DECONSTRUCTION OF THE MURCHANA PROCESS TO GET INSIGHTS ON THE STABILIZATION OF FUNCTIONAL GHEE.

A THESIS SUBMITTED TO

THE UNIVERSITY OF TRANS-DISCIPLINARY HEALTH SCIENCES AND TECHNOLOGY

FOR THE PARTIAL FULFILLMENT OF THE AWARD OF THE DEGREE OF
M.Sc. LIFE SCIENCES (AYURVEDA BIOLOGY)

BY

SABRINA ELSA EAPEN

UNDER THE GUIDANCE OF

DR. GURMEET SINGH
Professor & Dean (Research & Outreach)
The University of Trans-Disciplinary
Health Sciences and Technology,
Bengaluru, Karnataka

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THE UNIVERSITY OF TRANS-DISCIPLINARY HEALTH SCIENCES AND TECHNOLOGY
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DECLARATION BY THE CANDIDATE

I declare that this thesis "**Deconstruction of The Murchana Process to Get Insights on The Stabilization of Functional Ghee.**" submitted for the award of Master of Science to THE UNIVERSITY OF TRANS-DISCIPLINARY HEALTH SCIENCES AND TECHNOLOGY, Bengaluru, is my original work, conducted under the supervision of Dr Gurmeet Singh. I confirm that no part of the work reported herein has been submitted for a degree or examination at any other university. References, funding and material obtained from other sources have been duly acknowledged, and no part of this dissertation has been plagiarised.

Place: Bengaluru, Karnataka **Signature of the Candidate**

Date: 15 June 2023

Name of candidate: Sabrina Elsa Eapen

Reg. No.: 2021MSCAB10

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CERTIFICATE FROM THESIS SUPERVISOR

This is to certify that the work incorporated in this thesis "**Deconstruction of The Murchana Process to Get Insights on The Stabilization of Functional Ghee.**" submitted by Sabrina Elsa Eapen was carried out under my supervision. No part of this thesis has been submitted for a degree or examination at any other university. References, help and material obtained from other sources have been duly acknowledged. I confirm the originality of the work and that there is no plagiarism in any part of the thesis.

Name, Designation	Role	Signature, Date
Dr Gurmeet Singh Professor & Dean (Research & Outreach) The University of Trans-Disciplinary Health Sciences and Technology, Bengaluru, Karnataka	**Supervisor**	

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Last but not least, I would like to extend my gratitude to my friends and family for their unwavering support and encouragement. Their constant presence and belief in my abilities have been a source of strength and motivation.

DEDICATION

I dedicate this thesis to my husband, Giejo, my family, and my in-laws whose love, encouragement, and steadfast belief in my abilities have been the driving force behind my academic pursuits. Their unwavering support have been the foundation upon which I have built my dreams.

This work is also dedicated especially to my father, whose influence and inspiration continue to guide me every step of the way. His wisdom and unwavering belief in the power of knowledge have left an indelible mark on my life and have shaped me into the person I am today.

Finally, I dedicate this thesis to all the ambitious researchers and scholars who wants to make a difference in their respective fields. May this work serve as a stepping stone and provide insights that contribute to the advancement of knowledge and the betterment of society.

SUMMARY

Ghee, a popular clarified butter, is known for its nutritional properties and longer shelf life. However, its unsaturated fatty acids make it susceptible to oxidation, leading to rancidity and negative health effects. This study aimed to evaluate the oxidative stability of ghee and the effects of synthetic as well as the traditional Ayurvedic method of ghee processing called Ghrita Murchana.

Different samples of ghee were prepared, including untreated ghee, ghee treated with synthetic antioxidants (BHA, BHT, tBHQ), and Murchita ghee prepared with various herbal combinations. The oxidative stability of the samples was assessed using the Rancimat apparatus at 3 different temperatures 160, 180 and 200˚C. Additionally, the fatty acid profiles of the samples were analysed before and after oxidation using gas chromatography-mass spectrometry (GC-MS).

The results showed that the in-house ghee samples were more susceptible to oxidation compared to store-bought ghee, potentially due to variations in the production methods or synthetic antioxidants being already added in store-bought products. However, Murchita ghee with Amla and Vibhitaki exhibited improved oxidative stability without increased conductivity, suggesting Ghrita Murchana as a natural alternative to synthetic antioxidants. Murchita ghee showed reduced breakdown of unsaturated fatty acids during high-temperature oxidation and variations in oxidative degradation compounds.

Overall, incorporating natural antioxidants and Ghrita Murchana, especially with Amla, can enhance ghee quality and shelf-life by preserving unsaturated fatty acids. These findings support the use of traditional Ayurvedic practices for improved oxidative stability and highlight alternatives to synthetic antioxidants.

PERSONAL REFLECTION

Undertaking a thesis project is a significant milestone in one's academic journey, providing an opportunity for personal growth, skill development, and self-reflection. Throughout my thesis, I had the privilege of working on various aspects, such as operation and handling of instruments, report writing, data analysis, time management, and literature collection.

One of the key areas where I expanded my expertise was in the operation and handling of scientific instruments. Through hands-on experience, I became proficient in using instruments such as pH meter, moisture analyser, and Rancimat. I learned the importance of calibrating instruments, ensuring accurate measurements, and maintaining proper protocols to obtain reliable and reproducible results. Although under supervision of experts, I got hand-on experience for preparing the samples for a GC-MS analysis and also to run a GC-MS.

Another crucial aspect of my thesis journey was the development of report writing and presentation skills. Through incorporating the help of AI tools, I understood the importance of not only knowing, but also utilising all the resources available to give out my best. I honed my ability to articulate complex concepts clearly and concisely, ensuring that my findings were effectively communicated. Through continuous practice, I became proficient in organizing information, structuring my reports, and incorporating visual aids to enhance the clarity and impact of my presentations.

A thesis project necessitates a deep understanding of existing research and the ability to contextualize one's own work within the broader scientific landscape. I improved my skills in literature collection, utilizing various databases and search engines to find relevant sources. Additionally, I learned how to critically evaluate research articles, extract key information, and appropriately cite references in my work.

Perhaps one of the most significant lessons I learned during my thesis was the importance of avoiding unwanted complications in research. Through careful planning, and rigorous methodology, I minimized experimental errors and unforeseen obstacles. This experience taught me the value of foresight, attention to detail, and the significance of troubleshooting skills in scientific research.

My thesis journey was a transformative experience that equipped me with a diverse set of skills and valuable insights. Through hands-on experience with instruments, improved report writing and presentation abilities, enhanced literature collection and referencing skills, I have emerged as a more well-rounded and capable researcher. These acquired skills will undoubtedly prove invaluable as I continue my academic and professional journey in the field of scientific research.

LIST OF ABBREVIATIONS

BHA	Butylated hydroxy-anisole
BHT	Butylated hydroxy-toluene
tBHQ	tert-butyl-hydro-quinone
IT	Induction Time
GC-MS	Gas Chromatography- Mass Spectrometry
FAME	Fatty Acid Methyl Esters
IHG	In-house Ghee
SBG	Store-bough Ghee
MG5	Murchita Ghee with 5 herbs
AbG	Abhaya Ghee
VibG	Amalaki Ghee
AmG	Vibhitaki Ghee
MuG	Musta Ghee
HaG	Haridra Ghee

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1 INTRODUCTION

Fats and oils are an integral part of our daily diet, providing essential nutrients and energy. However, the processing and oxidation of fats and oils can form harmful compounds, posing potential hazards to human health. Lipid oxidation is a complex set of free radical reactions between fatty acids and oxygen, which results in oxidative degradation of lipids, also known as rancidity. The intermediate products (free radicals) and end products (reactive aldehydes) of lipid oxidation may interact with other food constituents, such as proteins, sugars, and vitamins, and negatively affect their properties.(Mozuraityte et al., 2015)

To prevent oxidative changes, fats and oils are added with antioxidants- synthetic and natural. BHA (butylated hydroxy-anisole), BHT (butylated hydroxy-toluene) and tBHQ (tert-butylhydroquinone) are few of the most commonly used synthetic antioxidants. Even though these antioxidants are efficiently increasing the stability of fats and oils, regular consumption of these have been associated with many negative impacts on health like cytotoxicity, DNA damage and carcinogenicity (Nagai et al., 1993).

Natural antioxidants are now being widely explored due to this reason. The natural antioxidants currently being used are rosemary extract, green tea extracts and combinations of different tocopherols. While being a safer alternative, these natural antioxidants are still not as effective as synthetic antioxidants in controlling oxidation and are also difficult to extract (Xu et al., n.d.).

Ghrita Murchana is a traditional Ayurvedic method of preparing for medicinal purposes. It involves heating ghee with certain herbs to infuse their therapeutic properties into the ghee. It is a comparatively less explored process as for its effect on the oxidative stability of ghee.

This study aims to understand the effect of the ayurvedic process called murchana in increasing the oxidative stability of ghee. Using the instrument Rancimat, this study tries to get more insights to the process of murchana by exploring the role of the each herb used in murchana in increasing the heat and oxidative stability of ghee and also comparing it with common synthetic phenolic antioxidants BHA, BHT and tBHQ. It also tries to understand the changes in the fatty acid profile of ghee and murchita ghee before and after oxidation.

1.1 Ghee: -

Ghee, is a kind of clarified butter which is used in the Indian subcontinent from very early times. The distinct aroma and nutty flavour of ghee makes it stand out from other types of fats and clarified butters. When compared to other fats and oils, ghee is said to have higher shelf life due to lesser moisture content and also the presence of antioxidants produced during manufacture of ghee (Kumar & Naik, 2018). But this only stays true to storage of ghee. As all other fats and oils, ghee too is prone to oxidation during any kind of cooking or heat processing. The presence of unsaturated fatty acids increases the chances of ghee getting oxidised during cooking (Zhuang et al., 2022).

1.2 Effects of heat processing: -

Heat processing and oxidation of fats and oils can result in the formation of trans fats. Trans fats are unsaturated fatty acids that have undergone a chemical transformation known as hydrogenation. Consumption of trans fats has been linked to an increased risk of heart disease, elevated cholesterol levels, and inflammation. Health organizations worldwide recommend minimizing the intake of trans fats due to their adverse effects on cardiovascular health.(Grootveld et al., 2001)

Exposure to heat and subsequent oxidation of fats and oils can also result in a loss of essential fatty acids, such as omega-3 and omega-6, which diminishes their health benefits. It can destroy heat-sensitive vitamins, such as vitamin E and K, which play crucial roles in various bodily functions. Consuming heat-processed and oxidized fats and oils may therefore lead to inadequate nutrient intake and compromise overall health.(Falade et al., 2017)

Heat processing, particularly during frying or baking, can lead to the formation of acrylamide, a chemical compound that forms when carbohydrates and amino acids react at high temperatures. The International Agency for Research on Cancer (IARC) has classified this substance as a potential human carcinogen. Acrylamide is commonly found in fried foods, snacks, and baked goods, and its consumption in significant amounts over time has been linked to an increased risk of developing certain types of cancer.(Ehling et al., 2005)

More that all these effects of heat processing, when fats and oils are exposed to high heat, air, or light during processing or cooking, they undergo oxidation, forming oxidized lipids. Oxidized lipids are characterized by the breakdown of fatty acids and the formation of free radicals. These compounds have been associated with cellular damage, inflammation, and an increased risk of chronic diseases such as cancer and cardiovascular disorders. Regular consumption of oxidized lipids, which can be found in deep-fried foods and repeatedly heated cooking oils, should be minimized to maintain optimal health.(Zeb & Uddin, 2017)

1.3 Antioxidants:

More studies on lipid oxidation and oxidative products and its effect on human health, led to the discovery of antioxidants. Antioxidants are substances, which when present in food, has the ability to prevent or delay oxidative changes. Widely used antioxidants such as BHA, BHT and tBHQ, although seemingly effective in reducing oxidative degradation, are considered to have negative impacts on health. Natural antioxidants seem to be better alternatives and hence are studied upon extensively.

