample taken

3.4 Analysis of fatty acid profile of clarified butter samples by Thin Layer Chromatography (TLC)

In this study, the TLC procedure was meticulously standardised to ensure reproducibility and accuracy of results. The standardisation process involved optimising solvent systems, developing consistent spotting techniques, and establishing uniform chromatographic conditions.

3.4.1 Sample Preparation

0.1 g of clarified butter was weighed into a clean, dry vial and 10 ml of hexane was added to dissolve the sample.

3.4.2 Preparation of solvent system

A mixture of hexane:diethyl ether:acetic acid in the ratio 70:30:1 (v/v/v) was prepared and mixed well. It was then added to a glass chamber and covered with a lid and left for saturation.

3.4.3 Preparation of visualisation chamber

Few iodine crystals were put into a glass chamber and left until they sublimed to form iodine vapour.

3.4.3 Marking of TLC plate

The silica-coated TLC plate was taken and the spotting line was marked 1.5 cm away from the bottom.

3.4.3 Spotting of samples

Using a capillary tube, the prepared clarified butter samples were spotted onto the TLC plate with some space between each sample. Spotting was done carefully to ensure small, well-defined spots.

3.4.4 Development of the TLC Plate

The spotted TLC plate was placed in the developing chamber containing the solvent system. The solvent was allowed to ascend onto the plate until it reached three fourth level of the plate.

3.4.5 Visualisation

After development, the TLC plate was removed from the chamber and allowed to dry. The separated spots were visualised by placing the plate in the visualisation chamber containing iodine. The iodine vapour was allowed to react with the fatty acids, producing visible spots.

3.4.6 Documentation and Analysis

The solvent front and the positions of the spots on the TLC plate were marked. The distance traveled by each spot from the origin was measured and the Rf (retention factor) values were calculated and compared with those of fatty acid standards to identify the fatty acids present using the formula:

$$Rf = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent front}}$$

CHAPTER 4: ANALYSIS AND CONCLUSIONS

4.1 Preparation of clarified butter

It was observed that in case of the traditional method (Fig. 1), the amount of inoculum (curd) added to the milk to convert it to curd was important and the longer the curd was churned, more the butter was produced which was then used to produce ghee.

In the direct cream method (Fig. 2) and cold cream method (Fig. 3), the quantity of cream collected from the milk had to be sufficient enough and it had to be churned well in the mixer grinder at regular intervals to form butter which was then melted to prepare ghee.

In the creamery butter method (Fig. 4), the butter had to be melted on a medium flame which led to bubble and froth formation, indicating the formation of ghee.

In all the methods, the amount of heat supplied to melt the butter to convert it to ghee had to be taken care of as slight increase in the flame would lead to charring of the ghee and a different flavour would be produced.

4.1.1 Traditional Method

Fig. 1: Preparation of clarified butter by Traditional Method. A: Boiling of milk, B: Addition of inoculum (curd) to the milk, C: Formation of curd, D: Churning of curd, E: Separation of butter and buttermilk, F: Heating of butter, G: Formation of clarified butter, H: Clarified butter after filtering I: Storage of clarified butter after filtration

4.1.2 Direct Cream Method

Fig. 2: Preparation of clarified butter by Direct Cream Method. A: Collection of cream after boiling milk daily for 7 days, B: Churning of cream in the mixer, C: Formation of butter, D: Heating of butter, E: Formation of clarified butter, F: Filtration of clarified butter, G: Storage of clarified butter

4.1.3 Cold cream method

Fig. 3: Preparation of clarified butter by Cold Cream Method. A: Accumulation of cream from chilled milk stored in the refrigerator for 7 days, B: Churning of cream in the mixer, C: Separation of butter D: Heating of butter E: Formation of clarified butter F: Filtration of clarified butter G: Storage of clarified butter

