Mangan Chakku Nanditha

Extraction and Characterization of Oils from Selected Exotic Fruit Seeds

UNIVERSIDADE DO ALGARVE
INSTITUTO SUPERIOR DE ENGENHARIA

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Mestrado em Tecnologia de Alimentos

Trabalho efetuado sob a orientação de:

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[Candidate’s signature goes here]

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Resumo

A alimentação é uma das necessidades básicas da humanidade e a exigência de alimentos aumenta paralelamente com o aumento da população que dá origem a um aumento da agricultura, produção alimentar e transformação de alimentos gerando uma elevada quantidade de resíduos e sub-produtos.

O aumento diário dos subprodutos alimentares tornou-se um dos principais fatores para o colapso do equilíbrio de sustentabilidade que deu origem a novas legislações de gestão de resíduos, permitindo assim encontrar novas técnicas para protocolos de gestão de resíduos rentáveis e valorizar o seu elevado potencial económico.

Embora a prevenção, redução, reciclagem, recuperação e reutilização dos subprodutos alimentares seja o principal objetivo da legislação da UE em termos de resíduos e plano de ação para a economia circular, a disponibilização de valores eco-conscientes cientificamente comprovados pelos subprodutos alimentares, estes atualmente são o principal alvo da valorização nas indústrias alimentares..

Os investigadores têm-se centrado na valorização verde dos subprodutos vegetais para obter várias vantagens, como a extração de produtos dietéticos, nutricionais, farmacêuticos e industrialmente valiosos, mercados verdes com novas oportunidades de emprego, uma alternativa aos combustíveis fósseis, redução das emissões de gases com efeito de estufa e redução do fosso entre a produção e consumo de alimentos.

As crescentes preocupações dos consumidores com a saúde tem provocado um aumento do consumo de frutas nas suas refeições diárias. A produção de frutas exóticas mostra um aumento contínuo com um crescimento anual previsto de 3% que será de 116 milhões de toneladas até ao ano 2024 devido às suas vantagens em termos de saúde e nutrição em relação aos frutos temperados, e os subprodutos de frutas exóticas têm sido preocupados devido à possibilidade de uma fácil recuperação de compostos bioativos benéficos, especialmente óleos saudáveis e gorduras que têm potencial a nível dietético, aplicações industriais e nutricionais, medicinais e farmacêuticas. O contributo das sementes de frutos exóticos é superior ao dos frutos produzidos em climas temperados, contendo uma grande parte significativa não utilizada que justifica o esforço de valorização.

Annona, manga e papaia estão entre as frutas exóticas mais consumidas globalmente e encontram-se numa fase de crescente produção devido à preferência demonstrada pelos consumidores mundiais. A fração do peso do fruto representada pelas sementes é de 13%, 10-85%, e de 6,5 a 20% para annona, manga e papaia, respetivamente. As sementes de annona, manga e papaia são ricas em ácidos gordos insaturados, aminoácidos e minerais.

O óleo de semente de annona é semi-seco à temperatura ambiente com aplicações funcionais e farmacológicas comprovadas. E é uma fonte potencial para a indústria do

biodiesel. Por outro lado, o óleo de miolo de manga - um semissólido de cor amarela dourada a castanho à temperatura ambiente - é um óleo comestível com elevada estabilidade e propriedades antimicrobianas e anti-oxidantes que tem aplicações na indústria de cuidados cosméticos e de pessoal, indústria alimentar e de confeitaria, bem como na indústria de cuidados de saúde e farmacêuticos, bem como na indústria de biodiesel. Finalmente,o óleo de sementes de papaia – um cor castanho-avermelhado líquido à temperatura ambiente – é um óleo comestível com elevada palabilidade e com aplicações nas indústrias de plástico e biodiesel.

A extração de óleos é uma forma corrente de valorização das sementes oleosas. A introdução de novas técnicas de extração “verde” decom abordagens de refinaria e biorefina proporciona uma recuperação fácil e valiosa de compostos funcionais. O pré-tratamento das sementes, o método utilizado e a otimização dos parâmetros associados são factores-chave do rendimento e da qualidade dos óleos extraídos. O pré-tratamento pode ser conjugado com novas técnicas de tratamento, tais como ultrassons, micro-ondas e aplicações de pressão às sementes antes do processo de extração para uma extração mais exequível e valiosa. E ultrassom, micro-ondas e pressão podem ser associados á obtenção de melhores resultados. Mas o desenvolvimento, a escala e a comercialização da combinação destes métodos para um desempenho mais elevado e melhor ainda requerem mais pesquisas a serem realizadas. No processo de extração de solventes, devem ser tomados em consideração solventes solventes orgânicos solúveis, tais como clorofórmio, benzeno, acetona, hexano e cicloxexano para romper as paredes celulares e a solubilidade, ponto de ebulição, toxicidade, disponibilidade, custo, polaridade e reutilização do solvente adequados para escolher o solvente adequado.

Para impulsionar a economia circular, o conceito de desperdício zero, e o conceito eco verde, foi estudada a possibilidade de recuperar compostos bioativos através da extração de óleos de sementes de frutos exóticos selecionadas (annona, manga e papaia) com um rendimento razoável de forma rentável. Os óleos foram extraídos no aparelho Soxhlet utilizando éter de petróleo como solvente de acordo com o método AOAC 948.22 (AOAC 2000) e a extração foi efetuada durante 9 horas. Os rendimentos do óleo obtidos foram de 28,9 ± 1,6% para as sementes de annona, 5,0 ± 0,0% para as sementes de manga e 24,0% ± 0,9% para as sementes de papaia. Neste estudo, os rendimentos dos óleos das sementes de annona e papaia obtidos encontram-se na gama que foi relatado na literatura, mas o rendimento do óleo de semente de manga foi menor do que o mencionado por outros autores.

Os óleos extraídos foram esterficados e os perfis de ácidos gordos (FA) foram estudados através da cromatografia gasosa-espectrometria de massa (GS-MS) equipada com uma coluna capilar. A GS-MS é uma ferramenta analítica multidimensional com elevada seletividade, sensibilidade e precisão que pode ser aplicada para analisar matrizes complexas e é amplamente utilizada na deteção e identificação de ácidos gordos. O teor total de ácidos gordos foi de 76,7%, 76,9% e 56,1% para as sementes de annona, manga e papaia, respectivamente. Os ácidos palmítico (17,68%), oleico (42,39%) e linoleico

(30,22%) foram os principais ácidos gordos no óleo de sementes de annona. Os ácidos linoleico (30,30%), oleico (25,00%), palmítico (22,01%) e esteleórico (14,62%) destacaram-se no óleo de manga, enquanto o ácido de oleico (66,52%) e o palmítico (15,2%) foram os ácidos gordos maioritários nas sementes de papaia.

O efeito da origem geográfica dos frutos e a presumível influência do solo e das condições climáticas, bem como o impacto do pré-processamento e as condições de pós-extração no rendimento de óleo, perfil de ácidos gordos e de componentes bioativos devem ser estudados mais detalhadamente. No entanto, a comercialização, aceitação pelos consumidores, a sustentabilidade do processo e os desafios práticos no processo não foram estudados, mas devem ser analisados experimentalmente para posterior eventual comercialização destes óleos com características especiais.