1.3.1 Synthetic antioxidants: -

Synthetic antioxidants such as BHA and BHT gained popularity in the mid-20th century, for their effectiveness in preventing lipid oxidation. BHA and BHT *(Figure 1)* are petroleum-derived antioxidants that have been widely used to preserve the quality and stability of fats and oils. They are effective at inhibiting free radical formation and slowing down the oxidation process. TBHQ *(Figure 1)* is another synthetic antioxidant commonly used in cooking fats and oils. It has a high resistance to heat and oxygen, making it effective in extending the shelf life of processed foods. TBHQ is often added to oils and fats used for deep frying to prevent rancidity and improve stability.(Santos et al., 2012)

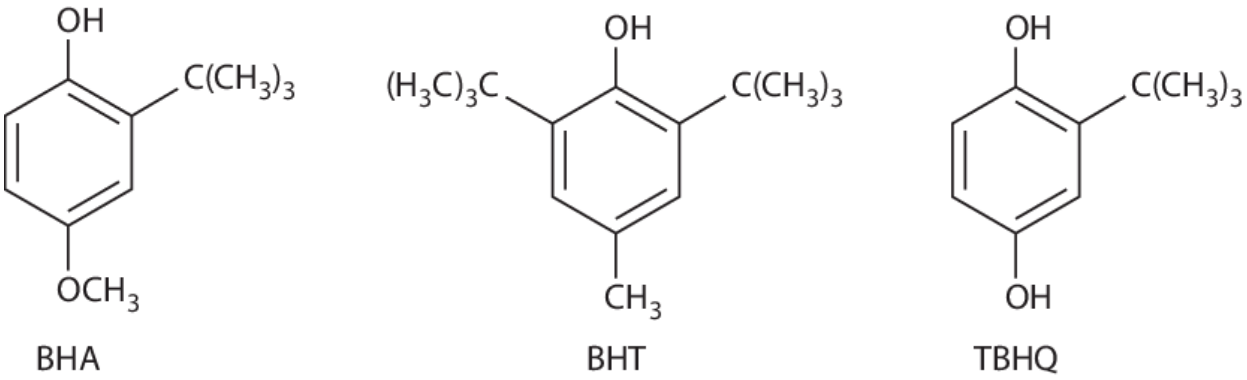

Figure 1- Structures of BHA, BHT, tBHQ (Almeida, 2015)

Although deemed effective antioxidants and also safe for use in low quantities, BHA, BHT, and TBHQ have been the subject of scrutiny due to their potential toxic effects. Studies have shown that these synthetic antioxidants can accumulate in the body over time, raising concerns about their long-term safety (Kupfer et al., 2002). While the approved levels of these antioxidants in food products are considered safe for most individuals, excessive or prolonged consumption may pose a risk, particularly for sensitive individuals or those with underlying health conditions.

BHA and BHT have been associated with allergic reactions and sensitization in some individuals. Allergies to these synthetic antioxidants can manifest as skin rashes, hives, itching, or respiratory symptoms. There are evidences showing exacerbation of allergic symptoms like urticaria by BHA and BHT(Goodman et al., 1990)

Concerns have been raised regarding the potential carcinogenic effects of BHA, BHT, and TBHQ. While studies on the carcinogenicity of these antioxidants in humans have yielded mixed results, some animal studies have shown an increased risk of tumours and cancer development (Ito et al., 1985). The International Agency for Research on Cancer (IARC) has classified BHA as a possible human carcinogen (Group 2B). Although the evidence for the carcinogenicity of these antioxidants in humans is limited, their long-term consumption and potential accumulation in the body have raised concerns about their safety.(Carocho et al., 2014)

Some studies suggest that BHA may interfere with hormonal balance. It has been found to exhibit endocrine-disrupting properties, affecting the normal functioning of hormones in the body.(Pop et al., n.d.) Although the extent of their endocrine-disrupting effects in humans is

still being studied, the potential risks associated with hormonal imbalance warrant further investigation and caution.

While BHA, BHT, and TBHQ have been widely used as antioxidants in various food products, their potential hazards should not be overlooked. Excessive or prolonged consumption of these synthetic antioxidants may pose risks such as toxicity, allergic reactions, potential carcinogenicity, and hormonal disruption. As the understanding of these compounds evolves, it is essential to consider alternative natural antioxidants and ensure the safety and well-being of consumers.(Xu et al., n.d.)

1.3.2 Natural antioxidants: -

In recent years, natural antioxidants, such as rosemary extract, have gained attention as a safer alternative to synthetic antioxidants.(Rahila et al., 2018) Rosemary extract contains natural compounds, including rosmarinic acid and carnosic acid, which exhibit antioxidant properties. It is commonly used in cooking fats and oils due to its ability to inhibit oxidation and preserve the quality of the products.(Zhang et al., 2010)

Natural antioxidants derived from plant-based sources offer a range of health benefits. These antioxidants possess anti-inflammatory, antimicrobial, and anticancer properties, which can help combat oxidative stress, reduce chronic inflammation, and potentially protect against various diseases. Unlike synthetic antioxidants, natural options provide additional health-promoting compounds, such as phytochemicals and micronutrients, which contribute to a more holistic approach to nutrition and health.(Griffiths et al., n.d.)

One key advantage of natural or naturally derived antioxidants in cooking fats and oils is their ability to preserve the nutritional value of the food. Natural antioxidants help maintain the integrity of essential fatty acids and fat-soluble vitamins, from degradation during cooking, ensuring that the cooked food retains its healthful properties. This is especially important for fats rich in polyunsaturated fatty acids, which are susceptible to oxidation. (Forell et al., n.d.)

Natural or naturally derived antioxidants offer the added benefit of enhancing the flavour and aroma of cooked food. Many natural antioxidants, such as herbs, spices, and fruits, possess distinct aromatic and taste profiles which can be used to infuse dishes with complex flavours and enticing aromas and reduce the reliance on artificial flavour enhancers and additives. The result is a more authentic and pleasurable culinary experience that celebrates the diversity of natural flavours. (De et al., 2018)(Nutraceuticals et al., 2017)

Natural antioxidants derived from medicinal plants have been gaining more popularity in recent years. Many studies have used the ethanolic extracts of Ayurvedic medicinal herbs like Shatavari (*Asparagus racemosus*) and Vidarikand (*Pueraria tuberosa*) to understand its antioxidant activity in ghee and it was found that the induction times were significantly improved when compared to the control ghee samples (Gandhi et al., 2013; Pawar et al., 2011). A study shows the blend of pomegranate peel and Moringa (*Moringa oleifera*) leaves to have an effect of increasing the induction time of ghee and hence its shelf life.(More et al., 2022)

This can be taken as an urge to study more about Ayurvedic methods or Ayurvedic herbs as an alternative to addition of synthetic antioxidants. Ayurveda, with its extensive use of fats and oils as both food and medicine, has a concept of processing fats and oils before it is used for making medicines. This is called murchana.

1.3.3 Murchana: -

18th CE Ayurveda text Bhaishajya Ratnavali, introduced the concept of processing ghrita, called as murchana, before it was used for the preparation of medicinal ghrita and the benefits of the process is similar to increasing the stability of ghee.

The shloka goes as-

"पथ्याधात्री विभीतैर्जलधररजनीमातलुंगद्रवैश्च ।
द्रव्यैरेतैः समस्तैः पलकः परिमितैर्मन्दमन्दानलेन ॥
आज्यप्रस्थम् विफेनम् परिचपलगतम् मूर्च्छयेद्वैद्यराज-
स्तस्मादामोपदोशम् हरति च सकलम् वीर्य्यवत् सौख्यदायि ॥"

768mL of ghee should be taken. 48g each of Haritaki (*Terminalia chebula*), Vibhitaki (*Terminalia bellirica*), Amalaki (*Phyllanthus emblica*), Musta (*Cyperus rotundus*), and Haridra (*Curcuma longa*) should be mixed with 48g of Matulunga (*Citrus medica*) swarasa and made into a paste. This paste should be added to the ghee and cooked in low heat until the frothing and ripple formation in the ghee disappears. (Kaviraj Govindadas Sen, 2006)

(* Note: - Commentaries of Bhaishajya Ratnavali, by Brahma Shankara Mishra and Ambika Dutta Shastri also suggest the addition of 750mL of water into the ghee mixture and then cooking it.)

This *murchana* process is said to remove the *ama dosha* in ghee and also improve its potential and health benefits. The text also claims that it makes the ghee more absorptive, and fragrant.

Although the addition of extra moisture content in murchana may lead to higher chances of rancidity, studies show murchita ghrita to have a lesser iodine value, lesser refractive index, and a lesser acid value which are all indicators of lesser chances of oxidation.(V et al., 2020)

It can be summarised that the need for pre-processing ghee using murchana is to remove the potential formation of toxins or *Ama,* and also to enhance the flavour and aroma of the ghee and all this indicates towards increasing the oxidative stability of ghee.

1.4 Methods to Study Oxidative Stability and Oxidative Changes: -

The increased interests in understanding the process of oxidation and oxidative changes led to the discovery of many methods to understand the oxidative stability of fats. This starts from basic methods like monitoring weight gain in fats during exposure to oxygen, quantification of oxidative products like peroxide value, various spectroscopic and calorimetric tests to Accelerated Oxygen Methods (AOMs).(Shahidi et al., 1996)
The time constraints of this study led us to select an AOM called Rancimat as the method for investigating oxidative stability of fats

1.4.1 Rancimat: -

There are several methods to check the oxidative stability of fats, like the Active Oxidation Method (AOM), Schaal Oven Test, Differential Calorimetry Test, Fourier Transform Infrared Spectroscopy etc.(Şahin, 2019)
Among these, Rancimat is an AOM method of checking the stability of different types of fats. It uses an accelerated aging technique by passing air through the sample at constant high temperatures so as to bring about the oxidation of fatty acids. Oxidation of fatty acids produces volatile secondary reaction products, which are carried and condensed into the measuring solution (deionised water). The rate of oxidation can be calculated by measuring the electrical conductivity of this measuring sample. Conductivity is a measure of the ability of water to pass an electric current. Hence, the conductivity of the measuring sample increases with the addition of the reaction products. The time until these products are

detected in the measuring sample is known as “induction time”. “Induction time” is an indicator of the stability of the fat *(Figure 2).*(Läubli & Bruttel, 1986)

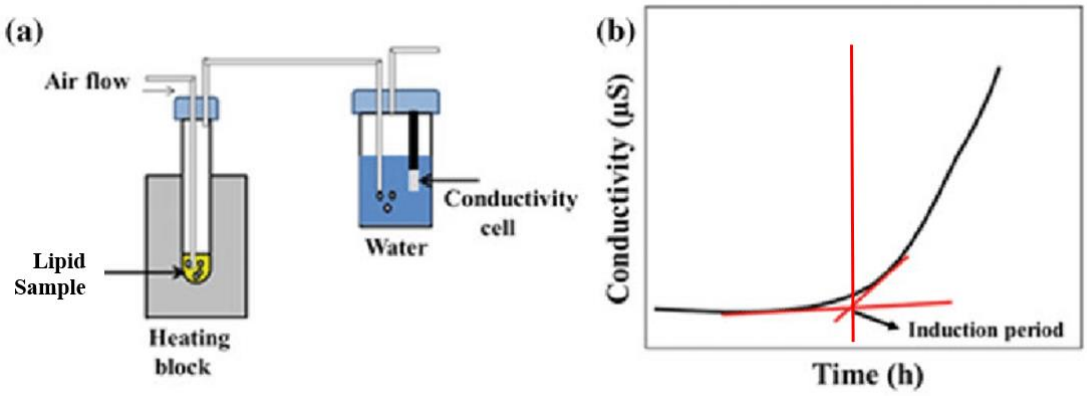

Figure 2- General Working Principle of Rancimat. (Martins et al., 2018)

Although Rancimat provides insights on how susceptible a fat is to oxidation, it does not provide any insights on the changes that occur in a fat during oxidation. To get a better understanding of these oxidative changes, Gas Chromatography-Mass Spectrometry (GC-MS) was chosen. This allows us to get insights on the fatty acid profiles of the samples before and after oxidation.

1.4.2 Gas Chromatography-Mass Spectrometry (GC-MS): -

Gas Chromatography-Mass Spectrometry (GC-MS) is a powerful analytical technique widely used to understand the fatty acid profile of ghee. GC-MS combines the principles of gas chromatography (GC) and mass spectrometry (MS). GC separates the individual components of a complex mixture, such as the fatty acids in ghee, based on their volatility and affinity for the stationary phase which allows for the identification and quantification of each fatty acid present.

The mass spectrometer detects and measures the mass-to-charge ratio (m/z) of ionized molecules, allowing for accurate identification of individual fatty acids. The mass spectra generated during the analysis can be compared to mass spectral libraries, which contain reference spectra of known fatty acids. This comparison helps in the identification of specific fatty acids and their corresponding structures. By using appropriate calibration standards and internal standards, the concentration of each fatty acid can be determined. This quantitative information helps in understanding the composition and relative abundance of different fatty acids within the ghee samples. The fatty acid profile of ghee can provide valuable insights into its nutritional properties, stability, and sensory characteristics.(Zeb & Uddin, 2017)

In this study, GC-MS helps understand the changes brought about in the fatty acids before and after oxidation at different temperatures. It helps to understand how effective are the store-bought used synthetic antioxidants in protecting the ghee from thermal and oxidative degradation. It will also help in understanding the changes brought about by the process of murchana and also to find out if murchana protects ghee from the oxidative changes better than synthetic antioxidants (Amrutha Kala, 2013).