4.1.4. Creamery butter method

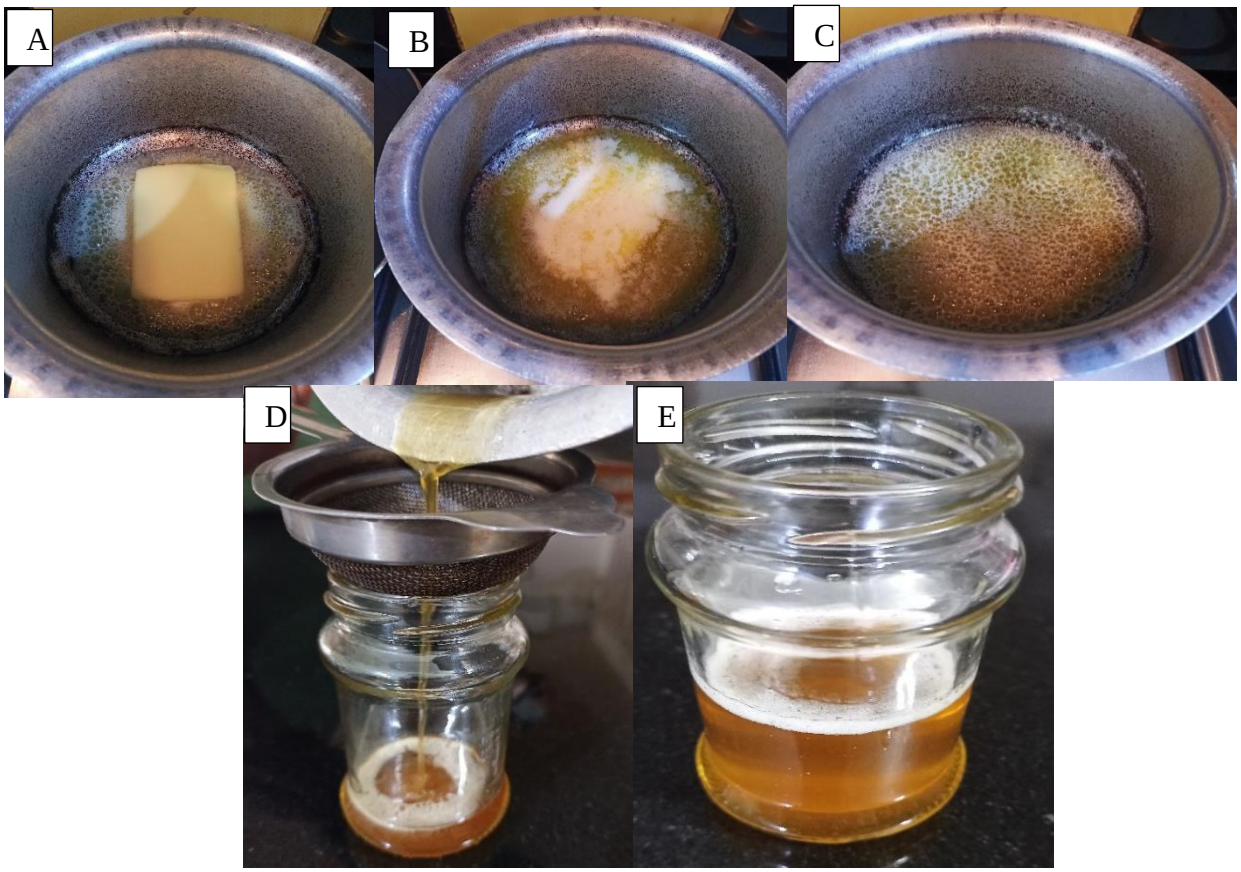

Fig. 4: Preparation of clarified butter by Creamery Butter Method. A: Heating of butter, B: Melting of butter, C: Formation of bubbles and frothing, D: Filtration of clarified butter, E: Storage of clarified butter

4.2 Analysis of organoleptic characters of clarified butter

According to literature, the quality of clarified butter depends on the milk, the cream of the milk formed, preparation method, clarification temperature, storage conditions and type of animal feed. The most important factor controlling the flavour intensity of clarified butter is the temperature of clarification. When prepared at 120˚C or above, clarified butter is referred to as cooked or burnt. On the other hand, clarified butter prepared at 110˚C has a mild flavour. The most desirable flavour is usually produced by the traditional (desi) method. The texture varies from smooth to grainy. The appearance can be transparent or opaque. Clarified butter generally has a sweet taste and the odour is typical and strong (Pena-Serna & Restrepo-Betancur, 2020).

