Mango Butter Extraction with n-Hexane Using a Soxhlet Apparatus

Graduation Internship Report

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Abstract

This research paper addresses the research aim of determining the most effective and sustainable method for mango butter extraction using a Soxhlet extraction method. The project focuses on meeting the goals of the start-up "Natural Waste", and utilizing discarded mango seeds from a Dutch juice processing company

The Netherlands is a major mango fruit consumer and large quantities of mango waste is being produced as a result of that, such as peels, seeds, and flesh. This project explores the potential of mango waste utilization and production of mango kernel butter for application in the cosmetics industry. Further, the goal of the project is to estimate the yield of mango butter that is possible to be achieved from the mango waste and create a viable and environmentally friendly production process.

It was concluded that the use of a thimble as a filter for the Soxhlet proved superior to cotton-wool filter. During the experiments Soxhlet extractions with n-hexane were done, testing Kent variety mango kernels (fresh and boiled version), Alphonso, and Kesar varieties were also tested. Alphonso and Kesar showed a similar butter yield of 8% out of 20g of dried kernels, in comparison to Kent where the average percentage was 6%. Moreover, longer extraction time of 6h resulted in higher butter yield than 4h of extraction. The maximum amount that was obtained was 1.65g of mango kernel oil, 8.18% out of 20g fresh mango kernel powder extracted in 4 hours, which might be due to higher solvent concentrations in the product. The higher iodine number of Kent mango butter indicated higher unsaturated fatty acids than the Alphonso type, making it more susceptible to oxidation and faster degradation.

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Chapter 1
Introduction

1.1 Food Waste Problem

Yearly one-third of edible food is wasted globally, which makes food waste one of the largest global problems of our century. Food waste is generated often when edible products, intended for consumption, are discarded by consumers and retailers due to unpleasant appearance and shapes. As a result, this has a severe impact on the economics, environment, and people. [2]

Moreover, population growth, rapid urbanization along with industrial development and changes in lifestyle and economic situation have resulted in the generation of a large amount of food waste. According to the FAO report, every year about 1.3 billion tons of food is wasted, equivalent to more than half of the world's total cereal production. Whenever it is discarded, this waste creates many economic, social and environmental concerns. When thrown into landfills, food waste produces harmful greenhouse gasses like methane and carbon dioxide, which contribute to global warming and climate change. Natural resources such as land, fresh water, fossil fuels and human resources are also wasted. Therefore, to manage this food waste, innovative and sustainable recovery technologies are needed within the framework of recycling and waste recovery in food waste management. [3]

One of the largest food waste generators are households, nearly two-thirds of the generated waste around the globe and a study suggests that managing household food waste and agricultural losses appropriately, can be decreased to 86%.[2]

1.2 Natural Waste

To tackle the problem of household waste ending up in landfills in the Netherlands, the start-up company Natural Waste was established. The company's primary focus is the utilization of the discarded household food products such as fruit and vegetable peels, seeds, coffee grounds, and more. Moreover, they wish to create natural products, which are beneficial , for example, create supplements from fruit and vegetable peels, formulate natural cosmetics and skin care products from peels and coffee grounds, or extract enzymes, oils and vitamins. Currently, Natural Waste has focused on mango waste utilization, which is derived from a mango processing company "Vezet", based in the Netherlands.

1.3 Mango waste in the Netherlands

According to data from the Dutch Central Bureau of Statistics (CBS), the Netherlands imported 186 million kilograms of mangoes in 2020. The Netherlands is a major importer of mangoes, with the majority of imports coming from countries such as Peru, Costa Rica, and Brazil. [4] However, it is estimated that globally, up to 50% of mangoes are wasted due to factors such as overproduction, transportation issues, and consumer preferences. [5]. Other than that, juice processing companies discard the mango seed/kernel and mango peel as a waste, which still have a significant amount of consumable mango flesh present.

1.4 Mango butter

The oil present in mango kernels, ranging from 7% to 14%, has been proven to be valuable in various industries such as cosmetics, food, pharmaceuticals, and energy. Despite its usefulness, mango kernel oil (MKO) has been neglected commercially due to its poor yield. Therefore, optimizing the extraction procedure to increase the yield is a highly anticipated path that may encourage future use. The choice of the extraction method becomes important to achieve this [6].

Mango butter has been extracted from mango kernels and other substrates using various techniques, including hydraulic pressing, solvent extraction, solvent extraction with a Soxhlet, ultrasound-assisted extraction, supercritical CO_2 extraction, and others [6]. The growing need for edible oils, driven by the increasing world population, has led to a search for plant sources that offer acceptable sensory and nutritional properties for extraction and production. Mango kernel butter is known to possess physicochemical qualities comparable to cocoa butter, shea butter, kokum butter, illipe butter, and sal butter. Furthermore, the unique fatty acid composition of mango kernel butter is similar to that of commercial cocoa butter, making it an ideal substitute. The antioxidant characteristics, phenolic content, and free radical-scavenging activity of mango kernel butter contribute to the rising interest in this unconventional lipid source [7].

1.5 Goal of the project

Since the Netherlands is a large consumer and processor of mangoes and a great amount of it is being wasted in the form of peels, seeds, and mango flesh, Natural Waste wants to focus on utilizing the discarded mango seeds and reusing them for mango butter production, which is planned to be applied in the cosmetics production. The current paramount aim is to estimate the amount of butter yield possible to obtain from the available mango waste. Additionally, Natural Waste aims to create a viable and green mango kernel butter production process from discarded mangoes.

Furthermore, the world is moving towards a circular economy, and the waste from one process can be turned into a valuable product. Throughout the Chemical Engineering program, we have been thought to optimize, reuse, and reduce the waste from the production processes, consequently, in this case, reducing the amount of biogas emission into the atmosphere from mangoes.

With a view to stick to this goal, a research question has been constructed to guide the project: ***What is the most effective and sustainable way of mango butter extraction by the Soxhlet extraction method?***

Chapter 2
Background and Theory

2.1 Mango (Mangifera Indica)

One of the most popular fruits in the world, mangoes (Mangifera Indica) are grown in over 100 nations across tropical and subtropical latitudes. Among the significant by-products generated during mango fruit processing is the mango seed (MS). The seed consists of a large, hard endocarp that encloses the kernel. In terms of weight, the seed typically represents between 10% and 25% of the fruit, while the kernel makes up approximately 45% to 85% (w/w) of the seed and 20% of the entire fruit. Every year, an estimated 35% to 60% of the fruit's weight, including the seed and peel, goes to waste, amounting to approximately 540,000 metric tons of leftovers. If not properly managed, this volume of waste can lead to environmental damage such as water contamination, greenhouse gas emissions, and the development of unpleasant odors caused by microorganism activity. One of the main alternative applications of the discarded mango seed is the extraction of mango oil, which finds use in various industries, including cosmetics and food [5].

2.1.1 Mango butter composition and application

The mango kernel is considered a natural source of high-quality edible oil, with the three main fatty acids in its lipid fraction being palmitic, stearic, and oleic acid, which make up around 15% of the entire seed. [8]
The fat composition in mango kernels ranges between 3.7 - 12.6% on a dry basis, varying depending on the type of mango fruit. Based on the research conducted by Bhattacharya, K., et al., the fatty acid content of mango butter consists mainly of stearic acid (C18:0), typically between 26 and 49%, and oleic acid (C18:1), between 38.9 and 45%. Additionally, linoleic acid (C18:2) constitutes 2% to 12% of the fatty acids. [9]

The iodine value (IV), which relates to its chemical properties, ranges from 40 to 61g/100g, and its melting point (MP) value varies from 25 to 39 °C, rendering it a semi-solid fat at room temperature.