Overall this study aims to understand more about the oxidative stability of ghee, the effect of addition of synthetic and natural antioxidants to ghee and also to find out the true potential of the Ayurvedic method of ghee processing called Ghrita Murchana.

2 MATERIALS AND METHODS

2.1 Antioxidant Sample Preparation: -

Store-bought ghee was purchased from a local market and stored at room temperature until use. Ghee was also prepared at the scientific kitchen using butter extracted from whole-fat cow milk (procured from local dairy farm). Different types of antioxidant mixed ghee samples were prepared by adding a 40 g of ghee with 8mg (200 ppm) of BHA, BHT, or TBHQ respectively. Like this the following samples of ghee were prepared-

1. Untreated store-bought ghee (SBG).
2. Untreated in-house ghee (IHG).
3. In-house ghee treated with BHA (IHG-BHA).
4. In-house ghee treated with BHT (IHG-BHT).
5. In-house ghee treated with tBHQ (IHG-tBHQ).

2.2 Murchana Process: -

Murchana was done using the in-house ghee. 6 samples of murchita ghee were prepared in the following steps-

1. 250mL of melted ghee was taken and its pH and moisture content were recorded. A paste was made with 15.6g each of Haritaki (*Terminalia chebula*), Vibhitaki (*Terminalia bellirica*), Amalaki (*Phyllanthus emblica*), Musta (*Cyperus rotundus*), and Haridra (*Curcuma longa*) and 15.6g of Matulunga (*Citrus medica*) swarasa *(Figure 3)*.

Figure 3- Ingredients for Murchana of Ghee and paste made with the herbs and citrus juice.

2. This paste was added to the ghee and then it was added with 250mL of RO purified water *(Figure 4).*

Figure 4- Ghee mixed with herb paste and water

3. The mixture was cooked until the water content evaporated *(Figure 5)*.

Figure 5- Cooking of the ghee and herb mixture

4. After cooling, the ghee was filtered through a muslin cloth and stored in an airtight glass jar *(Figure 6)*.

Figure 6- Filtration of the murchita ghee.

5. Moisture content and pH before and after murchana was measured using the RADWAG MA 50.R moisture analyser *(Figure 7)* and EUTECH pH tutor *(Figure 8)*.

Figure 7- RADWAG MA50.R Moisture Analyser

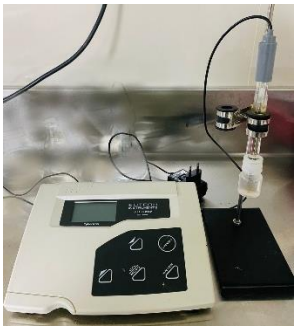

Figure 8- Eutech pH tutor

The classical signs of attaining perfect processing of ghee in Ayurvedic literature was observed towards the end of cooking as follows-

- The mixture of ghee and herbs had stopped frothing.
- Wick rolling test- The paste of the herbs in the heated ghee when taken and rolled between index and thumb fingers was not sticking the fingers and also was rollable into a perfect wick shape *(Figure 9)*.

Figure 9- Wick Rolling Test

- Flame test- This wick made of the heated herb paste, when exposed to a flame and lighted on fire, does not produce any sparks or crackling sound *(Figure 10)*.

Figure 10- Flame test

- Water content evaporation was also made sure by using the moisture analyser. The process was stopped when the supernatant ghee moisture (after attaining all of the above signs) was lesser than the initial moisture content before starting the process.

The process was repeated with 250mL of ghee and using 15.6 g of each single herb individually and 15.6 g of juice of *Citrus medica*.

Like this, 6 samples of murchita ghee were made-

1. Murchita ghee containing all 5 herbs and citrus juice (Murchita ghee) (MG5).
2. Murchita ghee containing Haritaki or Abhaya (*Terminalia chebula*) and citrus juice (Abhaya ghee) (AbG).
3. Murchita ghee containing Vibhitaki (*Terminalia bellirica*) and citrus juice (Vibhitaki ghee) (VibG).
4. Murchita ghee containing Amalaki (*Phyllanthus emblica*) and citrus juice (Amla ghee) (AmG).
5. Murchita ghee containing Musta (*Cyperus rotundus*) and citrus juice (Musta ghee) (MuG).
6. Murchita ghee containing Haridra (*Curcuma longa*) and citrus juice (Haridra ghee) (HaG).

2.3 Rancimat Sample Preparation and Process: -

For Rancimat evaluation, all types of ghee were melted and homogenized by stirring, and then divided into equal aliquots of 4 g each. The oxidative stability of all these ghee samples was evaluated using Metrohm 892 Professional Rancimat apparatus *(Figure 11)* following the standard method (*International Organization for Standardization: Animal... - Google Scholar*, n.d.) at 3 different temperatures- 160°C, 180°C, and 200°C.

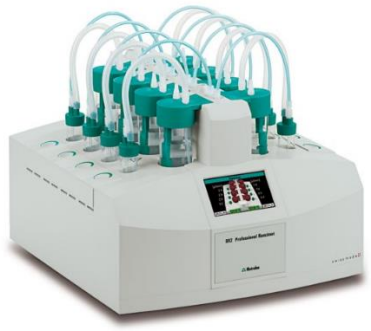

*Figure 11- Metrohm 892 Professional Rancimat (*Rancimat | Metrohm*, n.d.)*

2.3.1 Rancimat Analysis methodology: -

All parts of the apparatus needed for the analysis were washed with hot water and detergent, followed by distilled water rinse.

1. Heating Block Preparation:
    - The heating block is heated up to the desired temperature for the analysis.
2. Measuring Vessel Preparation:
    - The measuring vessel was taken and filled with 50 mL of deionized water and fitted with its cover.
    - The measuring vessel was placed on the openings on the Rancimat and the electrode plugs are connected to its respective connector.
3. Reaction Vessel Preparation:
    - The sample was weighed directly into the reaction vessel.
    - The air inlet tube was placed by immersing it into the sample and then was connected to the reaction vessel cover.
    - The foam barrier was connected to the air tube and the reaction vessel was covered.
4. Determination:
    - The temperature of the heating block was confirmed to be stable. And all the measuring vessels were confirmed to have the initial conductivity of less than 2 µS/cm.
    - The tubing between the reaction vessel and the Rancimat, and the one between the measuring vessel and the reaction vessel was connected.
    - The reaction vessel containing the sample was kept into the heating block and measurement was started immediately.

The induction period, which is the time taken for the formation of volatile oxidation products to reach a predetermined level, was measured for each sample. The induction time is the time to the break point of the conductivity vs. time curve recorded using the instrument. It is a characteristic for the oxidation stability of the sample being examined. For the automatic determination of the induction time, the 2nd derivative of the measured curve is used, which exhibits a maximum at the break point *(Figure 12)*.

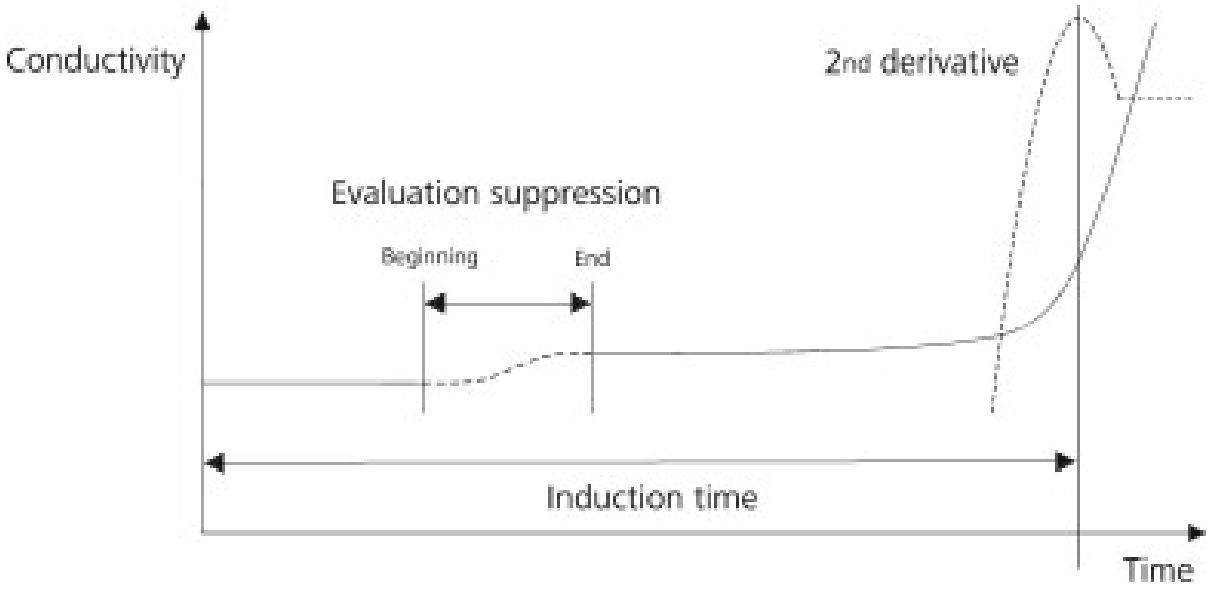

Figure 12- A model graph explaining Induction Time Calculation.

2.3.2 Shelf-life Calculation using Induction Times: -

The obtained IT of each sample at each of the three temperatures were used to calculate the shelf life at 25˚C, using the formula-

$$t_e = t_m \,.\, Q_{10}^{[(Tm - Te)/\,10]}, \text{ where,}$$

T_m - Measuring temperature (˚C or K)

t_m - Measuring time (h)

T_e – Target temperature (˚C or K)

t_e – Target time (h)

After Rancimat evaluation, the samples were collected for GC-MS evaluation.

2.4 GC-MS Sample Preparation and Process: -

Compositional profiles of control ghee sample (ghee without any treatment), synthetic antioxidant treated ghee samples (in-house ghee treated with BHA, BHT and TBHQ; and store-bought ghee treated with BHA, BHT and TBHQ), Murchita ghee samples treated with all 5 herbs and Murchita ghee samples treated with each herb individually (Abhaya ghee, Vibhitaki ghee, Amla ghee, Musta ghee, Haridra ghee) was analysed and compared with their oxidized counterparts (ghee sample treated at different elevated temperatures in Rancimat instrument) to study the changes in the fatty acid profile before and after oxidation by performing Schimadzu Gas Chromatography (GC) *(Figure 13)* of their respective fatty acid methyl esters (FAMES). Each lipid samples were derivatized into their respective FAMES by 0.25 M Sodium methoxide and extracted with hexane (Milinsk et al., 2008). The derivatized methyl esters were then analysed in GC-MS liquid injection mode with RT 2560 column (100 m, 0.25mm id, 0.20 µm df, 250˚C max temperature) with Helium as carrier gas (flow rate 1ml/min). Oven temperature 100°C, in a temperature ramp program raised to final temperature of 220 °C. A 36 FAMES standard mix was run at similar conditions to quantify the unknown amounts of fatty acids in the ghee samples.

Figure 13- Shimadzu GC-MS

3 RESULTS AND DISCUSSION

3.1 Murchana Results: -

All murchita samples were observed to have a lower pH than the control ghee due to the addition of Citrus juice. The moisture content was observed to be lower than the control which might also be adding to the stability of murchita ghee *(Table 1)*. The pH of Amla ghee was found to be 2.16, which was the lowest among the murchita ghee samples. This can be attributed to the acid content of Amla.

Table 1-pH and Moisture content of Murchita Ghee Samples

Sl. No.	Name of ghee	pH	Moisture content (%)
1	IHG	5.55	0.296
2	MG5	2.73	0.261
3	AbG	2.65	0.256
4	VibG	2.58	0.145
5	AmG	2.16	0.106
6	MuG	2.46	0.235
7	HaG	2.84	0.185

3.2 Results of Rancimat Analysis: -

3.2.1 Ghee Degradation with Increase in Temperature: -

When seeing the time vs. conductivity curve of the in-house ghee control ghee at the temperatures 160, 180 and 200˚C, it is seen that ghee degrades rapidly with increase of temperature. The Induction Time (IT) of IHG at 160, 180, and 200˚C is 0.58 h (~35 min), 0.21 h (~13 min), and 0.14 h (~8 min) respectively (*Chart 1, Chart 2, Chart 3)*.