Palavras-Chave

Sementes exóticas de frutas, Annona, Manga, Papaia, Extração de Óleo, Perfis de ácidos gordos

Abstract

Food is one of the basic needs of humankind and the requirement of food increases parallelly with the increase of population, thus giving rise to an increase in agriculture, food production, and food processing generating a heap of wastes and byproducts. The daily increase of food byproducts has become one a major issue when striving for food processing sustainability, demanding “new” legislation related to wastes management as well as requiring the study and development of new or alternative techniques for costeffective waste management protocols, that recapture its economic value. To value this effort, the possibility of recapturing bioactive compounds by extraction of oils from Annona, mango, and papaya seeds at a reasonable yield level in a cost-effective manner have been studied. Oils were extracted in the Soxhlet apparatus using petroleum ether as the solvent according to AOAC 948.22 method (AOAC 2000) and extraction was carried out for 9 h. The oil yields obtained were 28.92% ± 1.6% for Annona seeds, 5.00 % ± 0.004% for mango seeds, and 24.85% ± 0.90% for papaya seeds. The oils have been esterified and fatty acid (FA) profiles have been obtained through Gas ChromatographyMass Spectrometry (GC-MS) equipped with a capillary column. The total fatty acid content was 76.67%, 76.99%, and 56.11% for Annona, mango, and papaya seeds, respectively. Palmitic (17.68%), oleic (42.39%), and linoleic (30.22%) acids were the main fatty acids in Annona seed oil. Linoleic (30.30%), oleic (25.00%), palmitic (22.01%), and stearic (14.62%) acids were prominent in mango seed oil, while oleic (66.52%), and palmitic (15.2%) acids were important in papaya seed oils. The effect of the geographical origin of the fruits, and putative influence of soil and climatic conditions as well as the impact of preprocessing extraction, and post-extraction conditions on the oil yield, fatty acids profile, and bioactive components should be studied further. Moreover, since the marketability, consumer acceptance, sustainability, and practical challenges in the process have not been studied, they should be addressed for further conclusions and eventual commercialization

Keywords

Exotic Fruit Seeds, Annona, Mango, Papaya, Oil Extraction, Fatty acids profiles

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AOAC: Association of Official Agricultural Chemists
AS: Annona Seeds
FA: Fatty Acid
FAME: Fatty Acid Methyl Ester
GS-MS: Gas Chromatography-Mass Spectrometry
MS: Mango Seeds
PS: Papaya Seeds
PUFA: Polyunsaturated Fatty Acid
SFA: Saturated Fatty Acid
UFA: Unsaturated Fatty Acid

Chapter 1
Introduction

The world population is facing the problems of depletion of natural resources, energy crisis, pollution, and climate change that should be addressed immediately. Finding proper and accelerated ways for sustainable development has become a priority of all nations (Arancon et al., 2013). Agriculture has become one of the major sources to reduce worldwide poverty, hunger, and malnutrition guaranteeing adequate nutrition and health (Duque-Acevedo et al., 2020). With the increasing requirements, soil expansion for agricultural uses, and innovative technical contribution for good productivity, the average daily food production has been increased dramatically over the last fifty decades creating negative impacts on the environment subsequently affecting health and sustainability, and agriculture has become one of the highest biomass production sectors becoming a major input for the bioeconomy (Duque-Acevedo et al., 2020). Food wastes occur due to poor management, inadequate infrastructure, lack of knowledge, and unfavorable weather conditions resulting in a lack of resources, reduced food insecurity, and negative environmental impacts (Ebikade et al., 2021). While the prevention, reduction, recycling, recovery, and reuse of food wastes is the main purpose of the EU Waste Legislation and Circular Economy Action Plan, providing scientifically proven eco-conscious values to food wastes is the main target of wastes valorization (Bayram et al., 2021, Faustino et al., 2019).

Though landfilling, incinerating, and composting are uprooted technologies, due to the generation of toxic gasses, bad odor, high energy consumption, and slow reaction kinetics, these methods seem to be unsatisfactory and give food waste valorization the optimal approach to environmental sustainability (Akturk et al., 2020, Arancon et al., 2013). Food byproducts are renewable resources (Akturk et al., 2020). Availability and low cost of food wastes provide more valuable and economical industrial-scale production (Selo et al., 2021). Green valorization of such byproducts is a must factor to mitigate negative environmental impacts and to motivate a sustainable bioeconomy (Sabater et al., 2020). Valorization of vegetable byproducts contributes to value-added products, green markets, new job opportunities, reduced fossil fuel usage, reduced greenhouse gas emissions (Duque-Acevedo et al., 2020), and reduced gap between production and consumption (Villacis-Chiriboga et al., 2020).

Valorization of food byproducts can be carried out in different ways under different conditions for different end products (Arancon et al., 2013) and it should be done in a manner to get the best possible benefits without making monetary losses or damaging the environment (Omre et al., 2018) with optimal sustainable applications, (Shirahigue et al., 2020) and resource efficiency (Duque-Acevedo et al., 2020).

It is reported that around 25% of the total vegetal wastes are oilseeds, tubers, and roots that provide the importance and justification for transforming it into a value-added product. Vegetal seeds, as well as peels and pomace, are good sources of bioactive compounds that can be revalued in different ways (Bayram et al., 2021).

The ever-increasing population, changing dietary patterns, and increasing medicinal and nutritional requirements supply prime importance to fruits in daily meals, and these are consumed raw or processed generating a huge amount of highly perishable organic solid wastes (Sagar et al., 2018, Pathak et al., 2017). Some tropical and subtropical fruits originate from many botanical families (Cornara et al., 2020) often called exotic fruits that are growing in warm and humid regions between the Capricorn and Cancer tropics, are prominent in beneficial bioactive compounds compared to temperate fruits (Alwarez-Rivera et al., 2021, Villacís-Chiriboga et al., 2020), and have future potential but not yet completely available in the global market with 3% predicted annual growth rate that will reach to 116 million tons production by 2024 (Villacís-Chiriboga et al., 2020). These fruits such as avocado, papaya, rambutan, jack fruit, durian, mango, orange, and annona among many others are readily available in huge quantities in some tropical and subtropical countries (Kumoro et al., 2020). and they are spreading all over the world due to their ability and global tendency to satisfy consumers' preferences for diverse tastes, exotic flavors, and seasonal availability while providing their energy, medicinal and nutritional values (Bhat et al., 2016, Dabas et al., 2013). It is a fact that ethno-pharmacological evidence reveals the use of such fruits for medicinal purposes (Dabas et al., 2013). Traditional knowledge promotes the regular intake of exotic fruits (Bhat et al., 2016) and some Asian countries include local fruits directly in their medicines. The benefits of bioactive components in exotic fruits are not limited to enhancing human immune and liver function or preventing inflammation, diabetics, chronic diseases, and obesity (Joshi et al., 2018). Exotic fruits are a good source of natural antioxidants that reduce oxidative stress (Nowak et al., 2018) thereby reducing the risk of non-communicable diseases such as cancers, chronic diseases, mental abnormalities (Pem et al., 2015), and age-related functional disorders

(Kumoro et al., 2020). And they exhibit the ability to regulate extreme nutritional conditions by preventing malnutrition as well as overnutrition (Cornara et al., 2020).

Due to the seasonal nature, it should have a way to keep the excess seasonal production to use in non-productive seasons. Fruit preservation is a widely spread industry throughout the globe, processing fruits into preserved products such as jams, jellies, juices, marmalades, dried slices, and pickles among many others leading to a great volume of byproducts. Fruit byproducts commonly consist of peels, seeds, pomaces, and cores with a large amount of water giving the feasibility to easy fermentation. If not handled correctly these byproducts cause severe environmental pollution. Valorization of these byproducts provides the advantages of controlling environmental pollution (Romelle et al., 2016), minimizing post-harvest losses (Hossain et al., 2015), and adding an economical value by waste utilization. So the concept of valorization of fruit wastes has been gained more interest throughout the world (Romelle et al., 2016).

The aspect of sustainability, green technology, and zero waste concept leads to new process techniques to find out viable ways to transform these byproducts into value-added products providing an ongoing sustainable economy (Villacís-Chiriboga et al., 2020) and due to negative health effects and environmental impacts caused by synthetic phytochemicals, the interests of having natural origin alternatives are in high demand from medicinal, nutritional, and environmental aspects (Ge et al., 2018).

Almost every fruit has lipid parts and contains oils in one or more parts which can be extracted with a commercial value. These oils can be used either as edible oils in the food industry or can be used in other industrial applications such as in the synthesis of biodiesel, pharmaceutical products, health care, and sanitary products, lubricants, resins, polyols, and polymers. The demand for both edible and industrial oils is ever-increasing (Yusuf, 2018) due to higher consumption in the food industry and biofuel industry and also due to the higher demand for green technologies (Avram et al., 2014) leading to the efficient extraction of high-quality products with a high yield or recovery (Yusuf. 2018).

It is a fact that oils and fats of vegetable sources are high in unsaturated fatty acids (Awolu et al., 2019), and fruit-based oils are highly attractive due to their high nutritional, energetic, and chemical values (Avram et al., 2014). In most cases, these oils are extracted from oily seeds with a higher interest both on industrial and laboratory scales (Yusuf, 2018) due to their fatty acid contents especially their polyunsaturated fatty acids level and other health and nutritional compositions (Sousa et al., 2018).