On comparing the above prepared samples as well as branded market samples, it was found that all the samples were yellow to yellowish brown in colour. The best quality of clarified butter was that prepared by the traditional method using a wooden hand churner. The texture varied from smooth to grainy. All the samples were sweetish in taste and had specific smell of clarified butter (Tables 2 & 3).

Table 2: Organoleptic characters of different types of prepared clarified butter

Parameter	Clarified butter prepared by Traditional Method	Clarified butter prepared by Direct Cream Method	Clarified butter prepared by Cold Cream Method	Clarified butter prepared by Creamery Butter Method
Colour	Yellow	Yellowish brown	Yellowish brown	Yellow
Texture	Smooth	Grainy	Grainy	Smooth
Taste	Nutty, buttery flavour	Nutty, buttery flavour	Nutty, buttery flavour	Nutty, buttery flavour
Appearance	Translucent	Translucent	Translucent	Translucent
Odour	Specific odor of clarified butter	Typical smell of clarified butter	Typical smell of clarified butter	Characteristic smell of clarified butter

Table 3: Organoleptic characters of different types of branded ghee

Parameter	AML Ghee	GVRDN Ghee	BRTNA Ghee	A2 Ghee
Colour	Yellow	Yellow	Yellow	Yellow
Texture	Grainy	Smooth	Smooth	Smooth
Taste	Nutty, buttery flavour	Nutty, buttery flavour	Nutty, buttery flavour	Nutty, buttery flavour
Appearance	Translucent	Translucent	Translucent	Translucent
Odour	Typical smell of clarified butter	Characteristic smell of clarified butter	Characteristic smell of clarified butter	Characteristic smell of clarified butter

4.3 Analytical techniques for the assessment of physico-chemical properties of clarified butter

Studies reveal that the pH of clarified butter is in the alkaline range, i.e., pH 7.0 and above. (Battula *et al.*, 2020). In the present study, the pH of the clarified butter samples was found to be around neutral to mild alkaline (Table 4).

The average specific gravity for cow ghee is 0.9, specifically for ghee prepared by the desi method. The minor differences in specific gravity could be due to differences in fatty acid composition, density of separated fats and temperature used in ghee preparation (Nekera *et al.*, 2023). In this study, most of the samples exhibit specific gravity of 0.9 while others exhibit 0.8 owing to differences in fatty acids, temperature of preparation etc (Table 4).

The acid value refers to the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in one gram of ghee. It is a relative measure of rancidity as free fatty acids are generally formed during decomposition of triglycerides. The acid value is determined by directly titrating the ghee in an alcoholic medium against standard sodium hydroxide. Acid value <1 indicates good quality ghee (Nekera *et al.*, 2023**)**. In the samples used for this study, all the samples showed acid values <1, indicating that the samples are pure and not rancid (Table 4).

The peroxide value is a measure of the peroxides contained in a sample of fat and is expressed as milli-equivalents per 1000 grams of the material. Fresh ghee usually has a peroxide value below 10 meq/kg. A rancid taste is noticed when the value exceeds 20 meq/kg (between 20-40 meq/kg) (Tian *et al.*, 2023). The peroxide values obtained of the clarified butter samples were all below 10 meq/kg, thus indicating the absence of rancidity in all the samples and their safety for consumption (Table 4).