These ITs decrease by around 2-fold at each 20˚C increase of temperature. This is suggestive of the increased susceptibility of ghee to oxidation in high temperature like that in deep frying (200˚C) *(Chart 3*)

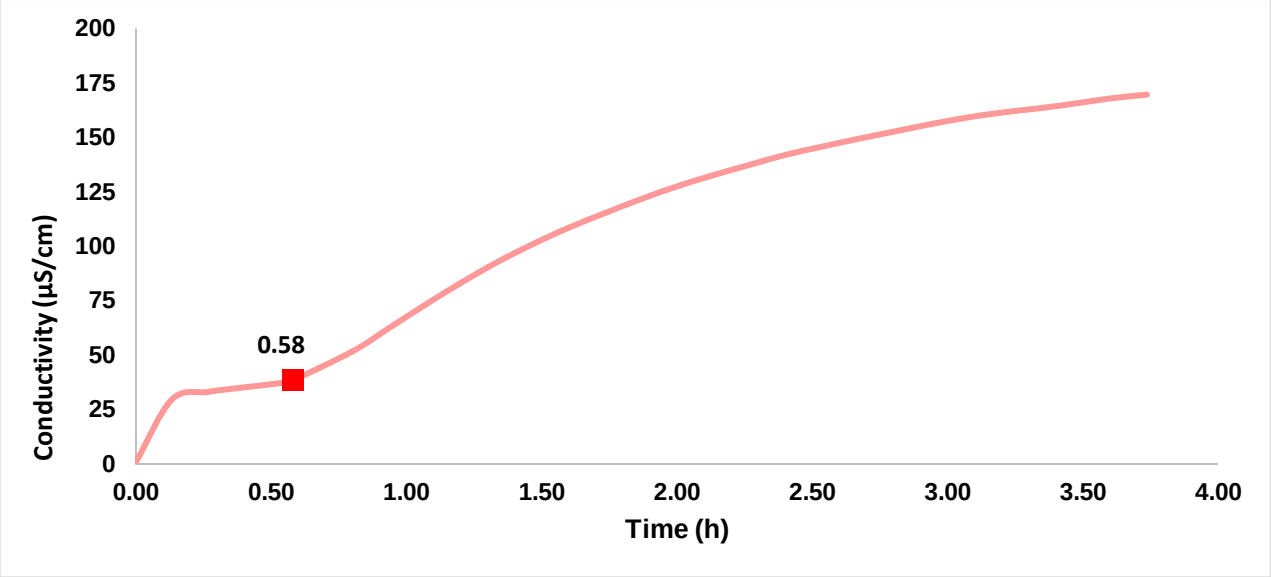

Chart 1- IT of Control In-house Ghee (IHG) at 160˚C

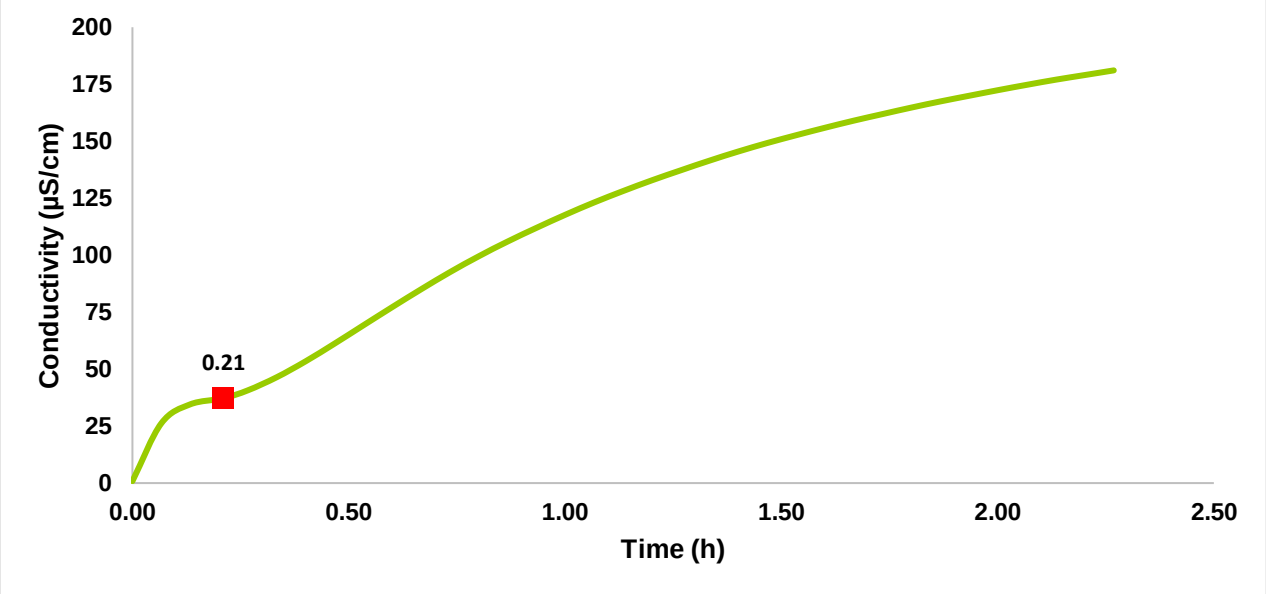

Chart 2- IT of Control In-house Ghee (IHG) at 180˚C

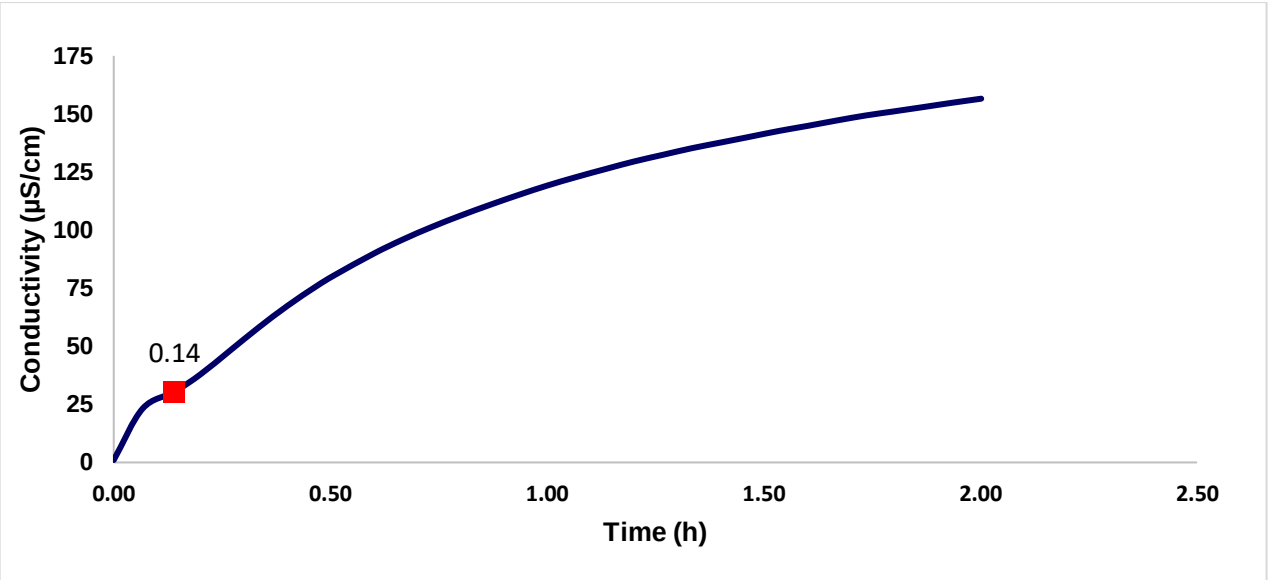

Chart 3- IT of Control In-house Ghee (IHG) at 200˚C

3.2.2 Comparison of In-house and Store-bought Ghee: -

When comparing the Rancimat results of In-house Ghee with that of Store-bought Ghee, the In-house Ghee was found to have a slightly lesser induction time than the Store-bought Ghee in all three temperatures. For instance, in 160˚C, In-house Ghee showed induction times of 0.58 h (~35 min), whereas the Store-bought Ghee, showed induction times of 1.07h (~65 min). The difference in the IT of the In-house and Store-bought samples decreased as the temperature increased and at 200C, the IT of In-house Ghee, 0.14 (~8 min) was slightly higher than that of Store-bought Ghee, 0.12 (~7 min) *(Chart 4)*.

As noticed in the case of In-house Ghee, IT of Store-bought Ghee also decreased drastically with increase in temperature suggesting the increased susceptibility for oxidative degradation in high temperatures.

The difference in IT of In-house Ghee and Store-bought Ghee might be the result of the differences in the process of making ghee adopted in both cases and also the possibility of Store-bought Ghee already having traces of added antioxidants in it. This uncertainty in the content of Store-bought ghee was taken into consideration and In-house Ghee was taken as the base for preparation of murchita samples and also as control for comparative results.

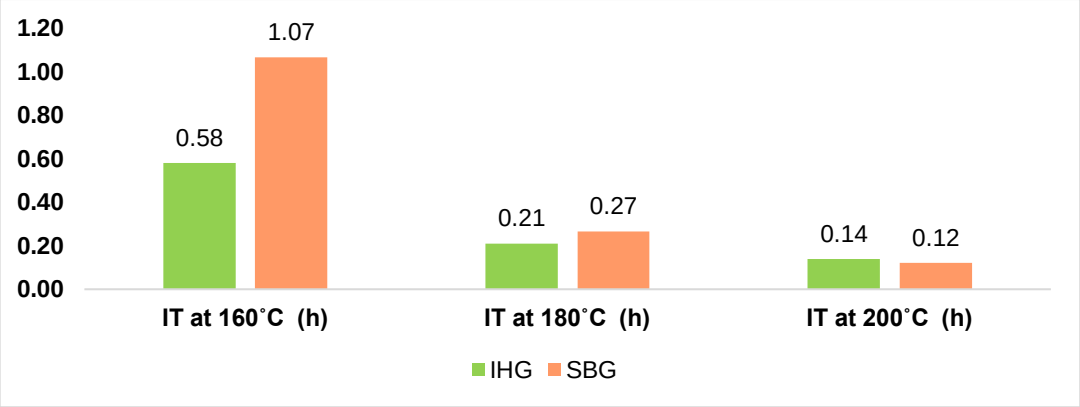

Chart 4- In-house ghee and store-bought ghee at all three temperatures

3.2.3 Shelf-life at 25˚C of In-house Ghee and Store-bought Ghee: -

When the IT at all three temperatures were used to calculate the shelf-life of the In-house Ghee and Store-bought Ghee, the In-house Ghee was shown to have a shelf-life of 2.3 days and around 3 months for Store-bought Ghee *(Table 2)*

Shelf-life is calculated by plotting the time vs. temperature using the IT at all three temperatures and extending it till 25˚C. As it can be seen in the graphs, *(Figure 14,Figure 15)* more IT at temperatures between 160 and 25˚C is needed to get more accurate calculation of shelf-life. Moreover, these values are theoretical calculations and just indicate the possible change in shelf-life at the target temperature.