These figures are comparable to those of other common industrial fats such as palm oil (IV: 50.6 - 55.1g/100g; MP: 30.8-37.6 °C) and cocoa butter (IV: 34 - 41g/100g; MP: 30-35°C).

Triacylglycerols (TAGs), similar to palm oil and cocoa butter, make up the majority of the substance (up to 93%), followed by free fatty acids, diacyl and monoacylglycerols, and micronutrients. The main difference between those oils lies in the presence of micronutrients and the composition of the triacylglycerols.

Furthermore, mango kernel fat can replace up to 60% of the total lipid fraction in cacao butter without significantly altering the distinctive characteristics of chocolate. This is particularly important as cacao butter needs to be precisely manufactured to prevent the typical fat bloom that occurs when the product is exposed to inappropriate storage conditions and high temperatures. [8]

Initially considered as an alternative to cocoa butter in confectionery products, mango kernel has also shown promise in cosmetic formulations due to its content of tocopherol, phytosterols, and triterpenes, which are skin-active ingredients. Additionally, mango butter is considered an effective substitute for paraffin-based emollients and offers protection against harmful UV rays, making it valuable in skincare products. [8]

2.2 Extraction types of fats and oils

2.2.1 Hydraulic pressing

Mango butter extraction can be achieved through various processes, and one commonly utilized method is hydraulic pressing. In this approach, the mango kernel is finely ground into particles, and the resulting paste is subjected to pressing to separate the liquid from the portion containing the butter. Hydraulic pressing is typically employed in small-scale operations due to its low investment and operational costs. However, it should be noted that the extraction yield is not exceptionally high, typically ranging from 45% to 55% of the total oil content [10].

Mechanical extraction methods, such as hydraulic pressing, offer advantages such as the production of high-quality oils and butters, particularly when cold-pressed. Additionally, the resulting cake from this process can be

further utilized since it does not contain harmful chemicals or solvents [7]. However, it is important to acknowledge that mechanical methods may not be suitable for effectively extracting oil from seeds with low oil content, like soybeans, and can result in a cake with high oil content.

To address the limitations of mechanical procedures, solvent approaches have been developed. By combining both mechanical and solvent extraction methods, it is possible to maximize extraction efficiency. Solvent extraction is also commonly employed to remove any residual oil that remains in the seed cake after mechanical extraction [11].

2.2.2 Solvent extraction (Soxhlet apparatus)

The solvent extraction method is a chemical process that involves the use of organic solvents for the separation of lipids. The choice of solvent is determined by the basic properties of the component, such as solubility, hydrophobicity or hydrophilicity, vapor pressure, molecular weight, and acid dissociation. The effectiveness of solvent extraction is influenced by factors such as particle size (lower particle size decreases the barrier between two phases), agitation speed, and extraction temperature [11].

Soxhlet extraction is a traditional method used to separate oils with volatile chemicals from solid components. It was introduced in the middle of the 19th century by Baron von Soxhlet and serves as a reference for many contemporary extraction techniques [1].

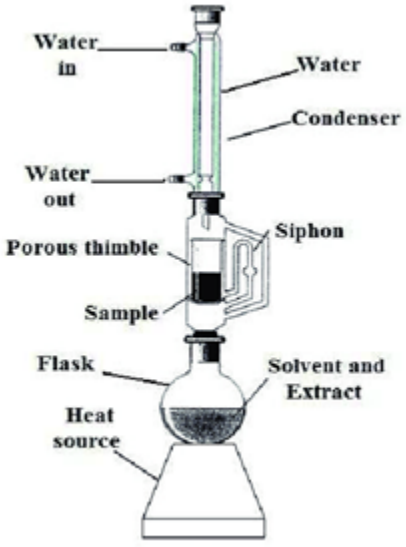

Figure 2.1: A representative figure of Soxhlet Apparatus. [1]

In Figure 2.1, a schematic representation of a typical Soxhlet setup is shown. The system consists of three parts: a thimble holder with a siphon mechanism and a side tube in the middle, a solvent vapor reflux condenser at the top, and a flask with a circular bottom connected to the bottom of the thimble holder. Soxhlet extraction is a slow method that can take anywhere between 6 and 48 hours due to the use of cooled, condensed solvents [1].

The process of mango butter extraction using Soxhlet involves heating the solvent (n-hexane) in the round-bottom flask to its boiling temperature. The vaporized solvent condenses and drips into the thimble containing the sample (mango kernel powder), where the extraction of lipids takes place. Once the extraction thimble reaches the level of the siphon tube, the extract drains back to the solvent flask, and the process is repeated until no further extraction occurs [11].

Soxhlet is a well-known technique often used as a standard for comparison with other approaches. However, it has a few drawbacks, including a lengthy extraction time and a large amount of solvent usage, depending on the extract [1].

Moreover, this method requires the use of a significant amount of organic solvent, which necessitates the removal of the solvent after extraction, resulting in high energy and operating expenses. The processing of food using

this method can lead to contamination of end products, raising concerns about environmental pollution and food safety. Alternative methods such as mechanical pressing, supercritical CO_2 extraction, and subcritical n-propane extraction have been proposed as less harmful ways to extract oil from seeds to address these issues. Supercritical and subcritical extraction processes operate at relatively high temperatures ($60°C$) and high pressures (12–40 MPA), requiring expensive reactors and raising safety concerns. Mechanical pressing results in only partial recovery of the oil, whereas these methods are advantageous for oil processing due to the use of a nontoxic solvent. Additionally, the conditions under which oil is extracted using the n-propane critical process greatly affect the production, purity, and profile of the oil [12].

2.2.3 Ultrasound assisted extraction (UAE)

Ultrasound-assisted extraction (UAE) is a recently developed technique that utilizes sound waves between 20 and 100 MHz, which are above the range of human hearing. UAE offers several benefits, including improved extraction efficiency, shorter extraction times, and reduced energy consumption. The primary characteristic of UAE is citation activity, which involves the production and collapse of bubbles. This cavitation activity destabilizes the material matrix, leading to enhanced solvent flow into the sample and the release of the extractable compounds and target chemicals.

The use of mechanical waves in combination with solvent extraction techniques, as demonstrated by UAE, addresses the limitations of traditional solvent extraction methods, such as prolonged extraction times, the use of toxic solvents, and low efficiency. Ultrasound probe or horn system (direct) can be employed for ultrasonic extraction. Industrial applications also exist, utilizing ultrasonic reactors.

Both the indirect and direct systems require a mechanical agitator and cooling bath to regulate the temperature increase during extraction. The bath system utilizes transducers attached at the bottom to provide ultrasonic waves, while the probe system incorporates an ultrasonic transducer installed in the extraction chamber.

During direct extraction, it is essential for the probe to come into contact with the solvent medium but not the substance being extracted to prevent blockage. Ultrasonography exposes the walls of glands or cells, primarily in plant-based materials, to mechanical stress, making them fragile and easily breakable. This facilitates the extraction of essential oils and lipids from various sources. By reducing the material size, the efficiency of the process can be further cavitation-assisted extraction.

The ultrasonication principle is based on the cavitation phenomena. When high-frequency ultrasonic waves (20 kHz) are delivered into a solvent medium containing the sample, bubbling occurs throughout the medium. The growing bubbles with negative pressure cause mechanical stress on the surface of cells. The collapse of cavitation bubbles at the cell surface leads to cell wall damage and the transfer of internal contents into solvent solution. UAE can be applied to both solid and liquid samples, although caution is advised when using solid samples to prevent analyte decomposition if trapped inside cavitation bubbles. Although conventional Soxhelt extraction is less effective and time consuming compared to UAE, it also utilizes hazardous and expensive organic solvents [11].