The saponification value is the number of milligrams of potassium hydroxide required for saponifying 1 gram of fat. The oil sample is saponified by refluxing with a known excess of alcoholic potassium hydroxide solution. The alkali required for saponification is determined by titration of the excess potassium hydroxide with standard hydrochloric acid. Milk fat differs from other fats and oils in terms of its fatty acid profile, including short chain (C4-C6) and medium chain (C8-C12) fatty acids, which is reflected in its high saponification value. Saponification value may be helpful in detecting adulteration of dairy products with cheaper fats and oils, because the addition of an oil/fat rich in C18 to a dairy product causes a decrease in saponification value (Ivanova *et al.*, 2022). The saponification values of the prepared clarified butter samples were found to be in the range of 225-229 mg/g, suggesting the presence of short chain and medium chain fatty acids (Table 4).

Table 4: Physico-chemical properties of clarified butter

Sample	pH	Specific Gravity	Acid Value	Peroxide Value	Saponification Value
Traditional method	7.53	0.9	0.575	1.547	225.312
Direct cream method	7.82	0.8	0.672	1.251	226.324
Cold cream method	7.21	0.8	0.573	0.613	225.973
Creamery butter method	7.99	0.9	0.732	1.346	227.436
AML Ghee	7.23	0.9	0.821	2.415	226.586
GVRDN Ghee	7.73	0.8	0.769	2.343	228.235
BRTNA Ghee	7.87	0.9	0.751	0.785	229.763
A2 Ghee	7.89	0.8	0.852	0.135	229.532

4.4 Analysis of fatty acid profile of clarified butter samples by Thin Layer Chromatography (TLC)

Upon completion of TLC, the solvent front and spots were marked and measured. The Rf values were then calculated.

Solvent front = 8.5 cm

It was observed that the fatty acids had separated according to their carbon chain length, i.e., short chain fatty acids travelled further than the long chain fatty acids (Fig. 7).

The Rf values of the clarified butter samples were found to be within the standard range of 0.4-0.7. The fatty acid Rf values also were within the similar range, indicating the presence of these fatty acids in the clarified butter samples. (Table 5 & Table 6). From the Rf values it was evident that arachidonic, linoleic and pentadecylic acids were dominant in the samples.

Table 5: Rf values of clarified butter samples

Sample	Distance travelled by sample (cm)	Rf value
Traditional method	2.6	0.305
Direct cream method	Spot 1 = 2.6 Spot 2 = 4.9	Spot 1 = 0.305 Spot 2 = 0.576
Cold cream method	Spot 1 = 2.6 Spot 2 = 4.8	Spot 1 = 0.305 Spot 2 = 0.564
Creamery butter method	Spot 1 = 2.5 Spot 2 = 4.9	Spot 1 = 0.294 Spot 2 = 0.576
AML Ghee	Spot 1 = 2.6 Spot 2 = 4.7	Spot 1 = 0.305 Spot 2 = 0.552
GVRDN Ghee	Spot 1 = 2.5 Spot 2 = 4.8	Spot 1 = 0.294 Spot 2 = 0.564
BRTNA Ghee	Spot 1 = 2.5 Spot 2 = 4.9	Spot 1 = 0.294 Spot 2 = 0.576
A2 Ghee	Spot 1 = 2.6 Spot 2 = 4.9	Spot 1 = 0.305 Spot 2 = 0.576

Table 6: Rf values of fatty acid standards

Standard	Distance travelled by standard (cm)	Rf value
Pentadecylic acid	4.8	0.564
Myristic acid	7	0.823
Linoleic acid	2.8	0.329
Palmitoleic acid	4.7	0.552
Arachidonic acid	2.4	0.282
Stearic acid	3.1	0.364
Oleic acid	3.4	0.4
Mixed standards	4.7, 3.4, 2.4	0.552, 0.4, 0.4