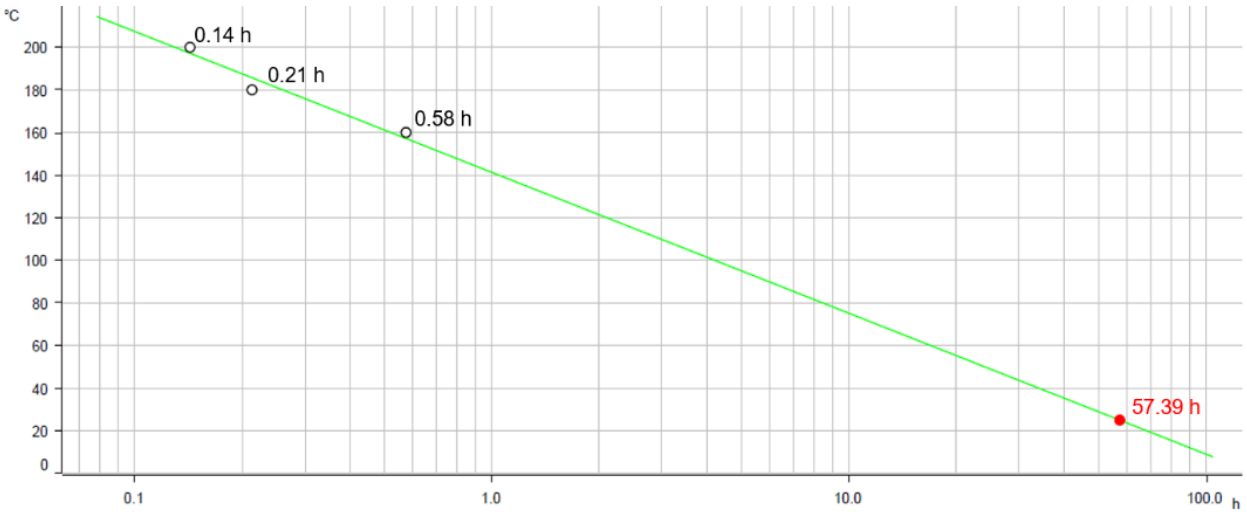

Figure 14- Shelf-life calculation of IHG at 25˚C

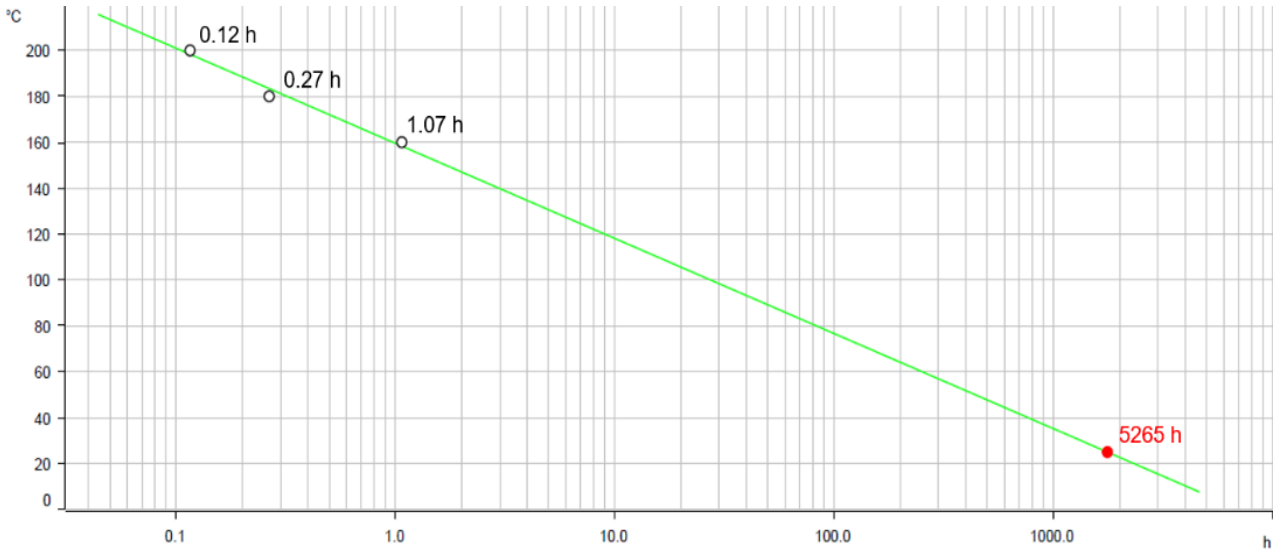

Figure 15- Shelf-life calculation of SBG at 25˚C

Table 2- Shelf-life of IHG and SBG at 25˚C

Sample Name	**Shelf life at 25˚C**
In-house ghee	57.3 h (2.3 days)
Store-bought ghee	1763 h (~3 months)

3.2.4 In-house Ghee added with Antioxidants: -

In-house ghee was used as the base to understand the effect of added synthetic antioxidants. When compared with In-house Ghee (control), In-house ghee added with BHA (IHG- BHA), In-house Ghee with BHT (IHG- BHA), and In-house Ghee with tBHQ (IHG-tBHQ), all antioxidants showed improvement in IT in 160˚C *(Chart 5)*. But in 180 and 200˚C, the improvement was very little. Among the three antioxidants tBHQ was performing the best and BHT was performing the least. Hence, IHG- tBHQ will be considered as the antioxidant control further onwards.

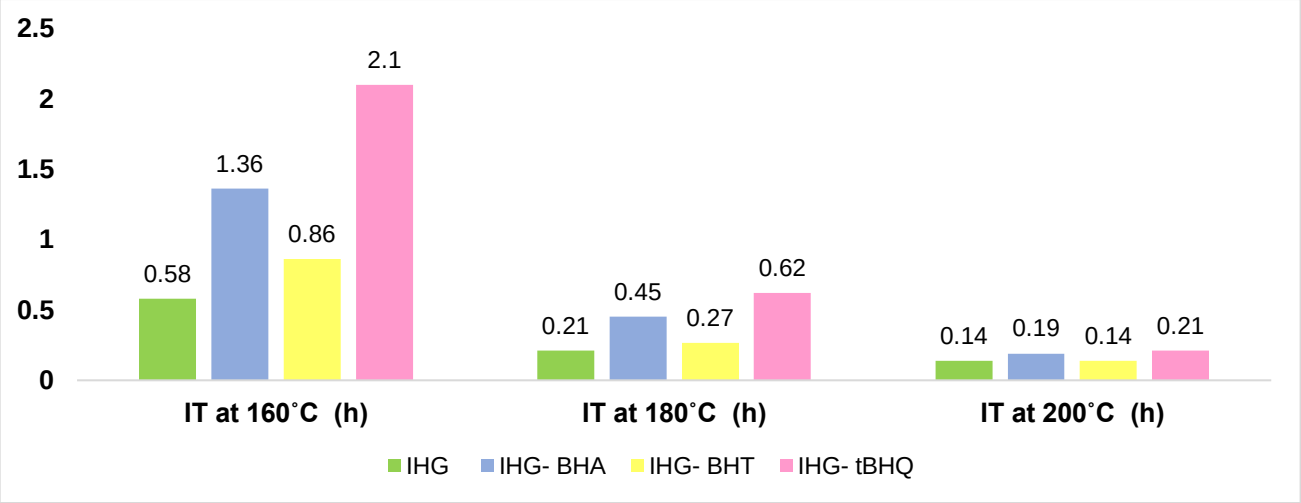

Chart 5- IT of IHG and IHG- Antioxidant Samples

3.2.5 In-house Control, Antioxidant-added and Murchita Ghee Samples: -

On comparison of the Rancimat results of In-house and murchita ghee samples, the murchita samples showed a general improvement in induction time. In 160˚C, 4 murchita samples, namely Murchita Ghee with all 5 herbs (MG5), Vibhitaki ghee (VibG), Amla Ghee (AmG), and Haridra ghee (HaG) showed IT as, 2.45 h (147 min), 2.59 h (~155 min), 2.78 h (~166 min), and 2.46 h (~148 min) respectively, and this was better than IT of IHG- tBHQ, which was 2.1 h (126 min) *(Chart 6)*. This trend was seen in the other two temperatures also. Amla Ghee showed the highest IT among all the murchita ghee samples and it was more than IHG, IHG-tBHQ. The samples MG5, VibG, AmG and HaG showed a 3-fold to 5-fold increase in the IT when compared to IHG (control). Abhaya ghee sample showed more or less the same induction time as the control in-house ghee sample. In 180 and 200˚C, Abhaya ghee showed a slightly increased induction time than control sample. In all 3 temperatures, Musta ghee showed induction time lesser than the control ghee induction time *(Chart 6)*.

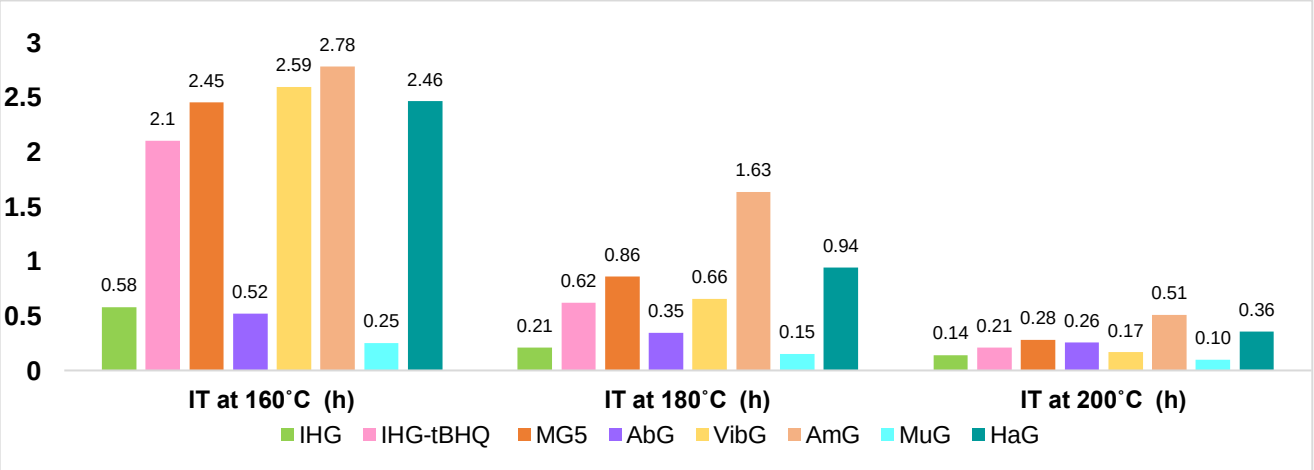

Chart 6- IT of IHG, IHG-Antioxidant and all Murchita Samples.

3.2.6 Shelf-life at 25˚C of In-house Ghee and Murchita Samples: -

Induction Times obtained for each sample from Rancimat analysis at all three temperatures were used to calculate the possible shelf life of the samples at 25˚C. Among the Murchita samples, MG5, VibG, AmG, and HaG showed shelf-life better than the IHG (control) sample. Among all these samples, VibG showed the longest shelf-life of around 3 years. Following the trend showed in Rancimat results, AbG and MuG showed the least shelf-life which comes around just 5 hours *(Table 3)*.

Table 3- Shelf-lives at 25˚C of IHG, IHG- Antioxidant and all Murchita Samples.

Sample Name	**Shelf life at 25˚C**
IHG	57.39 h (2.3 days)
IHG- tBHQ	5265h (~7 months)
MG5	3513 h (~5 months)
AbG	5.05h
VibG	25327 h (2 years 11 months)
AmG	939.8 h (~ 1 month)
MuG	5.51 h
HaG	1674 h (~2 months)

3.3 Results of GC-MS Analysis: -

3.3.1 Unsaturated Fatty Acids' Content: -

After GC-MS it was found that in all the ghee samples (untreated, antioxidant treated and herbs treated) the concentration of unsaturated fatty acids such as (Myristoleic, Palmitoleic, Oleic, Linoleic, Linolenic acids) were decreasing in their oxidized counterparts. Myristoleic acid, Palmitoleic acid, Oleic acid was constantly observed to be declining and it declined 3-fold, 4-fold, and 4-fold respectively in oxidized in-house and antioxidant treated, Murchita ghee, Abhaya ghee and Musta ghee samples as compared to their unoxidized counterparts at all temperatures *(Table 4; Table 5; Table 6; Table 7)*. Linoleic and Linolenic acid which were

detected in the unoxidized counterparts of ghee samples were absent in all the oxidized counterparts except for Haridra and Amla ghee in 200˚C *(Table 4; Table 6; Table 7; Table 7)*. The literature also suggests such depletion in unsaturated fatty acids which occurs due to oxidation at higher temperatures leading to breakdown of fatty acids into intermediates such as aldehydes, ketones, alcohols; (Rastogi et al., 2006).