Papaya (genus *Carica*, family Caricaceae), mango (genus *Mangifera*, family Anacardiaceae), and annona (genus *Annona*, family Annonaceae) are among those exotic fruits that have attracted attention worldwide. Seeds, peels, and stones of these fruits are being considered non-edible and un-utilizable waste products which lead not only to an environmental problem but also to an economic loss. These are economically important fruits that carry oily seeds. All these seed oils have proven nutritional and health values and the efficient oil extraction from these exotic fruit seeds provides economical, nutritional, and health benefits (Selvamuthukumaran et al., 2017).

So in the context of the research question - Is it worth extracting oils to get a reasonable oil yield with mentioned qualities in a cost-effective and eco-friendly manner from these seeds to overcome expanding requirements of oils for dietary and industrial applications? - this study was carried out to:

1) extract oil from selected exotic fruit (annona, mango, and papaya) seeds,
2) describe resulted oils in terms of oil yield and fatty acid profile, and
3) ponder the possibility of eventually scaling up to the commercial application.

Chapter 2
Literature Review

2.1 Importance of Wastes and Byproducts of Exotic Fruits

Due to the abundance in harvesting seasons, the price of seasonal fruits falls, and the producer concerns about the overall return and tries to transform the mass into a preserved value-added product (Salim et al., 2017) to maximize the profit and to minimize wastage of surplus fruits (Kumoro et al., 2020). Around 45-52% of the fruits are wasted in this scenario as peels, seeds, and pulps in commercially attractive and ready-to-have food processing industries (Villacís-Chiriboga et al., 2021). Due to large inedible parts, approximately half of exotic fruits is discarded generating a huge mass of wastes and byproducts more than temperate fruits that can be used as side streams of income-producing nutritionally and medicinally value-added products such as oils, essential oils, phenolic compounds, flavonoids, antioxidants, xanthones, tannins, carotenoids, bromelain, papain, acids, and lycopene (Schieber, 2019).

Especially these wasted seeds are rich in bioactive components with antioxidant properties, fats, proteins, and carbohydrates (Kumoro et al., 2020), and have been reported as potential sources of food preservatives, antioxidants, food colorings, foaming agents, texturing agents, and emulsifiers, that can be transformed into commercial production (Faustino et al., 2019).

The estimated global production of major exotic fruits in 2018 was 100 million tons and as predicted, it will be 116 million tons in 2024, that generates a huge mass to be thrown into the environment, and due to the huge amounts generated and proven and confirmed high functional and nutritional importance, the valorization of byproducts from exotic fruits has become essential in research and the industry (Villacís-Chiriboga et al., 2020, Ben-Othman et al., 2020).

2.2. Characterization of Byproducts from Exotic Fruits

Byproducts are unused parts that can be converted into value-added products (Villacís-Chiriboga et al., 2020), and it is a fact that byproducts from exotic fruits have a high potential for functional and nutritional benefits (Kumoro et al., 2020). Characteristics of fruits differ from one another, and past studies reveal that dietary fiber, polyphenols, and carotenoids are the major bioactive compounds in fruits deposited in varying percentages in various parts, and in most cases, those are found in higher concentrations in such byproducts than in the edible parts (Villacís-Chiriboga et al., 2020, Ben-Othman et al., 2020). The bioactive components, volumes, activities, and functional qualities extracted vary on the type of fruit, its byproducts, part (peels, seeds, ext.) used and its nature, pretreatment, and the extraction methods, parameters, and selected solvents (Ben-Othman et al., 2020, Kafkas et al., 2018). Various types of compounds can be extracted from various types of fruit matrixes. For example, apple, orange, kiwi fruit, pomegranate, and melon peels and rinds are good sources for the extraction of pectins (Guzel et al., 2019, Pathak et al., 2017).

Annona: Annona (Figure 2.2.1) is an equatorial and semitropical fruit from the family Annonaceae with high medicinal and nutritional values that exhibit its therapeutic values and it has been gained interest as a medicinal fruit due to its anti-malarial, anti-HIV, cytotoxic, immune impressive, anti-platelet aggregation, anti-oxidant, analgesic, anti-tumor, apoptosis-inducing, anti-diabetic and anti-inflammatory properties, containing more than 200 seeds in one fruit and discarding of seeds may cause a considerable burden to the environment (Eshra et al., 2019, Pinto et al., 2018).

Annona seeds are a good source for oil extraction that is composed of health-promoting polyunsaturated and monounsaturated fatty acids. Major polyunsaturated fatty acid detected is palmitoleic acid and major monounsaturated fatty acid is oleic acid. Saturated fatty acids such as palmitic and stearic acids are also present in annona seed oil (Elagbar et al., 2016). And similarly, Annona peels are rich in phytochemicals, phenolic compounds, and antioxidants, rich in carbohydrates, proteins, lipids, and minerals, and exhibit anti-microbial and anti-cancer properties (Shehata et al., 2021).

Figure 2.2.1: General View of Annona Fruit and Seeds

Mango: Mango (Figure 2.2.2) is one of the most popular exotic fruits from the family Anacardiaceae that originates byproducts with fats, carbohydrates, proteins, and so many bioactive compounds (Mwaurah et al., 2020). These byproducts are a good source for extracting valuable phytochemicals and its seed has gallic acid and its derivatives as phenolic acids, fisetin, quercetin, and isoquercetin as flavonoids, epicatechin gallate, epigallocatechin, and epicatechin as catechins, mangiferin as xanthanoids, carotenoids, and hydrolysable tannings that provides antioxidant, anti-tumor, anti-viral, anti-bacterial, and immune-modulatory properties. The peel has antioxidant properties and therapeutical properties for some disorders such as age-related vision problems and bone-homeostasis problems (Ben-Othman et al., 2020).

Figure 2.2.2: General View of Mango Fruit, Seed, and Kernel

Mango seed is a rich source of fatty acids and the major unsaturated fatty acids reported are oleic, linoleic, linolenic, and palmitoleic acids and stearic, palmitic, myristic, arachidic, and margaric acids have been revealed as saturated fatty acids (Yadav et al., 2017).

Studies reveal that mango peels are a good extraction source of anthocyanins, pectin, and polyphenols with high antioxidant and anti-fungal properties (Villacis-Chiriboga et al., 2020, Rojas et al., 2018), and are a good source to extract flavoring compounds that can be used in the food and cosmetic industries, but undesirable extraction conditions may lead to losses of valuable volatile components (Oliver-Simancas et al., 2020). In their study, Walia et al., (2013) report that mango peels are a viable source for bioethanol production.

Papaya: Papaya (Figure 2.2.3) is the third most consumed exotic fruit (Honoréa et al., 2020) available throughout the year (Sugiharto, 2020, Sultana et al., 2020) from the family Caricaceae, with proven medicinal uses and wide acceptance all over the world. Increasing consumption and processing of this fruit creates an environmental threat due to the large volume of byproducts that should be addressed critically (Kumar et al., 2017). Extraction of phenolic compounds with good anti-bacterial, antioxidant, anti-inflammatory, cytoprotective, renoprotective, and anti-carcinogenic properties from papaya seeds and peels have been recorded. The literature shows papaya seeds as a source for protein extraction and peels as a source for dietary fiber, absorbents, biofuels, and biomedicines extraction (Dotto et al., 2021, Villacís-Chiriboga et al., 2020, Pathak et al., 2018). Papaya seeds contain around 27-28% protein, 28-30% lipids, and 19-22% crude fibers and seed oil has greater oxidative stability than any other edible oil and is mainly composed of polyunsaturated fatty acids and nutraceuticals (Kumoro et al., 2020). Unsaturated fatty acids are dominant in seed oil and oleic acid contributes to around 75% of the fatty acids providing higher importance for the valorization (Li et al., 2015).

Figure 2.2.3: General View of Papaya Fruit and Seeds

2.3. Valorization from Exotic Fruit Seeds

While fruits provide functional properties to the meal, fruit seeds are among the major byproducts thrown into the environment by fruit processing industries. As a process of valorization of fruit wastes, extraction, and refining of bioactive components rich with high nutritional and pharmacological values deposited in exotic fruit seeds has become a smart way of utilizing byproducts (Kumoro et al., 2020). Experimental work and lab-scale projects have been carried out to find out new ways for the valorization of seeds that is a must factor for a sustainable circular economy by extracting bioactive compounds such as antioxidants, polyphenols, pigments, and oils with high economical values (Araujo et al., 2020). On the other hand, exotic fruit seeds are in high interest in the energy sector because oil extraction from non-edible oily seeds that are discarded as wastes is a promising way to generate biodiesel, reducing the competition for running out fossil fuels and also reducing the environmental pollution (Sultana et al., 2020).