2.2.4 Microwave assisted extraction (MAE)

Microwave-assisted extraction (MAE) is an innovative technique that has improved the yield, purity and efficiency of traditional solvent-based procedures. Over the years, MAE has emerged as a promising and practical method for rapidly separating specific molecules at higher rates. It is considered a green extraction method as it utilizes water or alcohols as solvents, along with controlled pressure and high temperatures.

Although water is typically not regarded as an extraction solvent for non-polar organic components due to its strongly polar nature, MAE has demonstrated that water can acquire alcohol-like properties under specific conditions. At increased temperatures and regulated pressure, water can dissolve various low-to medium-polarity compounds during extraction.

Microwave-based extraction techniques primarily focus on releasing trapped moisture from the extraction sample. By heating the moisture in the cells of the sample material with microwaves, the pressure builds up within the cells, causing swelling and straining the cell walls. Eventually, the intracellular contents are released into the solvent as the cell walls rupture, thereby increasing the extraction yield. To further enhance the efficiency of MAE,

solvents with a high heat dissipation capacity can be employed.

The effectiveness of MAE is influenced by several factors, including the nature and volume of the solvent, extraction period, microwave power, sample particle size, moisture content, temperature, and pressure. Notable advantages of MAE include reduced extraction times, lower solvent usage, cost effectiveness, and environmental friendliness. However, MAE may exhibit lower efficiency when dealing with non-polar or volatile analytes or extraction solvents. Additionally, due to the utilization of high temperatures, thermolabile chemicals can undergo decomposition. [11]

2.2.5 Supercritical CO_2 Extraction

The increased concern about the disposal of hazardous organic solvents and their negative impact on people and the environment has caused a shift towards a greener extraction technologies such as Supercritical CO_2 extraction. Carbon dioxide can be used as a non-toxic, non-flammable and safe to use solvent and it can provide solutions for difficult separation problems. [13]

Gasses like carbon dioxide, propane, toluene, ethylene, and water at supercritical conditions can be utilized as supercritical solvents. In supercritical conditions improvement in characteristics increase the selectivity, flow rate, and mass transfer compared to conventional solvents, further, making the extraction process more efficient and greener.[11]

Carbon dioxide is the most widely used solvent for lipid extraction and the compound reaches critical conditions at 304°K and pressure 73 atm. The definition of the extraction is when the supercritical compound as a solvent comes in contact with oleaginous sample, fats and oil dissolve in the solvent, additionally, by decreasing the pressure, the oil easily gets separated from the supercritical substance. Efficiency and oil purity are closely dependant on temperature, pressure, and extraction time. [11]

2.2.6 Enzymatic extraction

Enzymatic extractions have recently gained a lot of attention as a potentially effective technique for oil extraction. In extensively practiced enzyme-assisted solvent extraction, plant materials are first pretreated with enzymes before being extracted with organic solvents. While this technology extracts oil effectively, the use of organic solvents has a detrimental impact on the environment and the quality of the product. This restricts the use of this technique in the food processing sector. Aqueous enzymatic extraction, which utilizes water as the extraction solvent and eliminates the harmful impact of solvents, is suggested as a greener option for oil extraction. Enzymes play a significant role in this method by hydrolyzing the components of plant cell walls and the membrane structure of oil bodies, leading to cell disruption, improved structural permeability, and enhanced oil release in water during extraction. The extraction process is then made easier by the simple separation of the extracted oil from the aqueous phase, facilitating product purification. Additionally, studies have shown aqueous enzymatic extraction, compared to other chemical and mechanical extraction procedures, offers higher selectivity and product quality while requiring milder extraction conditions [12].

Table 2.1: Extraction Methods, Advantages, and Disadvantages

Extraction Methods	Advantages	Disadvantages
Hydraulic pressing	No solvent use, environmentally friendly Lower processing cost High quality extraction of lipids, with high content of fatty acids and antioxidants	Usually a lower extraction yield (45- 55% of the total oil content) Additional processing step for removal of impurities is required May not be suitable for mangoes with a lower oil content
Solvent/Soxhlet extraction	High yield of extraction can be obtained (50 - 70% of the total oil content) Can be scaled up for large-scale production The used solvent can be recycled up to 90% Green solvents can be used	Large amount of solvent is required Solvents may be hazardous to health and the environment Time and energy consuming (usually about 5 to 8 hours)

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Extraction Methods	Advantages	Disadvantages
Ultrasound-assisted extraction	Reduces extraction time up to 50% compared to conventional solvent extraction methods Energy efficient, does not require high temperatures Sustainable, requires low solvent volumes compared to the solvent method Can be scaled up Better quality of butter production compared to the conventional solvent method Oil recovery up to 80%	Requires specific equipment, costly Solvent is used A constant agitation is needed to prevent hot points and high energy build-ups
Microwave-assisted extraction	Extraction can be done without solvents Shorter extraction time Sustainable, requires low solvent volumes compared to the solvent method Shorter extraction time compared to conventional solvent extraction methods	Requires specific equipment Filtration is needed Potential heat degradation of the product
Supercritical CO_2 extraction	High-quality extract can be obtained with no solvent residue Extraction can be done at lower temperatures, preserving bioactive compounds Fast extraction Can be scale-up for large-scale production 80% to 95% oil extraction efficiency	High operational cost Expensive operational equipment
Enzymatic extraction	Green, no solvent is required Mild extraction conditions may preserve the bioactive compounds 30 to 90% of oil extraction efficiency depending on the enzyme choice Can be suitable for large-scale production Specific enzyme target specific compounds for extraction Enzymes can be reused	Enzymes are expensive Enzymes may be sensitive to environmental conditions and require careful handling Requires specialized equipment and expertise

2.3 Analytical methods for fats

Analysis of the oils and fats is important to understand the physical and chemical characteristics of the product, in order to know its benefits and behavior. An example for physical characterisation are melting point, color, fat content, density determination. Furthermore, the chemical analyses of fats and oils include free fatty acid determination, peroxide value, iodine number, saponification number analyses, which are described in the paragraphs below.

2.3.1 Peroxide value

The peroxide value provides information about the rancidity level in unsaturated fats and oils. It indicates the amount of peroxide oxygen present in 1 kg of fat or oil. Oils with high levels of unsaturation (double bonds) are more susceptible to autoxidation, a free radical reaction with oxygen that leads to the deterioration of fats and oils. This degradation results in the formation of off-flavors and off-odors. The peroxide value is determined by titrating the liberated iodine from potassium iodide with sodium thiosulfate solution [14].

2.3.2 Iodine number

The iodine number (IV) measures the degree of unsaturation in a fat or oil. It represents the number of grams of iodine absorbed by 100 grams of fat. Oils with high iodine values contain a higher concentration of highly unsaturated fatty acids, making them more prone to rapid degradative reactions such as autoxidation or polymerization. These reactions are particularly accelerated by high temperatures and the presence of dissolved oxygen from the air, especially during baking. Oils with iodine values exceeding 115 are referred to as “drying oils” due to their ability to polymerize into a tough, solid film upon exposure to air [15].

2.3.3 Saponification number

The saponification number (SN) or saponification value (SV) represent the amount of potassium hydroxide (KOH) required to saponify one gram of fat under specific conditions. It is a crucial parameter used to describe and assess the quality of edibles fats and oils. Additionally, the saponification value provides insights into the average molecular weight of the fatty acids present, as the molecular weight decreases with increasing saponification numbers [16].