The process of fatty acid degradation includes 3 steps-

1. Initiation- the abstraction of hydrogen-atom from the lipid by the peroxyl or alkoxyl radicals, which forms a lipid radical
2. Propagation- Lipid hydroperoxides keep forming due to the addition of oxygen to the lipid radicals
3. Termination- The formed radicals neutralize each other by radical-radical coupling *(Figure 16)*.(Fereidoon & Ying, 2010)

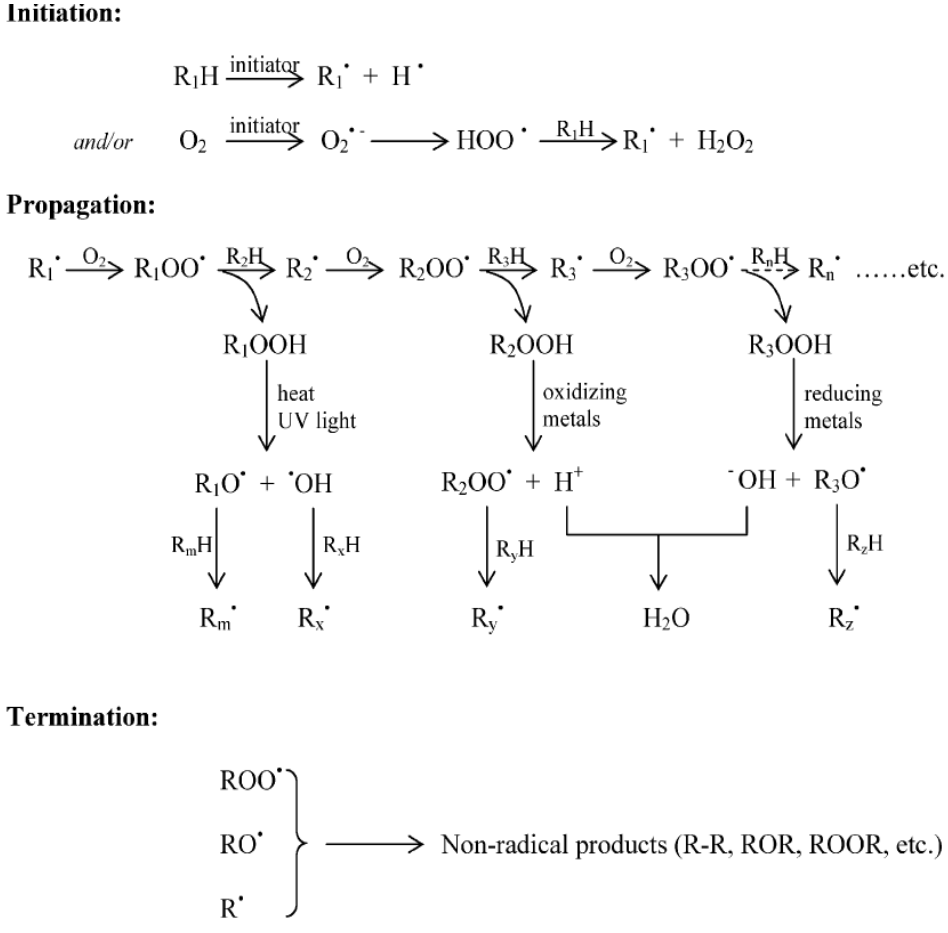

Figure 16- Lipid oxidation steps (Fereidoon & Ying, 2010).

However, the noteworthy observation was that Myristoleic acid, Palmitoleic acid, Oleic acid fatty acid content in murchita ghee samples (Amalaki ghee, Vibhitaki ghee, Haridra ghee) was seen to be relatively lesser declined as compared to the antioxidant treated counterparts' samples. This could indicate that the herbs responsible for increasing the shelf life of ghee

through delaying the oxidation time or increasing the induction time might be doing it by presumably providing protection/antioxidation effect to this unsaturated fatty acid which is why there was a relatively lower reduction of these fatty acids at high temperature oxidation compared to their antioxidant counterpart.

3.3.2 Short Chain Fatty Acid Content: -

The concentrations of saturated fatty acids especially short-chain fatty acids. did not decrease after oxidation compared to their unoxidized counterparts. On contrary the concentration of some of these fatty acids (Butyric, Caproic, Lauric, and Myristic acids) were observed to be increased in their oxidized counterparts which might be indicative of the notion that during oxidation the unsaturated fatty acids might be breaking down into intermediates and some of those intermediates could be short chain fatty acids as well *(Table 4; Table 5; Table 6; Table 7)*. Short chain fatty acids formation starts from a triglyceride containing an alkoxy radical. It occurs due to the homolytic β-scission of this alkoxy radical involving the carbon-carbon cleavage on either side of the oxygen-bearing carbon. This leads to the formation of a short chain fatty acyl and also different aldehydic products based on the fatty acyl it started with *(Figure 17)*.(Márquez-Ruiz et al., n.d.)

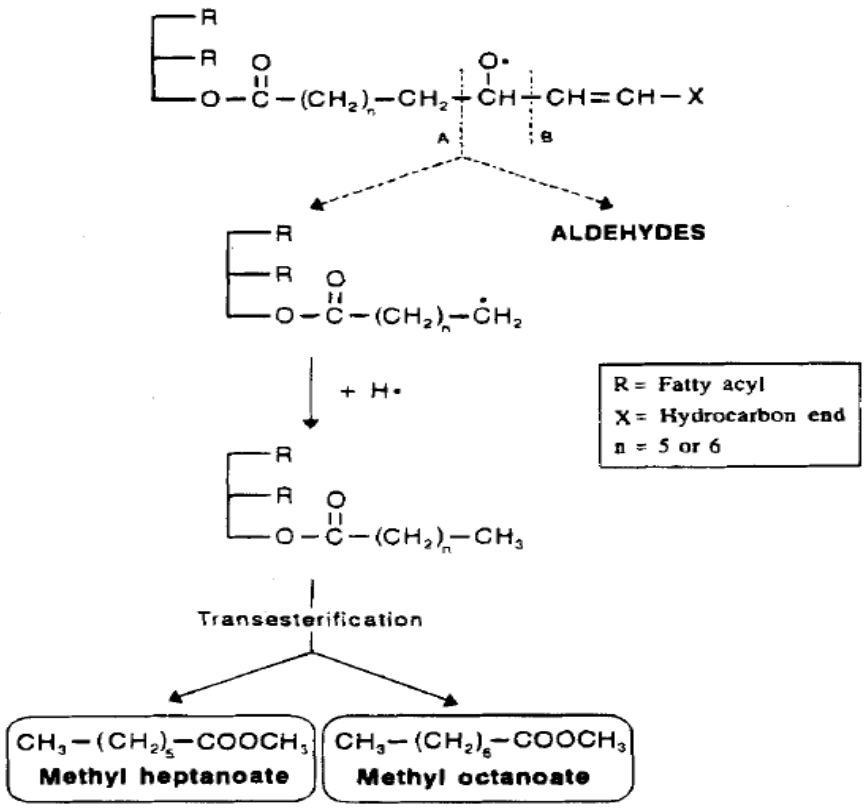

Figure 17- A representation of short chain fatty acid formation (Márquez-Ruiz et al., n.d.)

3.3.3 Oxidized Fatty Acid Counterparts: -

The presence of other compounds such as Ethyl para-ethoxy benzoate, Methyl 8-oxohexadecanoate, and cis 9,10-Ethoxystearic acid, which might be other oxidative degradation products was also observed in the oxidized ghee samples. In literature also mentioning of such compounds detected after oxidation has been found and this is in alignment to present study (Brühl & Matthäus, 2008); (Fullana et al., 2004)

Ethyl para-ethoxy benzoate was absent in all the 160˚C and 180˚C oxidised murchita ghee samples except Control In-house ghee and Vibhitaki ghee at 160˚C, and Murchita ghee at 180˚C *(Table 5;Table 6)*. Interestingly, at 200˚C, Ethyl para-ethoxy benzoate was present in all the murchita samples except Vibhitaki and Amla ghee *(Table 7)*.

Methyl 8-oxohexadecanoate was detected in almost all the three temperature conditions in almost all the samples, except in case of Amla ghee, where it was not detected in any of the three-temperature-oxidised Amla ghee samples.

The compound cis 9,10-Ethoxystearic acid was detected in all 160˚C oxidized samples, but it was absent in Murchita ghee sample and in lesser concentrations in the oxidized Vibhitaki and Haridra ghee samples compared to the oxidized control and antioxidant treated samples. Among 180 and 200˚C oxidized samples, cis 9,10-Ethoxystearic acid was only detected in the Control in-house ghee and tBHQ treated sample in both the temperatures and in the 180˚C Murchita sample *(Table 6;Table 7)*. Overall, there was either the absence or less concentration of oxidized fatty acid counterparts in the murchita samples, Vibhitaki ghee and Amla ghee *(Table 5; Table 6;*

Table 7).

Therefore, our findings are suggestive of the hypothesis that Murchana with herbs especially with amla has profound antioxidant effect which could be seen in fatty acid fingerprint data also.

Table 4- Before Rancimat-Oxidation Fatty acid profile of IHG (control), IHG-tBHQ, MG5, AbG, VibG, AmG, MuG, and HaG

Fatty acid Name	IHG (mg/g)	IHG-tBHQ (mg/g)	MG5 (mg/g)	AbG (mg/g)	VibG (mg/g)	AmG (mg/g)	MuG (mg/g)	HaG (mg/g)
Butyric acid (C4:0)	8.48	8.68	11.12	10.57	9.88	9.82	9.88	9.85
Caproic acid (C6:0)	6.03	6.10	6.67	6.15	6.81	7.30	7.32	7.13
Lauric acid (C12:0)	16.42	15.26	14.55	14.83	17.53	16.30	16.51	15.73
Myristic acid (C14:0)	89.93	87.13	87.96	88.22	94.91	86.85	87.37	86.84
Myristoleic acid (C14:1)	4.79	4.45	4.23	3.99	4.68	6.24	6.38	6.21
Palmitic acid (C16:0)	302.43	293.31	310.02	315.56	366.16	303.87	306.81	306.73
Palmitoleic acid (C16:1)	16.32	15.72	15.06	15.50	18.69	19.48	18.88	18.89
Oleic acid (C18:1)	38.74	37.07	37.31	37.13	24.04	34.42	34.75	33.82
Oleic acid, cis- (C18:1)	233.92	233.24	251.37	245.46	245.90	254.62	257.13	255.32
Linoleic acid (C18:2) (C18H32O2)	11.17	9.98	8.26	8.52	9.15	11.73	12.59	11.37
Linolenic acid (C18:3) (C18H30O2)	2.58	2.15	1.75	1.88	1.87	2.25	2.36	2.18
Ethyl para-ethoxy benzoate (C11H14O3)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Methyl 8-oxohexadecanoate (C17H32O3)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
cis 9,10-Ethoxystearic acid (C18H36O3)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 5- 160˚C Oxidation Fatty acid profile of IHG (control), IHG-tBHQ, MG5, AbG, VibG, AmG, MuG, and HaG

Fatty acid Name	IHG 160 (mg/g)	IHG-tBHQ 160 (mg/g)	MG5 160 (mg/g)	AbG 160 (mg/g)	VibG 160 (mg/g)	AmG 160 (mg/g)	MuG 160 (mg/g)	HaG 160 (mg/g)
Butyric acid (C4:0)	17.79	14.77	20.10	15.21	13.09	13.45	12.04	10.78
Caproic acid (C6:0)	11.22	9.71	13.09	9.11	8.78	10.00	8.99	8.05
Lauric acid (C12:0)	21.92	19.62	21.73	18.56	21.85	19.46	18.60	16.25
Myristic acid (C14:0)	117.49	110.01	126.22	115.18	113.15	107.87	105.64	93.73
Myristoleic acid (C14:1)	1.23	2.10	0.00	0.44	2.74	2.07	3.03	3.88
Palmitic acid (C16:0)	376.84	369.99	473.04	438.30	429.75	410.63	397.81	359.46
Palmitoleic acid (C16:1)	3.43	8.84	0.00	1.30	11.62	7.07	9.16	13.40
Oleic acid (C18:1)	9.12	16.53	0.00	4.18	13.25	11.08	14.31	22.31
Oleic acid, cis- (C18:1)	83.91	130.43	12.07	39.78	140.50	104.88	130.89	194.27
Linoleic acid (C18:2) (C18H32O2)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.07
Linolenic acid (C18:3) (C18H30O2)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ethyl para-ethoxy benzoate (C11H14O3)	2.32	0.00	0.00	0.00	0.73	0.00	0.00	0.00
Methyl 8-oxohexadecanoate (C17H32O3)	4.62	0.00	5.25	3.23	1.56	0.00	0.84	0.00
cis 9,10-Ethoxystearic acid (C18H36O3)	49.39	31.84	0.00	38.15	16.63	28.26	21.88	10.79

Table 6- 180˚C Oxidation Fatty acid profile of IHG (control), IHG-tBHQ, MG5, AbG, VibG, AmG, MuG, and HaG

Fatty acid Name	IHG 180 (mg/g)	IHG-tBHQ 180 (mg/g)	MG5 180 (mg/g)	AbG 180 (mg/g)	VibG 180 (mg/g)	AmG 180 (mg/g)	MuG 180 (mg/g)	HaG 180 (mg/g)
Butyric acid (C4:0)	13.31	13.55	14.74	15.11	15.25	10.15	15.15	12.46
Caproic acid (C6:0)	8.07	8.50	8.90	9.72	9.93	7.18	11.20	8.84
Lauric acid (C12:0)	18.48	18.61	17.24	18.00	23.42	15.31	21.10	17.97
Myristic acid (C14:0)	108.56	101.83	107.36	111.39	122.45	86.94	114.72	103.97
Myristoleic acid (C14:1)	1.14	3.19	1.45	0.97	2.28	5.14	1.76	3.17
Palmitic acid (C16:0)	373.03	344.86	421.04	429.11	461.08	341.95	432.67	395.18
Palmitoleic acid (C16:1)	7.77	12.64	5.47	4.45	8.27	15.41	5.00	9.79
Oleic acid (C18:1)	14.76	24.46	10.75	7.91	7.83	26.44	7.38	15.97
Oleic acid, cis- (C18:1)	109.72	192.43	99.34	70.24	90.24	239.16	66.66	145.26
Linoleic acid (C18:2) (C18H32O2)	0.00	0.00	0.00	0.00	0.00	3.39	0.00	0.00
Linolenic acid (C18:3) (C18H30O2)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ethyl para-ethoxy benzoate (C11H14O3)	0.00	0.00	1.55	0.00	0.00	0.00	0.00	0.00
Methyl 8-oxohexadecanoate (C17H32O3)	3.58	0.00	0.00	2.44	1.36	0.00	2.25	1.39
cis 9,10-Ethoxystearic acid (C18H36O3)	27.29	16.48	26.49	0.00	0.00	0.00	0.00	0.00