Annona: Annona seeds contribute to 13% of the fruit weight and show anti-microbial, insecticidal pesticidal, fungicidal (Pinto et al., 2018), anti-cancer (Shehata et al., 2021), and anti-tumor properties (Chen et al., 2016). Annona seed is a good source of carbohydrates, lipids, proteins, fibers, phytochemicals such as phenolic compounds, fatty acids (Pinto et al., 2018), tocopherols (Elagbar et al., 2016), and minerals such as K, P, Mg, Ca, and Na (Shehata et al., 2021, Eshra et al., 2019), and due to the high content level of lipids, annona seed is a valid source of extraction of oil for industrial and pharmaceutical applications (Pinto et al., 2018).

The defatted cake after oil extraction of annona seeds is rich in amino acids being glutamic, aspartic, tyrosine, phenylalanine, alanine, leucine, and arginine prominent. And also, it bears other amino acids such as proline, lysine, glycine, valine, threonine, serine, cysteine, methionine, isoleucine, tryptophan, and histidine. Amino acid content and essential amino acids (excluding tryptophan) profile of the defatted oil cake was higher than that of eggs (and some other proteinaceous seeds and nuts) giving the possibility of future applications in the food industry as a functional food (Eshra et al., 2019).

The unsaturated fatty acid content of annona seed oil recorded is 72% for *A. squamosa* (Eshra *et al* 2019*)* and 75% for *A. muricata* and similar fatty acid compositions were reported in *A. muricata* seed oil by different work done using different extraction methods (Pinto et al., 2018, Elagbar et al., 2016).

Mango: Mango seed contributes to 10-85% of the total weight of the fruit depending on the variety (Villacís-Chiriboga et al., 2020, Kumoro et al., 2020, Yadav et al., 2017, Selvemuthukumaran et al., 2017, Ebrahim et al., 2015), and bioactive assay and phytochemical characterization of seed kernel have been reported (Ballesteros-Vivas et al., 2019). It has gained interest for its folk medicinal applications in some countries (Kumoro et al., 2020). Fresh kernels are good for health problems such as asthma and dysentery, and the juice is used as a nasal drop for sinus troubles while dried seed powder is used as an anti-dandruff solution, and as a fortification agent for foods to supply necessary proteins, lipids, and antioxidants. Carbohydrates are the major component in mango seeds that can add value as an energy supplying food supplement (Kumoro et al., 2020) and extraction of economically viable good quality starch from mango seed kernels has been reported (Tesfaye et al., 2017). Scientific investigation reveals that the starch of the seed has been used as an alternative staple food in famines in India (Nithitanakool et al., 2013). Other than carbohydrates, mango seed kernels are composed of fat, protein, ash, fiber (Yadav et al., 2017, Nadeem et al., 2016), minerals such as K, Na, Ca, Mg, Co, Fe, Mn, and P (Mwaurah et al., 2020, Ebrahim et al., 2015), and vitamins A, E, K, C, and B. Major phytochemicals present in mango seed kernels are fatty acids, flavonoids, phenolic acids, tocopherols, phytosterols, carotenoids, and polyphenols (Mwaurah et al., 2020, Shirahigue et al., 2020). The total polyphenol compound content in mango seeds is around 15% higher than that of in the pulp (Ben-Othman et al., 2020). And amino acids detected from mango seed kernel are glutamic acid, leucine, alanine, aspartic acid, arginine, phenylalanine, valine, glycine, isoleucine, tyrosine, lysine, proline, serine, histidine, cysteine, threonine, and methionine (Mwaurah et al., 2020, Mahale et al., 2015).

Tonic, laxative, anthelmintic, and aphrodisiac properties have been found in extractions of mango seed kernel. The ethanol extract of seeds has exhibited anti-hepatotoxicity, anti-methicillin-resistant (for *Staphylococcus aureus*), antioxidant, anti-inflammatory, anti-tyrosinase, free radical scavenging, and cancer and diabetes-inducing properties giving the potential broad therapeutical and pharmacological applications for seed valorization (Nithitanakool et al., 2013).

Papaya: The seed content of papaya fruit is around 6.5-20% (Dotto et al., 2021, Kumoro et al., 2020, Sugiharto 2020, Anwar et al., 2019, Li et al 2015). The papaya seed contains fatty acids, crude protein, and crude fiber (Kumar et al., 2017). Kumoro et al., (2020) suggest around 28-31% lipid, 27-28% of protein, and 19-23% crude fibers content in papaya seeds. Oleic acid represents around 75% of the total fatty acid profile and linoleic, palmitic, stearic, and behenic acid are present in papaya seeds (Dotto et al., 2021, Anwar et al 2019). It is a rich source of vitamin A and C (George et al., 2018), rich in enzymes- myrosin, papain (Kumar et al., 2017), glutamic acid, leucine, aspartic acid, arginine, glycine, lysine, phenylalanine, alanine, isoleucine, serine, threonine, valine, histidine, proline, tyrosine, methionine, and cystine-*(*Sugiharto*, 2020),* carpaine, benzyl isothiocyanate, benzyl glucosinolate, benzyl thiourea, β-sitosterol, hentriacontane, giving the seed bactericidal, germicidal, and insecticidal properties (Dotto et al., 2021, Kumar et al., 2017). It is a rich source of K, Ca, P, and Mg. Na, Zn, and Fe also present in detectable amounts. (Dotto et al., 2021, Moses et al., 2018). Due to proven important chemical and medical properties such as anthelmintic, abortifacient, anti-implantation, anti-fertility, carminative, anti-amoebic (Dotto et al., 2021, Kumar et al., 2017), anti-inflammatory, and immunomodulatory activities (Sugiharto, 2020) papaya seed is a natural resource discarded without consideration of wide applicability in food, pharmaceutical, and other industries (Kumar et al., 2017). The functional and nutritional properties of oil extracted from papaya seeds are similar to that of olive oil. (Li et al., 2015). Muda et al., (2020) and George et al., (2018) report the possible application of papaya seeds as a natural coagulant in water purification.

Details about the composition of the seeds studied herein are summarized in Table 2.3.1 (next page).

Table 2.3.1: Summary of the Compositions of Seeds of Annona, Mango, and Papaya

	Annona Seeds	**Mango Seeds**	**Papaya Seeds**	**References**
Seed Contribution to the Total Fruit Weight (w/w)	13%	10% - 85%	6.5% - 20%	**AS-** Pinto et al. 2018 **MS-** Villacis-Chirboga et al., 2020 Kumoro et al., 2020 Yadav et al., 2017 Selvemuthukumaran et al., 2017 Ebrahim et al., 2015 **PS-** Dotto et al., 2021 Kumoro et al., 2020 Sugiharto, 2020 Anewar et al., 2019 Li et al., 2015
Major Components	Moisture Carbohydrates Lipids Proteins Fibers Ash	Moisture Carbohydrates Lipids Protein Ash Fiber	Moisture Lipids Proteins Fiber Ash	**AS-** Shehata et al., 2021 Pinto et al., 2018 **MS-** Kumaro et al., 2020 Tesfaye et al., 2017 Yadav et al., 2017 Nadeem et al., 2016 Nithitanakool et al., 2013 **PS-** Kumoro et al., 2020 Kumar et al., 2017
Amino Acids	Glutamic acid Aspartic acid Tyrosine Phenylalanine Leucine Alanine Arginine Proline Lycine Glycine Valine Threonine Serine Cystine Methionine Isoleucine Tryptophan Histidine	Glutamic acid Leucine Alanine Aspartic acid Arginine Phenylalanine Valine Glycine Isoleucine Tyrosine Lysine Proline Serine Histidine Cysteine Threonine Methionine	Glutamic acid Leucine Aspartic acid Arginine Glycine Lysine Phenylalanine Alanine Isoleucine Serine Threonine Valine Histidine Proline Tyrosine Methionine Cystine	**AS-** Eshra et al., 2019 **MS-** Mwaurah et al., 2020 Mahale et al., 2015 **PS-** Dotto et al., 2021 Sugiharto. 2020 Kumar et al., 2017
Phytochemicals	Fatty acids Phenolic compounds Tocopherols	Fatty acids Flavonoids Phenolic acids Tocopherols	Fatty acids Tocopherols Benzyl isothiocyanate	**AS-** Pinto et al., 2018 Elagbar et al., 2016