Table 2.2: Parameters of "Kent" mango type

Parameter	Value
FFA (oleic) (C18:1)	46.37 ± 2.39%
FFA (stearic) (C18:0)	30.47 ± 1.42%
FFA (palmitic) (C16:0)	10.57 ± 0.40%
FFA (linoleic) (C18:2)	10.4 ± 0.64%
FFA (arachidic) (C20:0)	1.64 ± 0.32%
FFA (linolenic) (C18:3)	0.60 ± 0.11%
Acid value	7.48
Peroxide value	0.75
Iodine value	51.08
Saponifiable matter	195.9
Unsaponifiable matter	2.74
Melting point (°C)	25-26 °C
Specific gravity at 25°C	0.9017

Having the pros and cons of each method, presented in Table 2.1 , it was decided that the most reasonable methods to be tested in the Hague University of Applied Sciences lab are the Soxhlet (solvent) extraction, Ultrasound-assisted and Microwave-assisted extractions with solvent, and Enzyme extraction methods. The mechanical pressing was decided not to be used. Even though it is the most environmentally friendly way, the extraction yield is not as high as the remaining methods. Furthermore, while supercritical CO_2 extraction appears to be the most effective extraction approach, it requires specialized equipment and cannot be performed in a university lab.

Additionally, the Soxhlet extraction method will be the main emphasis of the internship and be utilized to extract mango butter using the n-hexane solvent. Further, in Chapter 3 the materials and methods for the Soxhlet extraction, including the pre-treatment of the mango kernels will be described. In order to optimize the solvent extraction process, ultrasound-assisted extraction will be performed and the process and results will be compared with the Soxhlet extraction method.

Chapter 3
Methodology

3.1 Mango varieties and Preparation for Soxhlet Extractions

Discarded mangoes were provided from a Dutch juice-processing company "Vezet". The company primarily uses Kent and Keitt varieties of fibreless mangoes for their production, although the received mangoes are believed to be Kent variety. There were three deliveries of mangoes, first and second from the juice processing company, and a third one was obtained from an Indian store containing Alphonso and Kesar mango varieties.

For the Soxhlet extractions, mango flesh, mango seeds and kernels were carefully separated from the mango waste. Only the kernels were used for the mango butter extraction, which were cleaned with water, sliced into flat discs using a grinder, and dried for 2h at 55°C. The dried kernels were grinded into fine powder using a coffee grinder for about 15 seconds. The powder was run through a sieve size of 20 (0.38mm) and 30 (0.28mm) two times where the larger particles were put back in the coffee grinder.

3.2 Soxhlet Extraction Method

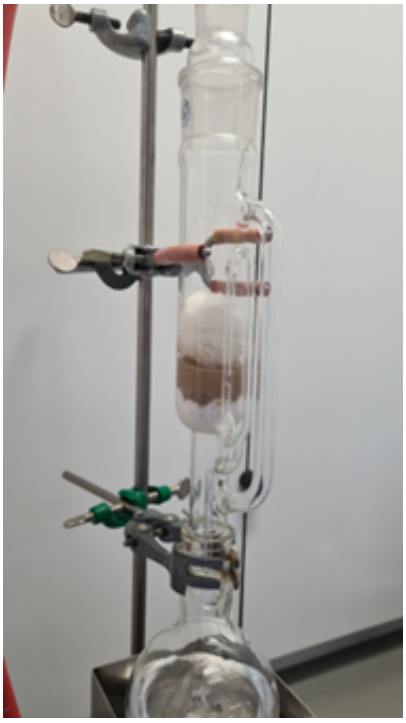
For each of the extraction, 20 grams of mango kernel powder were measured into the extraction chamber in the Soxhlet extractor. The round bottom flask of the set-up is filled with 300ml of n-Hexane solvent. A round bottom heating mantle was used to heat up the solvent until its boiling temperature of 68 $^{\circ}C$ and a magnetic stirring bar was placed in the round bottom flask for agitation.

Additionally, four different mango kernels were used: kernels extracted from fresh mango waste from the first delivery of mangoes, boiled mango waste kernels, which were part of the second mango delivery. Five duplicated Soxhlet extractions were performed, as shown in Table 3.1 , in order to confirm the accuracy of the obtained results, except the extraction with the Alphonso and Kesar mango types. For the first Soxhlet extraction, **1.1**/**1.2** cotton-wool was used as an extraction filter and the rest of the extractions were done using a thimble. Optimization of the extraction time was done by setting the extraction time of **2.1**/**2.2** to 6 hours and the **3.1** / **3.2** to 4 hours and the results were compared.

Table 3.1: Soxhlet extraction performed in the lab

Soxhlet extraction number	**Dried mango kernel powder**	**Type of Soxhlet filter**	**Kernel/solve ratio**	**Time of extraction**
1.1	Freshly extracted, 1st batch	cotton-wool	1:15	6h
1.2	Freshly extracted, 1st batch	cotton-wool	1:15	6h
2.1	Freshly extracted, 1st batch	thimble	1:15	6h
2.2	Freshly extracted, 1st batch	thimble	1:15	6h
3.1	Freshly extracted, 1st batch	thimble	1:15	4h
3.2	Freshly extracted, 1st batch	thimble	1:15	4h
5.1	Boiled mangoes, 2nd batch	thimble	1:15	4h
5.2	Boiled mangoes, 2nd batch	thimble	1:15	4h
6.1	Alphonso, freshly extracted	thimble	1:15	4h
7.1	Kesar, freshly extracted	thimble	1:15	4h

a) Soxhlet apparatus lab set-up.



b) Soxhlet with cotton wool.

c) Soxhlet with thimble for filtration.

FIGURE 3.1: Soxhlet Apparatus Setup

3.3 Rotary Evaporator Separation of the Solvent/Butter Mixture

In order to separate the extracted butter dissolved in the solvent (n-hexane), a rotary evaporator was used. The process continues for about 15 min, where the rotation is set to 20 rpm, the vacuum pump is turned on and the round-bottom flask with the solvent/butter mixture is emerged into a water bath of $35°C$, in order to avoid extreme boiling and escape of the butter into the condensed solvent.

After the initiation of the extraction, the temperature is increased to $50°C$, so that the temperature increases with $2°C$ per minute, since during evaporation the solvent becomes less concentrated and the boiling temperature becomes higher. Finally, the evaporated solvent is collected, the amount is measured, and stored in a 2L glass jar for future extractions.

When the solvent is completely evaporated and separated from the butter, the round-bottom flask is removed from the rotary evaporator. In order to transfer the butter into a jar container, a hair dryer is used to heat up a 5ml glass pipette and the round bottom flask containing the butter, since the butter is soft solid at room temperature. Moreover, the melted butter is transferred from the pipette into a small glass jar and a measure of the amount (ml) and weight (g) is taken using a weight scale.