Table 7- 200˚C Oxidation Fatty acid profile of IHG (control), IHG-tBHQ, MG5, AbG, VibG, AmG, MuG, and HaG

Fatty acid Name	IHG 200 (mg/g)	IHG-tBHQ 200 (mg/g)	MG5 200 (mg/g)	AbG 200 (mg/g)	VibG 200 (mg/g)	AmG 200 (mg/g)	MuG 200 (mg/g)	HaG 200 (mg/g)
Butyric acid (C4:0)	19.84	11.39	13.83	13.65	12.88	11.87	13.73	11.57
Caproic acid (C6:0)	12.73	7.50	8.45	8.58	8.50	8.29	9.74	8.26
Lauric acid (C12:0)	22.25	16.82	17.91	17.92	21.22	17.76	21.32	17.49
Myristic acid (C14:0)	117.55	98.14	100.75	108.50	111.90	96.60	114.22	100.68
Myristoleic acid (C14:1)	1.40	2.44	1.50	2.13	3.11	4.25	3.05	3.25
Palmitic acid (C16:0)	385.18	344.68	363.23	425.42	432.68	368.06	433.99	388.75
Palmitoleic acid (C16:1)	3.82	10.49	6.35	5.07	11.75	13.68	6.71	11.51
Oleic acid (C18:1)	7.88	24.75	10.70	14.38	14.77	22.14	10.15	18.67
Oleic acid, cis- (C18:1)	82.54	175.44	87.43	122.25	158.58	201.64	109.55	176.23
Linoleic acid (C18:2) (C18H32O2)	0.00	0.00	0.00	0.00	0.00	1.77	0.00	1.52
Linolenic acid (C18:3) (C18H30O2)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ethyl para-ethoxy benzoate (C11H14O3)	0.00	0.00	1.57	1.81	0.00	0.00	1.52	1.03
Methyl 8-oxohexadecanoate(C17H32O3)	4.65	2.05	1.53	0.00	1.78	0.00	0.00	0.00
cis 9,10-Ethoxystearic acid (C18H36O3)	38.34	8.72	0.00	0.00	0.00	0.00	0.00	0.00

*(*Note- GC-MS results of only selected samples are shown here. Complete master files of GC-MS results are shown in Appendix)*

3.4 Discussion

Ghee was seen to break down very rapidly with an increase in temperature. The stability of the In-house samples decreased by 2-fold in every 20˚C increase of temperature. Addition of synthetic anti-oxidants BHA, BHT, and tBHQ to the ghee improves the stability of ghee only in lower temperature of 160˚C. As the temperature increased, the increase in stability was very minimal. Among the synthetic antioxidants, tBHQ seems to be the most effective, followed by BHA. BHT is the least effective in increasing stability.

Comparison of In-house Ghee (IHG) with Store-bought Ghee (SBG) showed that IHG has lesser stability than SBG. Most likely, this would be because the commercial ghee making process is better in filtering off the solids than an in-house process. The presence of such solids in ghee could be a starting point for oxidation. There are also possibility of the SBG to have permissible contents of any synthetic antioxidant already added to it in its production.

Almost all murchita samples showed significant improvement in the stability of ghee when compared to the IHG and IHG-tBHQ samples. Among the 6 murchita samples, Murchita Ghee with all herbs (MG5), Amalaki Ghee (AmG), Vibhitaki Ghee (VibG) and Haridra Ghee (HaG) showed around 3-fold to 5-fold increase in the stability of ghee when compared to the IHG (control) sample. These murchita samples also showed better stability than the IHG-tBHQ sample, which was the best performing synthetic antioxidant sample. Abhaya Ghee showed little improvement in the stability and Musta Ghee showed a decrease in the stability when compared to In-house Ghee.

Shelf-lives of all the samples when calculated, again showed that the above 4 Murchita samples have increased the shelf-life of In-house ghee from days to many months.

After GC-MS analysis, the different oxidised samples when compared with the corresponding unoxidized samples showed a decrease in the contents on unsaturated fatty acids like, Myristoleic, Palmitoleic and Oleic acids. This might be due to the high susceptibility of unsaturated fatty acids to undergo oxidation and break down into other oxidative products. Interestingly murchita samples, Amalaki Ghee, Vibhitaki Ghee and Haridra ghee had a lesser decline in the concentration of these unsaturated fatty acids in the oxidised versions when compared to the unoxidized versions of. This might be

suggestive of some sort of protective action of these herbs on the unsaturated fatty acids preventing it from oxidative degradation.

The oxidised sample also had increase in the content of saturated fatty acids especially short chain fatty acids- Butyric, Caproic and Lauric acids. This again might be the consequence of break down of unsaturated fatty acids into shorter chain fatty acyls due to the action of free radicals.

Oxidised samples also showed the presence of some oxidised fatty acid counterparts like Ethyl para-ethoxy benzoate, Methyl 8-oxohexadecanoate, cis 9,10-Ethoxystearic acid. Here too, murchita samples Vibhitaki Ghee and Amalaki Ghee showed lesser concentrations or absence of these oxidised fatty acid counterparts suggesting a protection against oxidative degradation.

Overall, the murchita samples- Murchita ghee with all herbs, Vibhitaki Ghee, Amalaki ghee and Haridra ghee showed a protective action against oxidative degradation by increasing the Induction Time and by also preventing the breakdown of unsaturated fatty acids.

The herbs such as Musta and Abhaya displayed contradictory effect to our hypothesis, where either they did not increase induction time as (showing similar induction time as compared to their respective unoxidized counterpart or control ghee) or even reduced the induction time compared to the controls. This although negative but interesting observation in turn is serving the purpose of deconstruction study where the role of each herb is been clearly demonstrated. The data is presumably indicating either no antioxidant effect of these herb or even a potential pro-oxidant effect of Musta (increasing the oxidation). This collectively is explaining the reason why amla ghee displayed better antioxidation/higher induction time compared to all 5 herb murchita ghee sample.

4 CONCLUSION

- Murchita ghee samples, particularly those prepared with Amla and Vibhitaki, exhibited significantly improved induction times without increased conductivity, indicating the effectiveness of Ghrita Murchana as a natural alternative to synthetic antioxidants.
- Murchita ghee samples also demonstrated protection against excessive hydrolysis and breakdown of fatty acids during heating, which can lead to the production of volatile compounds.
- Even though, the concentration of unsaturated fatty acids decreased in all ghee samples after oxidation, Murchita ghee samples showed relatively lesser reductions in unsaturated fatty acids compared to antioxidant-treated samples, suggesting a protective effect of Murchana on these fatty acids during high-temperature oxidation.
- Various oxidative degradation products were identified in the oxidized ghee samples and the Murchita ghee samples showed a decrease in the presence and concentration of certain compounds, indicating the influence of herbal combinations used in Ghrita Murchana on oxidative degradation.
- Musta and Abhaya herbs displayed contradictory effects, suggesting either no antioxidant effect or potential pro-oxidant effects.
- When comparing the performance of in-house and store-bought ghee, in-house ghee samples showed faster oxidative degradation compared to store-bought ghee samples, possibly due to production methods or the addition of synthetic antioxidants in store-bought ghee.
- Overall, the results support the hypothesis that Murchana, especially with Amla, exhibits a significant antioxidant effect, as demonstrated by the preservation of unsaturated fatty acids during oxidation.
- Deconstruction of the murchana process has also clarified role of each herb pertaining to better performing and underperforming herbs in terms of imparting protection from oxidative degradation. This gave the clarity that may be the process can be simplified and instead of using all herbs, only better performing herbs could also be utilized for the antioxidant effects. This is an interesting research query which needs further investigation.

- These findings contribute to the understanding of natural alternatives to synthetic antioxidants and offer potential strategies for enhancing the shelf-life and quality of ghee. Further research is needed to explore the specific mechanisms and optimal herbal combinations in Ghrita Murchana for improving ghee stability and oxidative resistance.

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6 APPENDIX

6.1 Master table of IHG, IHG- Antioxidants and all Murchita Samples Before Rancimat

Fatty acid Name	IHG (mg/g)	IHG-BHA (mg/g)	IHG-BHT (mg/g)	IHG-tBHQ (mg/g)	MG5 (mg/g)
Butyric acid (C4:0)	8.48	10.15	9.49	8.68	11.12
Caproic acid (C6:0)	6.03	6.52	6.90	6.10	6.67
Caprylic acid (C8:0)	3.50	4.12	4.16	3.74	3.42
Capric acid (C10:0)	8.54	8.14	8.49	8.32	8.65
Lauric acid (C12:0)	16.42	15.14	16.13	15.26	14.55
Myristic acid (C14:0)	89.93	87.82	90.28	87.13	87.96
Myristoleic acid (C14:1)	4.79	4.71	4.83	4.45	4.23
Pentadecanoic acid, methyl ester (C15:0)	13.27	11.99	13.39	12.47	11.62
Palmitic acid (C16:0)	302.43	303.67	303.60	293.31	310.02
Palmitoleic acid (C16:1)	16.32	15.74	16.42	15.72	15.06
Margaric acid (C17:0)	10.21	8.41	8.96	8.84	8.27
Stearic acid (C18:0)	177.96	167.68	171.04	163.49	166.82
Oleic acid (C18:1)	38.74	0.00	38.65	37.07	37.31
Oleic acid, cis- (C18:1)	233.92	244.44	2.65	233.24	251.37
Ethyl para-ethoxy benzoate (C11H14O3)	0.00	0.00	0.00	0.00	0.00
Linoleic acid (C18:2) (C18H32O2)	11.17	8.81	9.43	9.98	8.26
Arachidic acid (C20:0)	5.03	3.43	3.95	3.55	3.77
Linolenic acid (C18:3) (C18H30O2)	2.58	2.09	2.16	2.15	1.75
Methyl 8-oxohexadecanoate (C17H32O3)	0.00	0.00	0.00	0.00	0.00
cis 9,10-Ethoxystearic acid (C18H36O3)	0.00	0.00	0.00	0.00	0.00
Fatty acid Name	**AbG (mg/g)**	**VibG (mg/g)**	**AmG (mg/g)**	**MuG (mg/g)**	**HaG (mg/g)**
Butyric acid (C4:0)	10.57	9.82	9.88	9.88	9.85
Caproic acid (C6:0)	6.15	7.30	6.81	7.32	7.13
Caprylic acid (C8:0)	3.45	4.19	3.55	4.19	4.62
Capric acid (C10:0)	7.47	9.44	8.68	10.40	9.60
Lauric acid (C12:0)	14.83	16.30	17.53	16.51	15.73
Myristic acid (C14:0)	88.22	86.85	94.91	87.37	86.84
Myristoleic acid (C14:1)	3.99	6.24	4.68	6.38	6.21
Pentadecanoic acid, methyl ester (C15:0)	11.53	13.08	10.39	12.86	12.70
Palmitic acid (C16:0)	315.56	303.87	366.16	306.81	306.73
Palmitoleic acid (C16:1)	15.50	19.48	18.69	18.88	18.89

Margaric acid (C17:0)	8.75	8.96	6.86	8.87	8.34
Stearic acid (C18:0)	175.33	156.24	24.04	156.21	156.70
Oleic acid (C18:1)	37.13	34.42	24.04	34.75	33.82
Oleic acid, cis- (C18:1)	245.46	254.62	245.90	257.13	255.32
Ethyl para-ethoxy benzoate (C11H14O3)	0.00	0.00	0.00	0.00	0.00
Linoleic acid (C18:2) (C18H32O2)	8.52	11.73	9.15	12.59	11.37
Arachidic acid (C20:0)	3.26	2.82	2.48	0.00	3.09
Linolenic acid (C18:3) (C18H30O2)	1.88	2.25	1.87	2.36	2.18
Methyl 8-oxohexadecanoate (C17H32O3)	0.00	0.00	0.00	0.00	0.00
cis 9,10-Ethoxystearic acid (C18H36O3)	0.00	0.00	0.00	0.00	0.00