		Phytosterols Carotenoids Polyphenols Tannin	Benzyl glucosinolate Benzyl thiourea Carpaine Carotenoids Hentriacontane B-cryptoxanthin Chlorogenic acid B-sitosterol	**MS-** Ben-Othman et al., 2020 Kumoro et al., 2020 Mwaurah et al., 2020 Shirahigue et al., 2020 Yadav et al., 2017 Nadeem et al., 2016 **PS-** Dotto et al., 2021 Kumoro et al., 2020 Anwar et al., 2019 Kumar et al., 2017
Major Fatty Acids	Palmitoleic acid Oleic acid Linoleic acid Palmitic acid Stearic acid	Stearic acid Oleic acid Palmitic acid Linoleic acid Linolenic acid Palmitoleic acid Myristic acid Margaric acid Arachidonic acid	Oleic acid Linoleic acid Palmitic acid Stearic acid Behenic acid	**AS-** Eshra et al., 2019 Pinto et al., 2018 Elagbar et al., 2016 Chen et al., 2016 **MS-** Awolu et al., 2019 Erwa et al., 2019 Kassi et al., 2019 Hiwot, 2018 Yadav et al., 2017 **PS-** Dotto et al., 2021 Oshin et al., 2021 Abubakar et al., 2020 Anwar et al., 2019 Barroso et al., 2016
Minerals	K, P, Mg, Ca, Na	K, Na, Mg, Ca, Co, Fe, Mn	K, P, Mg. Ca, Na, Zn, Fe	**AS-** Shehata et al., 2021 Eshra et al., 2019 **MS-** Mwaurah et al., 2020 Ebrahim et al., 2015 **PS-** Dotto et al., 2021 Moses et al., 2018
Vitamins		A, E, K, C, B	A, C	**MS-** Mwaurah et al., 2020 Shirahigue et al., 2020 **PS-** George er al., 2018

2.4. Extraction of Oil from Selected Fruit Seeds

Existing widely applicable conventional methods as well as the introduction of novel green techniques such as biorefinery and integrated biorefinery approaches (Ben-Othman et al., 2020) for seed pretreatment and extraction (Kaseke et al., 2021) using supercritical fluid, enzyme-assisted, ultrasound-assisted, microwave-assisted, pulse electric field-assisted, and pressurized liquid extraction methods (Saini et al., 2019) allows feasible and more valuable extraction of economical, nutritional, functional, and industrial promoting substances from these byproducts other than using them as fertilizers or animal feeds (Ben-Othman et al., 2020). However, the development, scale-up, and commercialization of the combination of these methods for higher and better performance still require more research to be performed (Selo et al., 2021).

Soil and environmental background of the resource, oil content of the seed, pretreatment and extraction method used are the major factors that contribute to oil yield (Yusuf, 2018).

Since we do not intend to study the origin or the spatial-temporal changes in the composition of the fruits and seeds, the latter two factors are of interest in this work and are examined next

2.4.1. Pretreatment of Fruit Seeds for Oil Extraction: Pretreatment provides more efficient and fast results (Selo et al., 2021) and provides the resulting oil to improve the yield, color, oxidative stability, antioxidant properties, recovery of bioactive compounds, and physicochemical attributes as well as the possible development of new functional compounds (Kaseke et al., 2021). Primary treatment of seeds is a necessary part of oil extraction before any other step. After defrosting, coat removing/dehulling, winnowing, and sorting, seeds are cleaned to ensure the removal of foreign particles and dust to get a quality product. After the cleaning and moisture removal, these are milled, crushed, or ground for a size reduction to get a high yield. Size reduction allows better penetration of solvents into oily cells by increasing the surface area and rupturing and breaking of oil-bearing cells ensuring a high yield (Satriana et al., 2019, Yusuf, 2018). Both large particles and very fine particles are not suitable for oil extraction. Large particles cause delay and reduction of oil yield and fine particles produce beds hindering the extraction process. Uniform particle size gives maximum extraction (Gopalasatheeskumar, 2018).

2.4.2. Extraction Methods: Mechanical expression extraction and solvent extraction are the widely applied conventional extraction methods in vegetable oil industries (Jahongir et al., 2019, Yusuf, 2018). Pressure-driven mechanical methods are used to extract oil from seeds with high oil content (Qin et al., 2016) and solvent extraction is carried out for low oil content seeds (Yusuf, 2018).

The (mechanical) pressure extraction method seems to be a moderate method to get quality oil at an affordable price. And the screw press method is said to be more advantageous for small-scale production due to its low cost, simple equipment, and high-quality product (Roncero et al., 2016).

Oil-soluble organic solvents such as chloroform, benzene, ethanol, acetone, hexane, and cyclohexane are used in the solvent extraction method to rupture the cell wall of the seed and extract lipids from the seeds. The quality of extracted oil depends on the solvent used. Solubility, boiling point, toxicity, availability, cost, and reusability of the solvent should be taken into consideration to choose the appropriate solvent (Satriana et al., 2019). It has been revealed scientifically the use of less polar solvents such as hexane and acetone for oil extraction gives a higher yield than that of high polar solvents (Sultana et al., 2020). Awolu et al., (2019) report the fatty acid composition varies on the solvent used.

Effective and efficient extraction processes are highly interesting to get an un-impaired quality oil with a high yield with minimum time and solvent consumption, and good quality residue (Araujo et al., 2020, Avram et al., 2014). Optimization of time, temperature, and solvent to matrix ratio, as well as the solvent used and particle size, determines the yield of oil extracted (Masime et al., 2017).

Petroleum ether and n-hexane are the most widely used solvents reported for oil extraction. Petroleum ether is a cheap, volatile, flammable, non-polar, non-toxic, and stable solvent with a higher extraction capacity, that does not affect the oil quality that can be easily used in oil extraction (Masime et al., 2017). N-hexane is widely applicable due to its high selectivity, low latent heat of vaporization, simple recovery, and non-polar nature (Kumar et al., 2017). Other organic solvents such as methanol, chloroform (Pinto et al 2018), diethyl ether (Elagbar et al., 2016), terpenes (Kumar et al., 2017), heptane (Ballesteros-Vivas et al., 2019) ethanol, and acetone (Awolu et al., 2019) have been reported in oil extraction from oily seeds.

Extraction efficiency, quality, and volume depend on the organic solvent used as well as on the temperature and agitation (Satriana et al., 2019). The effect of the solvent and the extraction period on the oil yield of mango seed kernels have been reported by Awolu et al., (2019), and the effect of particle size, temperature, extraction period, and solvent to sample ratio has been studied by Yadav et al., (2017).

Higher yield, good industrial performance with high reproducibility, and repeatability are the advantages related to the solvent extraction process. Relatively high time, energy and solvent consumption, the requirement of high purity solvents, possible thermal decompositions, low selectivity, high investment and operational costs, security threats, possible emissions of toxic gasses, and the number of processing steps are disadvantages of this process (Yusuf, 2018, Selvamuthukumaran et al., 2017) and the resulting oil from solvent extraction may contain residual solvent and may require further purification (Satriana et al., 2019).

To overcome the shortcomings of conventional extraction methods, novel highly efficient techniques such as supercritical fluid extraction have been introduced, and microwave-assisted extraction, ultrasonic-assisted extraction, and pressurized liquid extraction have been conjugated in oil extraction from fruit seeds but these methods do not seem to be cost-effective thus limiting industrial-scale application (Yusuf 2018, Roncero et al., 2016). But the potential advantages and disadvantages of these methods have been reported in the literature. For example, Kittiphoom et al., (2015) report microwave-assisted solvent extraction reduces extraction time by 80% without altering the oil quality, and Awolu et al., (2019) report that supercritical CO_2 extraction leads to a highly stable oil than soxhlet extraction but a lower yield in their work done using mango seed kernels.

Supercritical fluid extraction and ultrasound-assisted extraction of oil from papaya seeds were reported in recent literature by Barroso et al., (2016) and Samaram et al., (2014) respectively.
The oil yield of mango seed kernels using supercritical CO_2 extraction compared to Soxhlet extraction with hexane and the effect of temperature, pressure, and the flow rate has been studied by Akanda et al., (2015) and report that the Soxhlet extraction gives a slightly higher oil yield than that of supercritical CO_2 extraction.
Almost all the methods face challenges in upscaling of commercial extraction.. More commonly, application of lab scale production to industrial production is a challenge because

of the difficulties of controlling higher masses, optimising complex parameters, and setting up procedures (Villacis-Chiriboga et al., 2020).