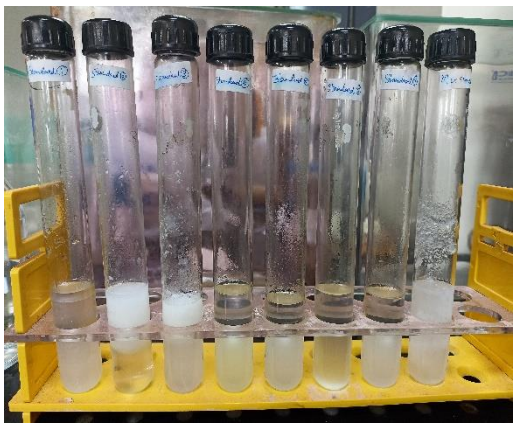

Fig. 5: Sample preparation of clarified butter for TLC

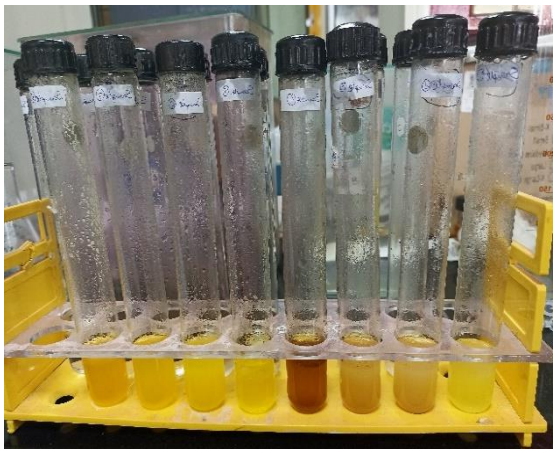

Fig. 6: Sample preparation of fatty acid standards for TLC

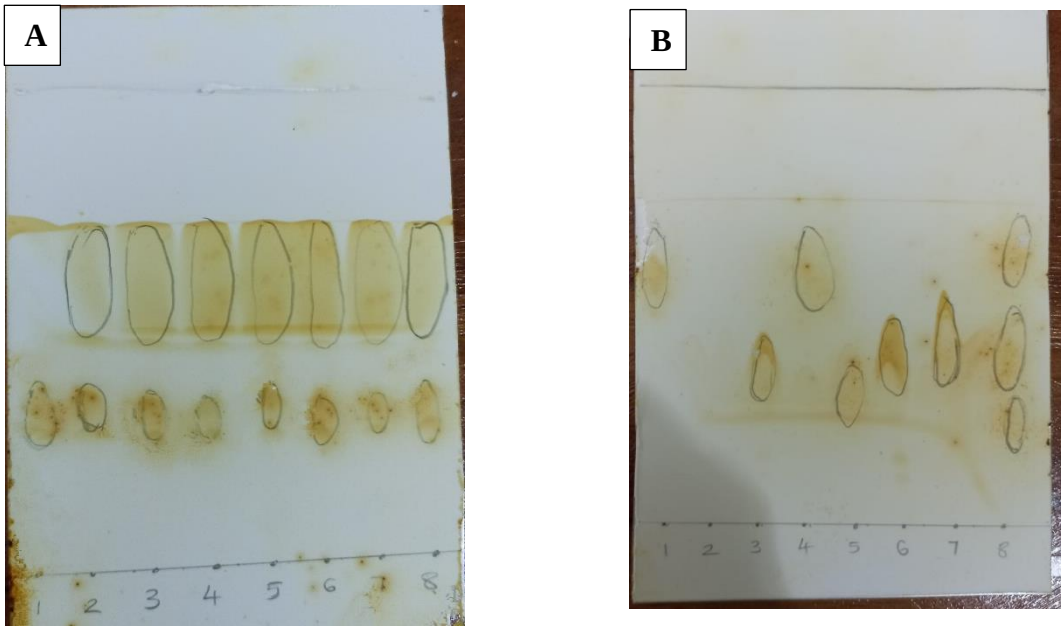

Fig. 7: Thin Layer Chromatography of clarified butter samples and fatty acid standards. A: Samples 1-8, B: Standards 1-7 and mixed standards