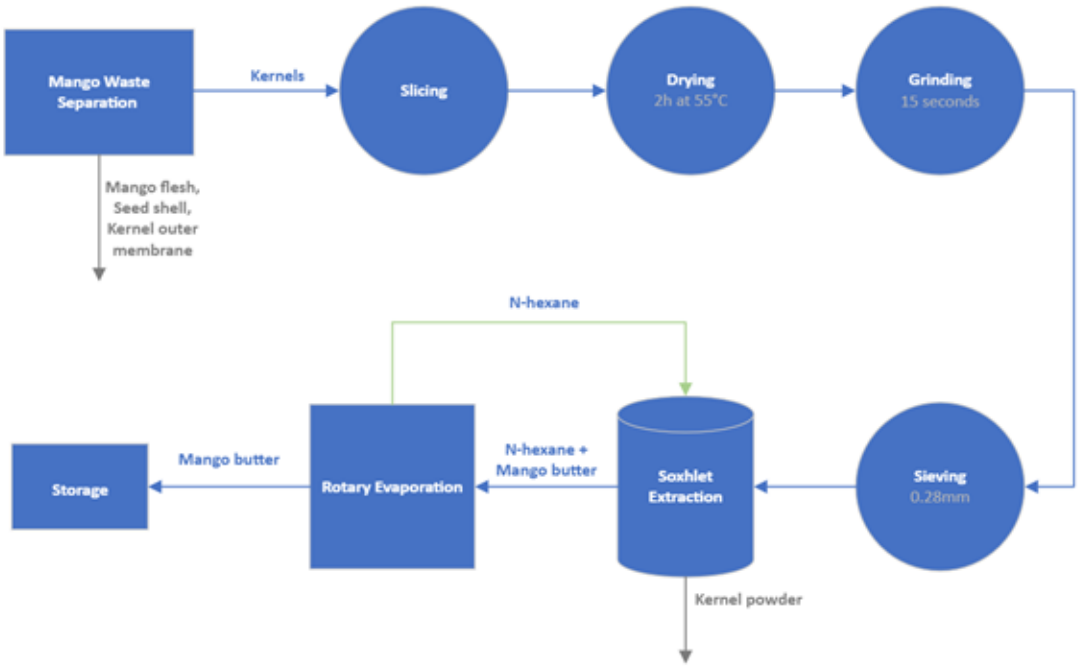


Figure 3.2: Block Flow Diagram of the Soxhlet Extraction.

In the figure above, the BFD of the whole process involving the pretreatment of the mango kernels, the Soxhlet extraction, and the rotary evaporation is presented. The same process was repeated for all the mango kernel types. During the rotary evaporation, the solvent n-hexane remains as a by-product, and it is collected and reused in the next Soxhlet extraction in order to make the process more sustainable.

Chapter 4
Experimental Results

4.1 Mango Waste Separation

4.1.1 Fresh Mangoes (First Batch)

In the Figure 4.1 below, the mango waste received from the juice company is shown. The mangoes were identified as Kent variety based on their green-yellow peel color with a bright red blush, which is a characteristic of ripened fruit. [17] After the separation of the flesh, seeds, and kernels of 10 mangoes, it was estimated that approximately 71% of the processed discarded mangoes is edible flesh, while about 15% of the whole (wasted) fruit was mango kernel Table 4.1.

Figure 4.1: Separation process of mango waste a) Waste mangoes received from "Vezet", first batch; b)Separation of the kernel from the seed; c)Drying the slides mango kernels d)Dried mango kernel powder, ready for extraction

Table 4.1: Mango separation values and relations to the whole mango fruit (waste) (10 mangoes)

	Total weight	**Flesh**	**Seed**	**Kernel**
Average (g)	**193.44**	**137.11**	**53.97**	**28.93**
Relation to the Total (%)		**71%**	**28%**	**14.84%**

4.1.2 Fresh and Boiled Mangoes (Second Batch)

The second delivery of mangoes, similar in size and appearance to the first batch, was also identified as the Kent variety. However, these mangoes were less ripe, making the separation of the flesh and kernel more challenging, requiring more strength.

4.1.3 Kesar and Alphonso Mango Kernel Separation

The Indian store bought Kesar and Alphonso mangoes' kernels were extracted fresh. The shell opening of both these varieties was much more challenging than the Kent variety. It required the use of a knife, a nutcracker, and took approximately 5 minutes to open one kernel, since the seed shells were very small and resilient. As it can be seen in the figures below, in comparison to the Kent variety, the Kenso type was about 70% of the size of Kent and the Alphonso type about 50%, furthermore, the Kesar mangoes were slightly longer and easier to open than the Alphonso type. The kernel behavior differed as well, with the Kesar type being similar to Kent and the Alphonso type being stickier.

Figure 4.2: Kesar and Alphonso Mango Kernel Separation.

4.2 Drying

The drying process of the mangoes was carried out using a dryer available in the university kitchen. All mango varieties were dried at $55°C$ for **2 hours**. The moisture content of the fresh Kent mango kernels before drying was estimated to be 51.25%, which dropped to 8.04% after drying, and this is comparable to literature. [18]

Other drying methods used were air drying of thin sliced mango kernels, which took about 3-4 days of air drying to be completely dry. Air drying of whole kernels was attempted as well, however, the drying of the kernels took much longer, approximately 2 weeks, with the risk of molding if not properly ventilated. Moreover, drying whole kernels in a dryer did not yield promising results, as the pits remained wet to touch after drying for 4 hours at $55°C$. Further, it was noticed that the kernel powders have different colors as can be seen on Figure 4.3

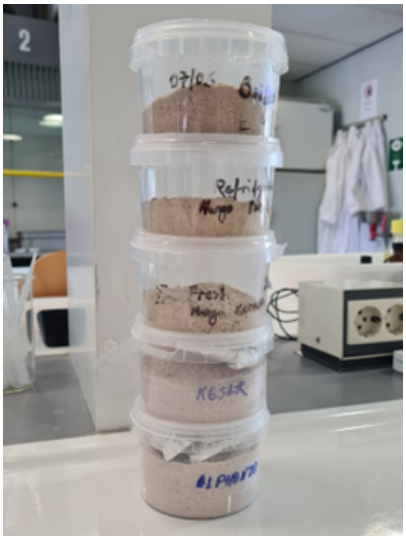


Figure 4.3: From top to bottom: Boiled, Refrigerated, Fresh, Kesar, Alphonso mango kernels.

4.3 Rotary Evaporation and Solvent Recovery

During the rotary evaporation, it has been estimated that the initial extraction temperature to be 35 $^{\circ}C$, since during the **1.1** and **1.2** the temperature was around 56 $^{\circ}C$, which caused extreme boiling and part of the solvent/butter mixture escaped into the flask containing only the condensed. Therefore, a slow increase of the water bath temperature was found to be successful in removing the n-hexane only, and the total removal takes approximately 15 minutes.

Figure 4.4: Rotary Evaporator.

Moreover, the solvent, n-hexane, was recycled after every Soxhlet extraction, with an estimated 80% recovery rate of the solvent. The remaining 20% was likely lost during the Soxhlet process.

4.4 Soxhlet Extraction

4.4.1 Fresh Mango Kernels (First Batch)

- Comparing extraction with cotton wool and extraction with thimble

A comparison was made between extraction with cotton wool and extraction with a thimble. Table 4.2 demonstrates the results obtained from the Soxhlet extraction 1.1 and 1.2 for which cotton-wool was used as a filter to separate the kernel powder from escaping into the round bottom flask of the Soxhlet, and Table 4.3 shows the amount obtained after using the thimble as a filter. In Figure 4.5, the difference between the two types of extracts is shown, and it can be seen that the sample where the thimble was used is a much clearer color.

Table 4.2: Mango butter extract from a cotton wool Soxhlet apparatus.

	Kernel powder (g)	**Solvent (ml)**	**Extractio time (h)**	**Extracted butter (g)**	**Extracted butter: Kernel (%)**
1.1				**1.3352**	6.68
1.2	20	300	6	**0.973**	4.86

Table 4.3: Mango butter extract from a thimble Soxhlet apparatus.