Low efficiency and high consumption of time, limit the industrial processing of mechanical expression. Seeping high volatile solvents into the sorrounding atmosphere, hazardous residues remaining in oils, high energy requirements, and high operation costs are challenges related with commercial application of solvent extraction (Yusuf 2018), and getting a good performance with economic returnes is one of the major challenges associated with combined novel green technologies (Villacis-Chiriboga et al., 2020).

According to the literature, annona seeds have a quite satisfactory level of oil content. Oil yield extracted from annona seeds has been recorded in the range of 15%-40% (Wong et al., 2019, Elagbar et al., 2016, Subramanian et al., 2016, Chen et al., 2016, Adepoju et al., 2014, Rana, 2014, Reyes-Trejo et al., 2014, Umaru et al., 2014, Kimbonguila et al., 2010). The highest of this range 20% - 40% has been reported in solvent extraction using n-hexane, (Wong et al., 2019, Elagbar et al., 2016, Subramanian et al., 2016) and petroleum ether (Kimbonguila et al., 2010) as solvents.

Quite diverging values have been reported in the yields of oils extracted from mango seeds, 1.2% to 26% for mango seeds in solvent extraction has been reported (Okonkwo et al., 2021, Awolu et al., 2019, Erwa et al., 2019, Kassi et al., 2019, Sikdar et al., 2017, Yadav et al., 2017, Nadeem et al., 2016, Ebrahim et al., 2015, Kittiphoom et al., 2013, Olajumoke 2013). Awolu et al., (2019) report ethanol and acetone are more effective than hexane and petroleum ether in soxhlet extraction giving 19% - 20 % oil yield from mango seed kernels, but Olajumuke et al., (2013) report 25.57% oil yield from Soxhlet extraction with petroleum ether as the solvent.

Oil yield extracted from papaya seeds recorded so far varies from 13%-38% (Oshin et al., 2021, Abunbakar et al., 2020, Sultana et al., 2020, Anwar et al., 2019, Zhang et al., 2019, Saha et al., 2018, Seshamamba et al., 2018, Chielle et al., 2016, Li et al., 2015, Agunbiade et al., 2014, Wong et al., 2014, Yanti et al., 2014). Li et al., (2015) report 26% and 29% oil yield using ultrasound-assisted and ultrasound-microwave solvent extraction synergistic methods respectively and suggest that ultrasound- microwave synergistic method is more effective for oil extraction from papaya seeds with the optimum time, temperature, and ratio of material to solvent parameters. Wong et al., (2014) reported the maximum oil yield 34% -38% by solvent extraction using n-hexane as the solvent.

The diagram in Figure 2.4.1 (next page) depicts the pretreatment steps, and oil extraction parameters, and methods that apply to seeds.

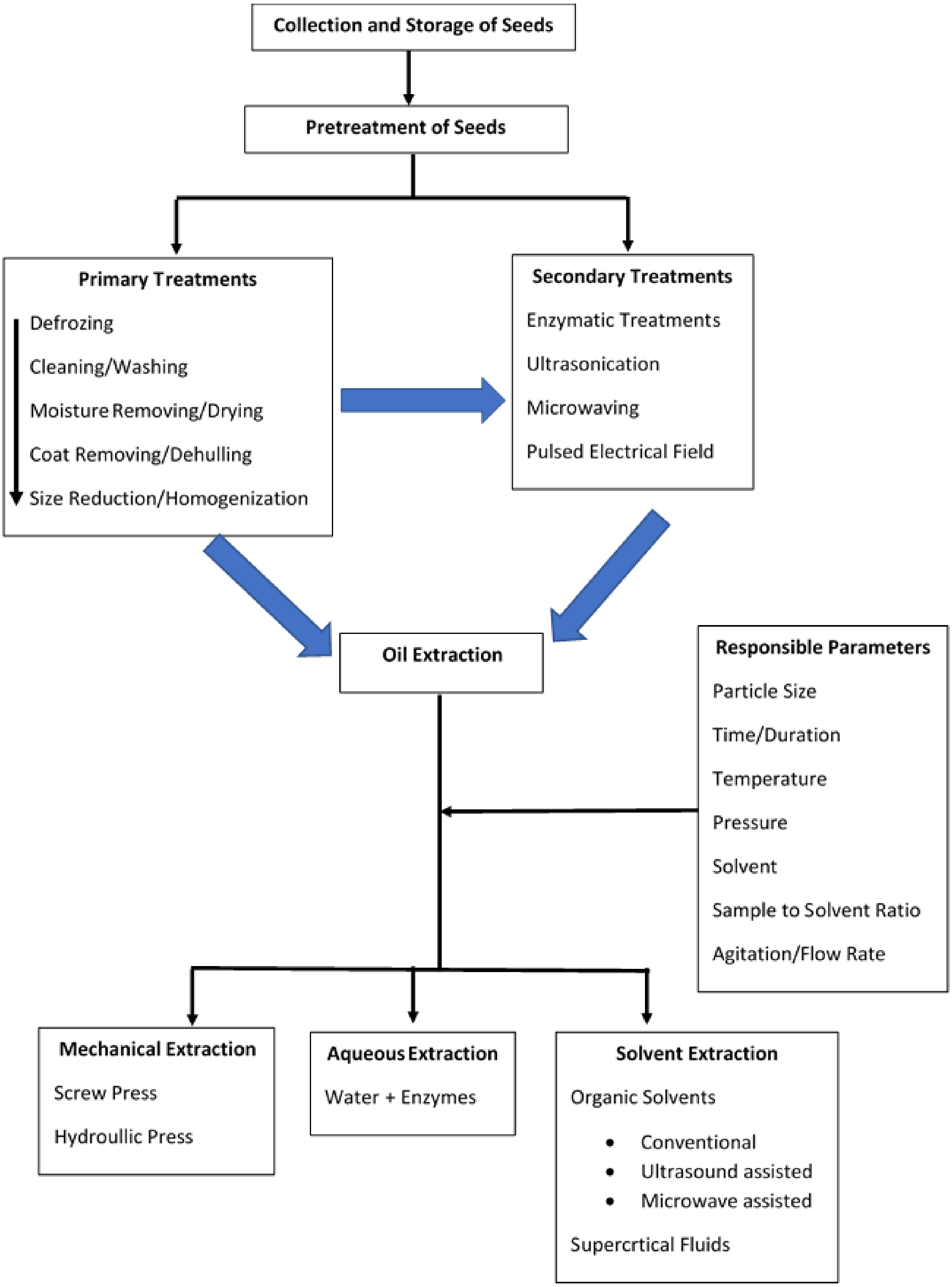

Figure 2.4.1: Seeds Pretreatment Steps, Oil Extraction Parameters, and Methods

2.4.3. Fatty Acid Analysis: Fatty acids are carboxylic acids with aliphatic chains in different lengths having saturated or one or more unsaturated bonds in different positions (Chiu et al., 2020). The human body can synthesize saturated and monounsaturated fatty acids but the most essential polyunsaturated fatty acids can`t be synthesized in human bodies due to the absence of required enzymes and can be synthesized only in plants (Roncero J. M. et al, 2016). These essential fatty acids should be taken into human bodies through dietary intake to keep and maintain health (Orsavova et al., 2015).

Gas Chromatography is one of the primary chromatographic analytical tools that has applications in so many analytical fields in the detection and quantification of components in sample mixtures (Shellie, 2013).

Mass spectrometry is the detection of molecules after ionization and separation. Detection of molecules is proceeded according to the mass to charge ratio of the ionized molecules and the results are recorded in a graphical representation of a mass spectrum (Smith, 2013), in which the no. of peaks represents the no. of components, and the area of the peak represents the quantity of the relevant molecule (Kaur et al., 2018).

Though there are some other analytical tools such as gas chromatography-flame ionization detection (GC-FID) and liquid chromatography-mass spectrometry (LC-MS) for fatty acid analysis, due to the ability to provide more structural information, better selectivity, and higher efficiency in the detection and identification of fatty acids, Gas Chromatography-Mass Spectrometry (GS-MS) has become the widely used tool for identification of fatty acids with analytical interest (Chiu et al., 2020).