Sample 1: Clarified butter prepared by traditional method
Sample 2: Clarified butter prepared by direct cream method
Sample 3: Ghee prepared by cold cream method
Sample 4: Clarified butter prepared from butter
Sample 5: AML Ghee
Sample 6: GVRDN Ghee
Sample 7: BRTNA Ghee
Sample 8: A2 Ghee
Standard 1: Pentadecylic acid (C15)
Standard 2: Myristic acid (C14)
Standard 3: Linoleic acid (C18)
Standard 4: Palmitoleic acid (C16)
Standard 5: Arachidonic acid (C20)
Standard 6: Stearic acid (C18)
Standard 7: Oleic acid (C18)
Mixed standards

FUTURE PROSPECTS

In the present study, clarified butter was prepared using different methods. Further research can be carried out for refining production methods to enhance the nutritional profile of clarified butter.

Various types of fatty acids present in the clarified butter samples were determined in this study. The amount of fatty acid content in the prepared clarified butter can be quantified. Producers can gain guidance to optimise their clarified butter products for both flavour and nutritional value.

Information for consumers can be gained to make dietary choices regarding the use of different types of clarified butter in their diet.

CONCLUSIONS

From the present study, it can be concluded that the traditional method of preparation of clarified butter yielded the best quality and texture based on physical analysis. The yield of the clarified butter prepared by traditional method was maximum.

Some samples were smooth in texture while others were grainy. All samples had specific smell of ghee and the taste was nutty and buttery. Some samples were translucent whereas some were opaque in nature.

Among the branded market samples used for the study, BRTNA Ghee, GVRDN Ghee and A2 Ghee were found to have the best quality as they were smooth in texture while AML Ghee was grainy.

The predominant fatty acids found in the samples, i.e., arachidonic acid, linoleic acid and pentadecylic acid are polyunsaturated, unsaturated and saturated fatty acids respectively. Unsaturated and polyunsaturated fatty acids are beneficial because they can improve blood cholesterol levels whereas saturated fatty acids are harmful as they cause an increase in low density liopoprotein (LDL) cholesterol. Hence, the consumption of ghee must be in appropriate amount as excess consumption can cause cardiovascular problems.

All the samples were alkaline in nature and showed specific gravity as per the standard value, i.e., ≤ 0.9.

The samples were pure and not rancid as determined by their acid and peroxide values.

The Rf values determined of the clarified butter samples and fatty acid standards showed considerable similarity and thus it can be concluded that the samples contained mainly arachidonic acid, linoleic acid and pentadecylic acid as is expected in clarified butter.

Moderation in consumption is crucial to maintain a balanced lipid profile and overall health.

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APPENDIX

Reagent preparation:

1. Phenolphthalein indicator solution: 1 g of phenolphthalein was dissolved in 100 ml of ethyl alcohol.
2. Standard aqueous sodium hydroxide solution (0.1 N): 1 g of sodium hydroxide was dissolved in 250 ml of distilled water.
3. Acetic acid: chloroform solvent mixture (3:2): 3 volumes of glacial acetic acid was mixed with 2 volumes of chloroform.
4. Saturated potassium iodide solution: 100 g of KI was dissolved in 100 ml of water with heat and stirring. 5 g of KI was continuously added until the solution no longer dissolved additional solid.
5. Sodium thiosulphate solution (0.1 N): 25 g of sodium thiosulphate was weighed and dissolved in 1 litre of distilled water. It was boiled, cooled and filtered.
6. Starch solution (1%): 1 g of starch was dissolved in 100 ml of distilled water.
7. Alcoholic potassium hydroxide solution: 35 g of potassium hydroxide was dissolved in 20 ml of distilled water, sufficient alcohol was added to make up the volume to 1000 ml. The solution was allowed to stand in a tightly stoppered bottle for 24 hours. The clear supernatant was then decanted into a reagent bottle for use.