	Kernel (g)	**Solvent (ml)**	**Extractio time (h)**	**Extracted butter (g)**	**Extracted butter: Kernel (%)**
2.1				**1.0781**	5.39
2.2	20	300	6	**0.969**	5.12

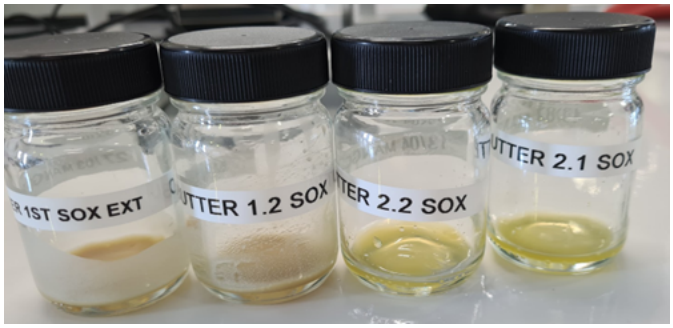


Figure 4.5: Mango butter Extraction with Soxhlet with Cotton Wool (1.1, 1.2) and Soxhlet with thimble (2.1, 2.2).

- Soxhlet extraction 3.1 and 3.2 of fresh dried mango kernels (first batch) for 4h

An attempt was made to try an optimization of the extraction time, since it was noticed that most of the butter is being extracted during the first 2 hours of the Soxhlet process, therefore, the extraction time was lowered to 4 hours. The results are two very different values Table 4.4, 0.889g and 1.656g of mango butter. Also, the difference of the two sample measurements of the "right after extraction" and "a few days later at room temperature" (3.1 = 0.03g and 3.2 = 0.194g) is probably the remaining solvent in the sample being evaporated.

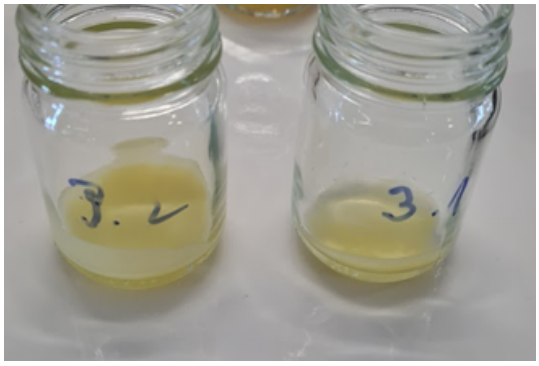


Figure 4.6: Mango Butter from Soxhlet Extractions 3.1 and 3.2.

Table 4.4: Results from the extraction of fresh Kent kernel butter for 4 hours.

	Kernel powder (g)	**Solvent (ml)**	**Extractio time (h)**	**Sample after extrac-tion (g)**	**Sample after time at room temp. (g)**	**Extracted butter: Kernel (%)**
3.1	20.123			**0.919**	**0.889**	4.41
3.2	20.1604	300	4	**1.85**	**1.656**	8.18

4.4.2 Boiled Mango Kernels (Second Batch)

The results from extraction 5.1 and 5.2 made from boiled mango kernels, as illustrated in figure 4.7 below, are different from the other extracts in terms of color, being slightly darker yellow. Sample 5.1 was liquid at room temperature, whereas sample 5.2 was solid. Also, after the extraction was done the solvent/butter mixture of 5.1 appeared very foggy-yellow, while 5.2 was slightly clearer.

Figure 4.7: Mango Butter from Soxhlet Extractions 5.1 and 5.2.

Table 4.5: Results from Boiled Kent Mango Kernel Extraction.

	Kernel powder (g)	Solvent (ml)	Extractio time (h)	Sample after extrac-tion (g)	Sample after time, at room temp. (g)	Extracted butter (after) : Kernel (%)
5.1	20.3402	300	4	**1.522**	**1.2976**	6.38
5.2	20.2854			**1.12**	**1.0488**	5.17

4.4.3 Soxhlet Extraction of Alphonso (6.1) and Kesar (7.1) mango butter for 4h

The extracts from both Alphonso (6.1) and Kesar (7.1) mangoes were similar in quantity and had a similar pale-yellow color. Additionally, the solvent/butter mixture of sample 7.1 (Kesar) was very clear with a tint of yellow, while sample 6.1 (Alphonso) was slightly blurry with the same color. The kernel scent of both butters was similar and slightly stronger than the rest.

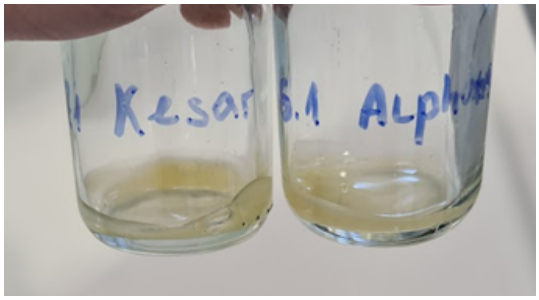


Figure 4.8: Mango butters from Kesar (7.1) and Alphonso (6.1) mango types

Table 4.6: Results from the extraction of Alphonso mango kernel butter (6.1).

Kernel powder (g)	Solvent (ml)	Extractio time (h)	Sample after extrac-tion (g)	Sample after time, at room temp. (g)	Extracted butter: Kernel (%)
20.484	300	4	**1.6694**	**1.641**	8.01

Table 4.7: Results from the extraction of Kesar mango kernel butter (7.1)

Kernel powder (g)	Solvent (ml)	Extractio time (h)	Sample after extrac-tion (g)	Sample after time, at room temp. (g)	Extracted butter: Kernel (%)
20.108	300	4	**1.7044**	**1.6228**	8.07

Figure 4.9: All the extracted mango kernels butters from bottom right to left (1.1, 2.1,... 7.1).

Chapter 5
Discussion

Mango Waste Separation

From the first mango delivery, the extraction of the kernel from the hard shell was relatively easy and possible from the more fibrous side of the pit, by pressing with a thumb or a hard thin object and pulling from both sides of the shell. The flesh was easy to remove by a knife, but time consuming, therefore if a scale-up of the process is planned an effective way of separating the flesh should be implemented.

Moreover, the " boiled" mangoes were as a result of boiling half of the mango waste from the second delivery, so that an alternative mango flesh separation can be tested. As a result, the mango flesh was easily separated from the seed by scraping with a spoon. Additionally, two hours of boiling made it easier to remove the flesh compared to one hour of boiling, however, the energy consumption is higher and if additional use of the flesh is not intended, the boiling may be costly. On the other hand, if the mango flesh is planned to be used as well, the boiling step can be a good option, since it will help remove the flesh from the seed and at the same time be a pasteurization step for the flesh processing.

Rotary Evaporator

It is important to leave the butter to be extracted for an additional 5 minutes after all the visible n-hexane is removed. The last amount of the trapped solvent takes longer to evaporate, so a good suggestion would be to stop the vacuum pump of the evaporator and let air in, then close the air valve again and turn on the rotary evaporator with the vacuum, in other words restart the process. This was noticed to work for the extraction of the remaining parts of the solvent.

Soxhlet Extractions

It was noticed that during the first Soxhlet extractions (1.1 and 1.2) that the mango butter obtained had a darker color, indicating impurities. A reason for the impurities was thought to be the cotton-wool used as a filter, to prevent the mango kernel powder from escaping into the solvent flask. Whereas, in the second extractions (2.1 and 2.2) the filtration was done using a thimble, which has a smaller pour size and prevents the fine solid particles from leaving the extraction chamber. Furthermore, the volume of the samples in the first extraction (1.1) is slightly higher (6.7%) from the rest (about 5%), the error is thought to be the high amount of solvent that remains with the extracted butter, due to lower evaporation time with the rotary evaporator. Additionally, initially the 1.1 was in a liquid form at room temperature, as opposed to the rest (1.2, 2.1 and 2.2), which were in solid form due to solvent present. As a result of the two kinds of extractions it was concluded that the thimble is a better filtration method than the cotton-wool and it was used for the following extractions.