6.2 Master table of IHG, IHG- Antioxidants and all Murchita Samples After 160˚C Oxidation

Fatty acid Name	IHG 160 (mg/g)	IHG-BHA 160 (mg/g)	IHG-BHT 160 (mg/g)	IHG-tBHQ 160 (mg/g)	MG5 160 (mg/g)
Butyric acid (C4:0)	16.99	17.49	14.77	17.79	20.10
Caproic acid (C6:0)	10.65	10.63	9.71	11.22	13.09
Caprylic acid (C8:0)	8.05	8.41	6.88	8.57	9.47
Capric acid (C10:0)	12.69	13.01	11.52	13.85	13.76
Lauric acid (C12:0)	21.33	22.46	19.62	21.92	21.73
Myristic acid (C14:0)	115.21	118.20	110.01	117.49	126.22
Myristoleic acid (C14:1)	1.69	1.55	2.10	1.23	0.00
Pentadecanoic acid, methyl ester (C15:0)	14.63	16.20	14.65	15.53	15.03
Palmitic acid (C16:0)	379.37	389.40	369.99	376.84	473.04
Palmitoleic acid (C16:1)	4.91	5.17	8.84	3.43	0.00
Margaric acid (C17:0)	9.49	10.10	9.67	9.83	10.33
Stearic acid (C18:0)	186.07	195.32	194.04	185.31	221.58
Oleic acid (C18:1)	12.76	9.39	16.53	9.12	0.00
Olieic acid, cis- (C18:1)	112.51	87.05	130.43	83.91	12.07
Ethyl para-ethoxybenzoate (C11H14O3)	0.00	0.00	0.00	2.32	0.00
Linoleic acid (C18:2) (C18H32O2)	0.00	0.00	0.00	0.00	0.00
Arachidic acid (C20:0)	3.81	2.82	3.61	3.51	3.29
Linolenic acid (C18:3) (C18H30O2)	0.00	0.00	0.00	0.00	0.00
Methyl 8-oxohexadecanoate (C17H32O3)	0.00	0.00	0.00	4.63	0.00
cis 9,10-Ethoxystearic acid (C18H36O3)	37.65	41.52	31.84	49.39	0.00
Fatty acid Name	**AbG 160 (mg/g)**	**VibG 160 (mg/g)**	**AmG 160 (mg/g)**	**MuG 160 (mg/g)**	**HaG 160 (mg/g)**
Butyric acid (C4:0)	15.21	13.45	13.09	12.04	10.78
Caproic acid (C6:0)	9.11	10.00	8.78	8.99	8.05
Caprylic acid (C8:0)	7.43	6.97	6.22	6.56	4.81
Capric acid (C10:0)	11.02	12.37	11.14	11.90	10.11
Lauric acid (C12:0)	18.56	19.46	21.85	18.60	16.25
Myristic acid (C14:0)	115.18	107.87	113.15	105.64	93.73
Myristoleic acid (C14:1)	0.44	2.07	2.74	3.03	3.88
Pentadecanoic acid, methyl ester (C15:0)	14.30	14.71	12.37	13.97	12.83
Palmitic acid (C16:0)	438.30	410.63	429.75	397.81	359.46
Palmitoleic acid (C16:1)	1.30	7.07	11.62	9.16	13.40
Margaric acid (C17:0)	9.72	9.00	8.18	9.52	9.33
Stearic acid (C18:0)	221.83	191.34	145.79	186.30	174.10

Oleic acid (C18:1)	4.18	11.08	13.25	14.31	22.31
Oleic acid, cis- (C18:1)	39.78	104.88	140.50	130.89	194.27
Ethyl para-ethoxy benzoate (C11H14O3)	0.00	0.00	0.73	0.00	0.00
Linoleic acid (C18:2) (C18H32O2)	0.00	0.00	0.00	0.00	1.07
Arachidic acid (C20:0)	3.13	3.05	2.54	2.86	3.27
Linolenic acid (C18:3) (C18H30O2)	0.00	0.00	0.00	0.00	0.00
Methyl 8-oxohexadecanoate (C17H32O3)	0.00	0.00	0.00	0.00	0.00
cis 9,10-Ethoxystearic acid (C18H36O3)	38.15	28.26	16.63	21.88	10.79

6.3 Master table of IHG, IHG- Antioxidants and all Murchita Samples After 180˚C Oxidation

Fatty acid Name	IHG 180 (mg/g)	IHG-BHA 180 (mg/g)	IHG-BHT 180 (mg/g)	IHG-tBHQ 180 (mg/g)	MG5 180 (mg/g)
Butyric acid (C4:0)	13.31	14.26	13.64	13.55	14.74
Caproic acid (C6:0)	8.07	9.33	8.03	8.50	8.90
Caprylic acid (C8:0)	6.27	6.67	6.07	5.50	6.21
Capric acid (C10:0)	10.40	10.48	10.53	10.29	9.82
Lauric acid (C12:0)	18.48	17.40	18.35	18.61	17.24
Myristic acid (C14:0)	108.56	101.04	101.91	101.83	107.36
Myristoleic acid (C14:1)	1.14	2.67	2.71	3.19	1.45
Pentadecanoic acid, methyl ester (C15:0)	14.32	13.06	14.00	12.93	12.57
Palmitic acid (C16:0)	373.03	340.19	345.36	344.86	421.04
Palmitoleic acid (C16:1)	7.77	12.21	11.08	12.64	5.47
Margaric acid (C17:0)	10.09	8.52	9.31	9.25	8.67
Stearic acid (C18:0)	210.46	177.91	190.98	187.75	200.86
Oleic acid (C18:1)	14.76	23.66	22.32	24.46	10.75
Oleic acid, cis- (C18:1)	109.72	189.42	172.23	192.43	99.34
Ethyl para-ethoxy benzoate (C11H14O3)	0.00	0.00	0.00	0.00	0.00
Linoleic acid (C18:2) (C18H32O2)	4.21	3.18	4.23	4.21	2.65
Arachidic acid (C20:0)	0.00	0.00	0.00	0.00	0.00
Linolenic acid (C18:3) (C18H30O2)	27.23	14.56	19.38	16.48	26.50
Methyl 8-oxohexadecanoate (C17H32O3)	0.00	0.00	0.00	0.00	0.00
cis 9,10-Ethoxystearic acid (C18H36O3)	0.00	0.00	0.00	0.00	1.56
Fatty acid Name	**AbG 180 (mg/g)**	**VibG 180 (mg/g)**	**AmG 180 (mg/g)**	**MuG 180 (mg/g)**	**HaG 180 (mg/g)**
Butyric acid (C4:0)	15.11	10.15	15.25	15.15	12.46
Caproic acid (C6:0)	9.72	7.18	9.93	11.20	8.84
Caprylic acid (C8:0)	7.38	5.21	7.14	9.09	6.33
Capric acid (C10:0)	11.24	9.70	12.22	13.36	11.81
Lauric acid (C12:0)	18.00	15.31	23.42	21.10	17.97
Myristic acid (C14:0)	111.39	86.94	122.45	114.72	103.97
Myristoleic acid (C14:1)	0.97	5.14	2.28	1.76	3.17
Pentadecanoic acid, methyl ester (C15:0)	12.92	11.84	13.35	15.03	13.86
Palmitic acid (C16:0)	429.11	341.95	461.08	432.67	395.18
Palmitoleic acid (C16:1)	4.45	15.41	8.27	5.00	9.79
Margaric acid (C17:0)	8.79	8.31	8.63	10.65	8.72
Stearic acid (C18:0)	215.37	160.31	152.12	191.44	181.55

Oleic acid (C18:1)	7.91	26.44	7.83	7.38	15.97
Oleic acid, cis- (C18:1)	70.24	239.16	90.24	66.66	145.26
Ethyl para-ethoxy benzoate (C11H14O3)	0.00	0.00	0.00	0.00	0.00
Linoleic acid (C18:2) (C18H32O2)	3.00	2.67	2.91	2.94	3.17
Arachidic acid (C20:0)	0.00	0.00	0.00	0.00	0.00
Linolenic acid (C18:3) (C18H30O2)	0.00	0.00	0.00	0.00	0.00
Methyl 8-oxohexadecanoate (C17H32O3)					
cis 9,10-Ethoxystearic acid (C18H36O3)	0.00	0.00	0.00	0.00	0.00

6.4 Master table of IHG, IHG- Antioxidants and all Murchita Samples After 200˚C Oxidation

Fatty acid Name	IHG 200 (mg/g)	IHG-BHA 200 (mg/g)	IHG-BHT 200 (mg/g)	IHG-tBHQ 200 (mg/g)	MG5 200 (mg/g)
Butyric acid (C4:0)	19.84	11.98	12.37	11.39	13.83
Caproic acid (C6:0)	12.73	7.39	7.63	7.50	8.45
Caprylic acid (C8:0)	10.10	5.38	5.86	5.01	6.96
Capric acid (C10:0)	14.06	10.02	9.80	9.44	10.91
Lauric acid (C12:0)	22.25	17.51	17.70	16.82	17.91
Myristic acid (C14:0)	117.55	100.32	102.40	98.14	100.75
Myristoleic acid (C14:1)	1.40	2.91	3.04	2.44	1.50
Pentadecanoic acid, methyl ester (C15:0)	15.18	13.52	13.26	13.42	12.59
Palmitic acid (C16:0)	385.18	342.96	348.19	344.68	363.23
Palmitoleic acid (C16:1)	3.82	10.49	9.08	10.49	6.35
Margaric acid (C17:0)	9.37	10.21	9.52	9.85	7.87
Stearic acid (C18:0)	192.36	194.28	197.43	199.69	177.19
Oleic acid (C18:1)	7.88	25.62	22.59	24.75	10.70
Olieic acid, cis- (C18:1)	82.54	174.48	166.53	175.44	87.43
Ethyl para-ethoxybenzoate (C11H14O3)	0.00	0.00	0.00	0.00	1.57
Linoleic acid (C18:2) (C18H32O2)	0.00	0.00	0.00	0.00	0.00
Arachidic acid (C20:0)	2.99	4.05	4.26	4.32	2.53
Linolenic acid (C18:3) (C18H30O2)	0.00	0.00	0.00	0.00	0.00
Methyl 8-oxohexadecanoate (C17H32O3)	4.65	0.00	0.00	0.00	0.00
cis 9,10-Ethoxystearic acid (C18H36O3)	38.34	11.32	13.23	8.72	0.00
Fatty acid Name	**AbG 200 (mg/g)**	**VibG 200 (mg/g)**	**AmG 200 (mg/g)**	**MuG 200 (mg/g)**	**HaG 200 (mg/g)**
Butyric acid (C4:0)	13.65	11.87	12.88	13.73	11.57
Caproic acid (C6:0)	8.58	8.29	8.50	9.74	8.26
Caprylic acid (C8:0)	6.24	6.19	6.19	8.71	6.17
Capric acid (C10:0)	10.42	11.18	10.33	13.39	11.06
Lauric acid (C12:0)	17.92	17.76	21.22	21.32	17.49
Myristic acid (C14:0)	108.50	96.60	111.90	114.22	100.68
Myristoleic acid (C14:1)	2.13	4.25	3.11	3.05	3.25
Pentadecanoic acid, methyl ester (C15:0)	13.25	13.29	12.06	15.33	13.67
Palmitic acid (C16:0)	425.42	368.06	432.68	433.99	388.75
Palmitoleic acid (C16:1)	5.07	13.68	11.75	6.71	11.51
Margaric acid (C17:0)	9.18	9.17	8.10	9.71	9.06
Stearic acid (C18:0)	206.83	168.57	146.86	190.05	181.18
Oleic acid (C18:1)	14.38	22.14	14.77	10.15	18.67
Olieic acid, cis- (C18:1)	122.25	201.64	158.58	109.55	176.23

Ethyl para-ethoxybenzoate (C11H14O3)	1.81	0.00	0.00	1.52	1.03
Linoleic acid (C18:2) (C18H32O2)	0.00	1.77	0.00	0.00	1.52
Arachidic acid (C20:0)	2.42	2.71	2.79	2.78	2.33
Linolenic acid (C18:3) (C18H30O2)	0.00	0.00	0.00	0.00	0.00
Methyl 8-oxohexadecanoate (C17H32O3)	0.00	0.00	0.00	0.00	0.00
cis 9,10-Ethoxystearic acid (C18H36O3)	0.00	0.00	0.00	0.00	0.00