Gas chromatography-mass spectrometry allows quality control, quality assurance, and method development, by separation, purification, identification, characterization, and quantification of complex sample matrixes. Other than in the food industry, it has wild applications in so many fields such as forensic science, criminal science, environmental science, astrochemistry, geochemistry, medicine, pharmaceutical science, and energy and fuel industries (Kaur et al., 2018, Al-Rubaye et al 2017).

The gas chromatograph is of a mobile phase (carrier gas), separation column, and stationary phase. The mobile phase or carrier gas is an inert gas that carries the sample to the detector through the separation column. The compounds should be with less polarity, high thermal stability, and high volatility with high vapor pressure. Less volatile samples should be derivatized to achieve sufficient volatility. A solid or liquid stationary phase should be in the separation column to perform chromatographic separation. If the stationary phase is solid, it is called gas-solid chromatography, and if it is liquid, it is called gas-liquid chromatography (Stashenko et al., 2014, Shellie, 2013). Column selection and resolution power of the spectrometry are among the key factors for more accurate and sensitive analytical results. (Chiu et al., 2020).

The separation of sample components occurs due to the difference between the equilibrium in the mobile and stationary phases. (Shellie, 2013).

The distribution constant can be expressed as (Equation 1):

$$K = \frac{Cs}{Cm}$$

Where Cs is the equilibrium concentration of the solute in the stationary phase and Cm is the equilibrium concentration of the solute in the mobile phase

The retention time of a compound is proportional to the distribution constant and the distribution constant depends on the temperature. So, the retention time of a solute depends on the temperature (Shellie, 2013). The ability to separate molecules based on their distribution constants is called the resolution and it is the most important factor of the system. The resolution power depends on many operational parameters (Stashenko et al., 2014).

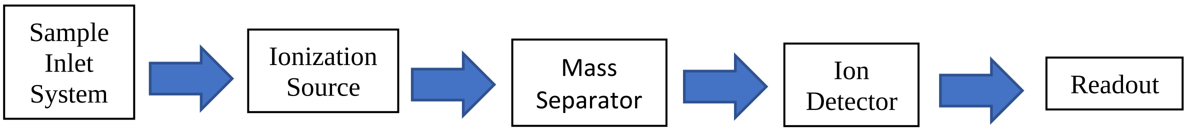

Figure 2.4.2: Block Diagram of Mass Spectrometer (adapted from Christian, 2004)

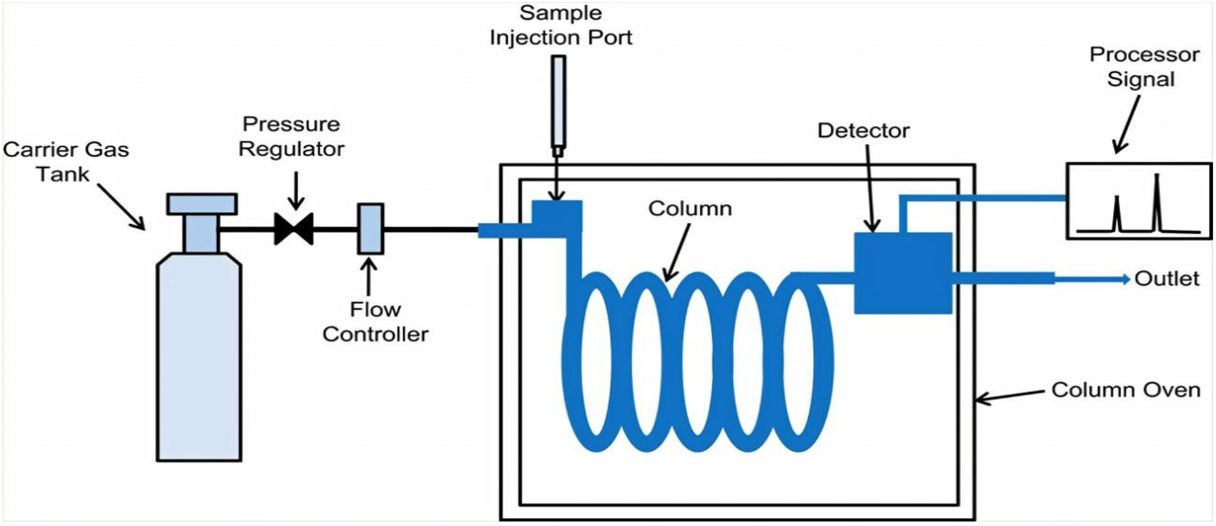

Figure 2.4.3: Schematic Diagram of Gas Chromatograph (adapted from Christian, 2004)

2.5. Applications of Selected Fruit Seeds Oils

The oils obtained from the seeds of annona, avocado, mango, and papaya have been shown to have different characteristics, properties, and applications.

Annona: Annona seed oil is a semi-dry oil (Eshra et al., 2019) that shows functional and pharmacological properties with reduced risk of metabolic syndromes and potential applications in cardiovascular health (Pinto et al., 2018, Elagbar et al., 2016). The potential application of this oil and the remaining mass of the seed after oil extraction (defatted oilseed cake) is more to be revealed (Eshra et al., 2019).

Mango: Mango seed oil is an edible, trans fatty acid-free (Solís-Fuentes et al 2020) golden yellow to brown color semi-solid at room temperature (Sikdar et al., 2017) with high stability (Ebrahim et al., 2015) that is rich with antioxidants and free radical scavenging compounds and, due to its ant-oxidant properties, mango seed oil can be used to preserve fats and oils that are more vulnerable to oxidation (Nadeem et al., 2016). This is used in the cosmetic and personnel care industry, food and confectionery industry, and health care and pharmaceutical industry (Sikdar et al., 2017). Adesina et al., (2013) reveal effective results of mango seed oil in the food industry as an anti-fungal agent to prevent food spoilage caused by *Aspergillus niger* and

Penicillium italicum. Ebrahim et al., (2015) show the possibility of this oil being used to produce lubricants.

Papaya: Papaya seed oil is a reddish-brown color liquid at room temperature with potential edibility (Li et al., 2015) rich with monounsaturated fatty acids and nutraceuticals mainly health-promoting phenolics, pigments, and tocopherol (Kumoro et al., 2020), and shows effects on vascular contraction and reduced risk of coronary heart diseases. It has proven increased palatability in frying and as a spray oil for snacks, crackers, and bakery products (Syed et al., 2012). Sinaga et al., (2021) report a possible application of papaya seed oil as a raw material for epoxy compounds that can be used as plasticizers. The defatted part of the papaya seed available after oil extraction is a good source of protein with foaming and emulsifying properties. It has been detected 32- 44% protein content in this cake with possible applications in food formulations (Yanti et al., 2014).

Besides oils for human consumption, the possibility of producing energy-efficient, eco-friendly, biodegradable, renewable, non-toxic, sulfur-free, and ester-based biodiesel has been studied (Anwar et al., 2019, Eshra et al., 2019, Wong et al., 2019, Hiwot, 2018, Reyez-Trejo et al., 2014, Wong et al., 2014) by using papaya seed oil (Oshin et al., 2021, Abubakar et al., 2020, Anwar et al., 2019, Agunbiade et al., 2014, Wong et al., 2014), annona seed oil (Reyes-Trejo et al., 2014, Eshra et al, 2019, Wong et al., 2019), and mango seed oil (Okonkwo et al., 2021, Gasma et al., 2020, Hiwot, 2018, Umaru et al., 2014) as sustainable sources with reduced levels of greenhouse gas emissions, unburned hydrocarbons, and particulate matter as an alternative to high polluting fossil fuels due to their similar characterization to fossil diesel (Anwar et al., 2019, Eshra et al., 2019, Wong et al., 2019, Hiwot, 2018, Reyez-Trejo et al., 2014).

Chapter 3
Materials and Methods

3.1 Raw Materials and Chemicals

The exotic annona (Annona *muricata*), mango (Mangifera *indica*), and papaya (Carica *papaya*) fruits were purchased at the local market in Faro, Algarve, Portugal. Mango and papaya were produced in Brazil and annona was produced in the Algarve region.

Petroleum ether was obtained from Merk. Fatty acid methyl ester (Supelco® 37 Component FAME-mix C8-C24) and FAME saturated and unsaturated standards used for gas chromatography analysis were obtained from Sigma-Aldrich.