For the extractions 3.1 and 3.2, the extraction time was 4 hours, 2 hours shorter than the previous ones. The results from 3.1 (4.57% butter from 20g of kernel powder) showed that the amount is slightly lower that the 2.1 and 2.2 (5.39% and 5.12%) showing that longer extraction time is more yieldy. However, the results from 3.2 gave much higher results (9.18%), which might be due to the presence of a lot of solvent and it is probably not correct when compared to the previous results.

Boiled mango kernel butter 5.1 and 5.2 has a darker color from the rest of the extracts (with thimble), this might be because during boiling (100°C) the high temperatures might have affected some of the chemical and physical properties of the kernel. The 6.1 Alphonso and 7.1 Kesar mango butters have a lighter color that the rest of the extractions, maybe their fatty acid composition has some differences than the Kent variety. On Figure 4.9 the difference in color can be easily seen. Furthermore, both varieties have 8% butter yield from 20g of kernel powder, concluding that the fat content in Alphonso and Kesar is higher than Kent. Judging by the study of Ohale,

P.E. et al., about "Solvent extraction of oil from three cultivars of Nigerian mango seed kernel: Process modeling, GA - optimization, nonlinear kinetics and comparative characterization"[19], about 16% of Alphonso mango kernel butter can be extracted by Soxhlet extraction.The iodine number of Alphonso is estimated to be 13.42 gI2/100g, compared to the iodine number of Kent variety 51.08g I2/100g of fat [18], meaning that Kent mango butter has a higher number of unsaturated fatty acids than Alphonso, making it more susceptible to oxidation and faster degradation. Information about the characteristics of Kesar variety butter was not found. Additionally, the difference in the sample weights is not very high, leading to the conclusion that solvent is present in low concentrations in the butter, therefore, the 15 minute rotary evaporation has successfully removed most of the n-hexane.

The color deviation from the other samples shows that the mango butter is dependent on the kernel powder coloring, as well, it can be seen from Figure 4.3 and Figure 4.9 the butter turned out almost the same color as the mango kernel powder it is extracted from.

Analysis

Analysis of the mango butter extractions was not possible due to limited laboratory equipment, further the butter samples were relatively small to perform peroxide value, iodine number, and saponification value determination tests by titration and the time was limited to extract larger amounts. Possibly, DSC analysis can be made for determination of the melting point of the butters, which might give a clear insight of the fatty acid composition and whether the obtained products are pure, depending on the range of melting.

Chapter 6
Conclusion and Recommendations

In conclusion, this graduation internship thesis was done to answer the research question: What is the most effective and sustainable way of mango butter extraction by the Soxhlet extraction method?, also to estimate the oil quantity of Kent mango waste, derived from the juice processing company "Vezet" in order for it to be used as a cosmetic product by the start-up "Natural Waste".

The project successfully utilized discarded mango seeds to produce mango butter while emphasizing sustainability. Through the separation of mango waste components and optimization of the Soxhlet extraction process, important insights were obtained. It was concluded that the use of a thimble as a filter for the Soxhlet proved superior to the cotton-wool filter. Moreover, longer extraction time of 6h resulted in higher butter yield that 4h of extraction. The maximum amount that was obtained was 1.65g of mango kernel oil, 8.18% out of 20g mango kernel powder extracted in 4 hours, which might be due to higher solvent concentrations in the product. However, further investigation and experimentation is needed to validate these results.
The experiments also revealed differences of color among different mango varieties, possibly indicating differences in the fatty acid composition or in the physicochemical characterization. Additionally, the Alphonso and Kesar varieties showed higher butter yields (8%) compared to the Kent variety (6%). The higher iodine number of Kent mango butter indicated higher unsaturated fatty acids, making it more susceptible to oxidation and faster degradation. Even though a full answer to the research question was not done.
For further research, it is recommended to test more times the time optimization of the Soxhlet extraction as well as the solvent/mango kernel use. Furthermore, I would recommend trying the ultrasound-assisted and microwave-assisted extraction with a solvent, so as to achieve a more sustainable process. The solvent/butter separation process can be researched more and analyzed for full removal of the solvent. Last but not least I would suggest a full analysis of the physicochemical characteristics of the butter Overall, this project provided practical knowledge on mango butter extraction and highlighted the importance of sustainability and circular economy in the industries.

Chapter 7
Appendix

Appendix A - Protocol 1: Mango butter extraction by Soxhlet apparatus using n-hexane with thimble

Materials

- Mango kernel powder (100g) 20g per extraction
- Soxhlet extraction apparatus
- Round bottom flask
- Heating mantle
- Weighing scale
- Thimble for the soxhlet chamber or cotton wool
- Tweezers
- Condenser or rotary evaporator

Chemicals

- n-Hexane - 500mL, 300mL for extraction

Procedure

1. Grind the dried mango kernels into a fine powder using a blender or coffee grinder.
2. Weigh 20 g of the grinded mango kernels and transfer them into the Soxhlet thimble in the extraction chamber (if a thimble is not available use cotton-wool to enable the mango powder to escape from the chamber).
3. Fill the round-bottom flask with 300ml of n-hexane. (15ml/g of grinded mango kernels)
4. Assemble the Soxhlet extractor with the round-bottom flask, condenser, and heating mantle.
5. Start the extraction process by heating the round-bottom flask $67^{\circ}C$. with the hexane to boiling point. As the hexane boils, it will vaporize and travel up the condensate, where it will cool and condense back into a liquid, dripping onto the powdered mango kernels. The liquid will dissolve the mango butter from the kernels and will drip back into the round-bottom flask.
6. After about 6 hours of extraction, turn off the heat and remove the Soxhlet extractor from the round-bottom flask.
7. Evaporate the hexane from the extracted liquid by heating it gently with a heat source such as a rotary evaporator and collect it in an appropriate glass jar for further reuse.
8. The remaining substance will be the mango butter extracted from the mango kernels.
9. Collect the mango butter and store it in a container.
10. Rinse the flask 2-3 times with ethanol or acetone then rinse 3-4 times with deionized water.

Appendix B - Protocol 2: Iodine determination of lipids

Materials

- Balance
- Burette
- Spatula
- Filler
- Pipette
- Filter paper
- Funnel
- Beaker
- Hot plate

Chemicals

- Starch Soluble
- Carbon Tetrachloride
- Potassium Iodine
- Wijs solution
- Sodium Thiosulfate

Procedure

Reagent preparation

1% starch solution For the preparation of 1% starch solution measure 50 ml of distilled water and take it in a 50 ml beaker. Place the beaker on a hot plate and heat the water to boil Take the weight of 0.5 gram of starch soluble and transfer into the boiling water. Stir the solution with a cleaned glass rod while boiling to dissolve the starch in water. After dissolving the starch, filter the solution immediately using a filter paper, collect the filtrate and use it in the test as a 1% starch indicator.

- 0.1N Sodium Thiosulfate

For the preparation of 0.1 N sodium thiosulfate add 2.5 grams sodium thiosulfate in 80 ml distilled water. Shake the flask to dissolve the chemical in water. Heat the solution to dissolve the crystal in water completely Cool the solution and add enough distilled water to make the final volume of 100. Standardize the solution.

Sample preparation

Take around 0.5g of mango butter. Note the sample weight. Prepare a Blank IV flask without taking a sample into it. Pipette 25ml Carbon Tetrachloride and pour it into the sample flask and close the flask with its lid immediately. Pipette another 25ml Carbon Tetrachloride into the blank flask and close it with a stopper immediately. Pipette 2x25 ml Wijt's Solution and pour it into the both flasks. Shake both flasks . Add Potassium Iodine crystal around the surface of the stoppers. Keep the flasks in the dark for 30 minutes.

- Titration of sample

Take 0.1N sodium thiosulfate solution in a burette. Take the initial burette reading Add 100 ml distilled water into the sample flask, washing the stoppers and mix well. Measure 1 ml of 1% starch solution and keep ready to use later in the titration. Start titration using 0.1N Sodium Thiosulfate. When the color changes to a lighter one add the pre-measured 1% starch solution. Resume the titration with vigorous mixing of the flask. Milky white color indicates the end point of the titration. Shake the flask for 30 seconds to ensure that the white color is unchanged. If the blue color appears again, titrate again. Note the final burette reading.

- Titration of Blank

Do the titration of the blank as the same as the sample. Add 100 ml distilled water into the sample flask, washing the stoppers and mix well. Measure 1 ml of 1% starch solution and keep ready to use later in the titration. Start titration using 0.1N Sodium Thiosulfate. When the color changes to a lighter one add the pre-measured 1% starch solution. Resume the titration with vigorous mixing of the flask. Milky white color indicates the end point of the titration. Shake the flask for 30 seconds to ensure that the white color is unchanged. If the blue color appears again, titrate again. Note the final burette reading.

Calculations

Iodine value =

$$\frac{12.69 \cdot (Vb - Vs) \cdot N}{W_s}$$

Sample weight (Ws) =
Normality of Sodium Thiosulfate (N) = 0.01N
Volume of Sodium Thiosulfate for Blank (Vb) = Final burette reading - Initial reading
Volume of Sodium Thiosulfate for Sample (Vs) = Final burette reading - Initial reading

Appendix C - Protocol 3: Peroxide Value Determination

Materials

- Beakers 50, 100ml
- Magnetic hot plate
- Glass roth
- Pipettes (1 ml, 5 ml, 10 ml, 20 ml)
- Measuring cylinders (25 ml, 100 ml)
- Stop watches
- Microburette (2 ml)
- Burette (50 ml)
- Erlenmeyer flasks (100 ml, 200 ml) with stoppers
- Balance with at least 0.1 g sensitivity

Chemicals

- Chloroform
- Acetic acid
- Starch Soluble
- Potassium Iodine
- Sodium Thiosulfate

Procedure

Acetic acid: chloroform 3:2

First label a reagent bottle acetic acid and chloroform should be mixed in a ratio of 3:2. Measure 90ml concentrated Acetic acid and add it into a pre-labeled 300ml bottle Measure 60ml chloroform and add it into the bottle.Mix well.

1% starch solution

50ml distilled water Take it to a beaker and heat to boil Weight 0.5 starch soluble and add it to the boiling water Stir with a glass rot and boil until a transparent solution is obtained Filter the solution while still hot, using a filter paper (30 min) Ready to use.

0.01N Sodium Thiosulfate solution preparation

Dissolve 0.25g Sodium Thiosulfate in 80 ml distilled water and add 0.02g sodium carbonate. Mix well by shaking If necessary heat the solution to dissolve the SOdium Thiosulfate and cool at room temperature Add distilled water to reach 100 ml volume Standardize the prepared solution with Potassium Dichromate

Saturated Potassium Iodine solution

Label a test tube with "Saturated KI" Pour 2 ml distilled water into the test tube. add potassium iodide crystal by a spatula and shake to dissolve the potassium iodide in water. Continue adding potassium iodide until the sediment of the potassium iodide is left in the bottom of the test tube.

Sample Preparation

Take 10 to 12 grams of oil sample in an erlenmeyer flask. Note the sample weight. Measure 30 ml Acetic acid Chloroform mixture and pour into the erlenmeyer flask containing the oil sample. Shake and rotate the flask to mix the sample with the chemical mixture. Now add 1 ml saturated potassium iodide in the same flask shake and rotate the flask clockwise and anti-clockwise for 1 minute to make a homogenous mixture. Now add 30 ml distilled water into the flask and shake the flask again for one minute. Mix well.

Titration

Take the 0.01N Sodium Thiosulfate in a burette. Take the initial burette reading. Add 0.5 ml of 1% Starch solution. Mix well by shaking. Start titration. Titration should be carried out with vigorous agitation of the flask to get the accurate results to stop the titration when the solution color is changed into yellow- white. Note the final burette reading.

Calculation

Sample Weight (Ws) =
Normality of $Na_2S_2O_3$ (N) = 0.01N
Volume of Na2s2O3 (V) = Final burette Reading - Initial Burette Reading
Peroxide Value =

$$\frac{V \cdot N \cdot 1000}{W_s}$$

Appendix D - Protocol 4 - Standardization of Sodium Thiosulfate - 0.01N Na2S2O3

Materials

- Beakers 50, 100ml
- Magnetic hot plate
- Glass roth

- Pipettes (1 ml, 5 ml, 10 ml, 20 ml)
- Measuring cylinders (25 ml, 100 ml)
- Stop watches Microburette (2 ml)
- Burette (50 ml)
- Erlenmeyer flasks (100 ml, 200 ml) with stoppers
- Balance with at least 0.1 g sensitivity

Chemicals

- Hydrochloric acid about 37%
- Potassium dichromate 99.5% (NA)
- Sodium Thiosulphate (Pentahydrate) 99%
- Potassium iodine crystals
- Starch soluble

Procedure

1% starch solution preparation

Measure 50 ml d water into a beaker. Place the beaker on a hot plate and boil the water. Weight 0.5g of Starch soluble. Transfer the starch into the hot water. Stir the solution and boil until everything is dissolved. Filter the solution while still hot. Transfer the solution into a beaker. It is ready for use.

15% Potassium Iodine solution

Dissolve 15 grams potassium iodide crystal (KI) into 100 ml distilled water. Swirl to dissolve the crystals completely. Store in the dark and use immediately after preparation.

0.01N Na2S2O3 solution preparation:

Weight 2.48 grams of sodium thiosulfate crystal into 100 ml volumetric flask fill the flask with distilled water up to the mark. Record the measured amount. Mix to dissolve the crystals. Label the flask as "Expected 0.1 molar sodium thiosulfate"

Potassium dichromate solution K2Cr2O3: In a fume cupboard

Transfer a spoon of the crystals into a glass dish. Dry the potassium dichromate crystals at 110 degrees celsius for 30 minutes. After drying, cool the potassium dichromate in a desiccator. Take 0.16 to 0.22 gram of dried potassium dichromate crystal into a conical flask and add 25 ml distilled water. Swirl the flask to dissolve the crystals. Now add 5 ml concentrated hydrochloric acid into the flask. Add 20 ml of 15% potassium iodide solution into the flask, swirl the flask again and wait for 5 minutes. Add 100 ml distilled water and swirl the flask to mix the content properly.

Sodium Thiosulphate Solution Standardisation

For the standardization take the newly prepared sodium thiosulfate solution in a buret. Record the initial burette reading. Titrate the potassium dichromate solution by liberating sodium thiosulfate solution from the burette. Carry out the titration until the yellow color has almost disappeared. Pause the titration when the yellow color fades out. Now add 1 ml of 1% Starch solution into the flask and resume the titration. Swirl the flask to mix the content. Resume the titration. Stop the titration when the blue color (when it turns green). Record the final burette reading.

Calculations

Weight of Potassium dichromate

(W) = (g) Volume of Sodium Thiosulfate solution

(V) = Final Burette Reading - Initial Reading
Molarity of the sodium thiosulfate solution (M)

$$M = \frac{20.394 \cdot (W)}{V}$$

Label the flask with the actual molarity.

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