All the solvents and reagents for the analysis were of chromatography or analytical grade.

3.2 Seeds Preparation for Oil Extraction

Seeds were removed manually from annona, mango, and papaya fruits and stored at -20 ^{o}C until oil extraction. Seeds from those exotic fruits were thawed, washed with potable tap water, and dried in a tray dryer where air at 45 ^{o}C was forced to circulate until a moisture content of 5% (w:w) was achieved. The dried seeds were dehulled by hand and crushed (Figure 3.2.1) in a hammer mill with a 0.75 mm sieve (Souza et al., 2018).

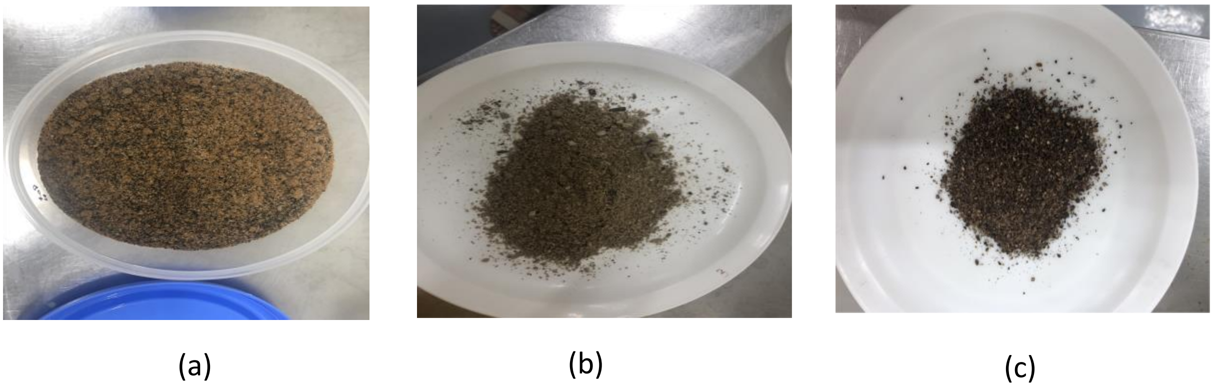
(a) (b) (c)

Figure 3.2.1: Crushed seeds of (a) annona, (b) mango, and (c) papaya.

3.3 Oil Extraction

A Soxhlet apparatus with four distillation units (Figure 3.3.1) was used for the seeds oil extraction. All the round bottom flasks with glass balls inside were kept in an oven preheated to 100 °C for 4 h and transferred to a desiccator for 30 min to decrease the temperature of the extracted oil until room temperature.

Figure 3.3.1: The Soxhlet Apparatus

3.4 Oil Extraction from Selected Exotic Fruit Seeds

Samples (5 g) of crushed seeds were kept in each thimble and each of them was covered with cotton. Petroleum ether (170 ml) in analytical grade was added to each sample container. The temperature of the Soxhlet apparatus was controlled at the boiling point of petroleum ether (40 °C to 60 °C) by an electrical heating mantle and the cold water has been supplied to each condenser. Oil extraction in a Soxhlet apparatus was performed according to AOAC 948.2 method (AOAC 2000) using a minimum extraction time of 9 h. The extracted oil mixed with the solvent in flasks was separated by evaporating the solvent at 70 °C using a rotary evaporator. The residual solvent was further removed from the flasks by drying in a preheated oven for one

h. The extracted oils were sealed in sample tubes, labeled, and stored at -20 °C for further analysis. The recovered solvent was stored for further recycling.

The oil extraction yield (Y, %) of oil obtained was calculated by (Equation 2):

$$Y = \frac{M_O}{M_S} \times 100\%$$

where M_O is the mass of oil extracted and M_S is the mass of seeds.

3.5 Fatty Acid Analysis

The extracted oils were esterified and the fatty acid methyl esters (FAME) were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS, Brucker SCION 456/GC, SCION TQ MS) using a capillary column GC-MS ZB-5ms (30 m × 0.25 mm internal diameter x 0.25 μm film thickness) obtained from Phenomenex. The temperature programming was: 60 °C held for 1 min, 30 °C/min until 120 °C, 4 °C/min until 250 °C and 20°C/min until 300 °C for 4 min. The injector temperature was kept at 300 °C. FAMEs were identified by comparing their fragmentation pattern with authentic standards from Supelco® 37 Component FAME Mix (Sigma-Aldrich).

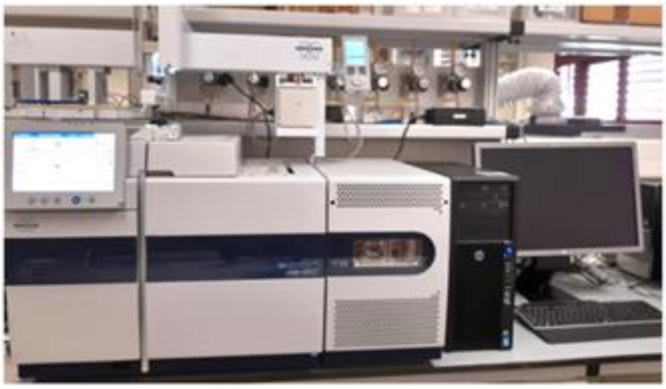

Figure 3.3.2: Illustrative Image of a GC-MS (www.ccmar.ualg.pt)

3.6 Data Analysis

Microsoft Excel Data Analysis Tool was used for the data analysis. Mean values and standard deviations were determined for oil yield.

Chapter 4
Results and Discussion

4.1 Oil Yield

The extraction yields were distinct among the seeds studied herein.

Oil yield from annona seeds was 28.9±1.6%, within the range so far reported. The yield found in this study is higher than the 22.7% reported by Chen et al. (2016) but less than 36.8% reported by Mondal (2015), and 40% reported by Kimbonguila et al., (2010) using the same method (Soxhlet extraction with petroleum ether as the solvent). The countries of origin of the seeds studied were Portugal (this study), China (Chen et al., 2016), India (Mondal, 2015), and Congo (Kimbonguila et al., 2010) respectively.

On the other hand, the oil yield from mango seeds reported so far in the literature was 1.2%-26%. In this study, it was quite low and was 5.00±0.01%. Olajumoke et al., (2013) reported 25.57% oil yield, Umaru et al., (2014) reported 14% oil yield, Erwa et al., (2019) reported 10.8% oil yield, Awolu et al. (2019) reported 8.77% oil yield, and Kittiphoom et al., (2013) reported 8.04% oil yield respectively, from mango seed kernels from different countries of origin of fruits using the similar extraction process (Soxhlet extraction with petroleum ether) proceeded in this study. However, Awolu et al., (2019) report 8.77% of maximum oil yield after 4 h and a decrease in yield with increased time.

The oil yield from papaya seeds in this study was 24.85±0.90% which is quite similar to the oil yield of 24.01% reported by Saha et al., (2018) using Bangladesh origin fruit seeds and for Indian origin papaya seeds by Seshambamba et al. (2018) that was 25.6%, but less than the oil yield reported by Syed et al., (2012) for Indian origin papaya seeds that was 30.1% using the same process (Soxhlet extraction with petroleum ether as the solvent).

So, the possible reasons for these differences in oil yields can be assumed as the variety, spatio-temporal parameters related to soil and climatic factors, or operational parameters.

4.2 Fatty Acid Profile

In this study, the oils obtained from annona, mango, and papaya seeds presented different fatty acid profiles (Table 4.2.1).

The annona seed oil contained palmitic (17.68%), oleic (42.39%), and linoleic (30.22%) acids as the main fatty acids. Fatty acid profiles of annona seed oils extracted in the same way (Soxhlet extraction using petroleum ether as the solvent) have been studied by Chen et al (2016), Mondel (2015), and Kimbonguila et al (2010). Chen et al used Chinese origin fruit seeds and reported 9.92% palmitic acid, 9.12% stearic acid, 20.49% linoleic acid, and 56.50% oleic acid, Mondel (2015) reported 12,4% palmitic, 52.4% oleic, and 19.6% linoleic acid in the study done using Indian-origin fruit seeds, and Kimbonguila et al (2010) reported 20.33% palmitic, 41.41% oleic, and 30.6% linoleic acid in their study using Congo-origin fruit seeds. Differences may be due to the distinct soils and climatic conditions influencing the compositional qualities of fruits.

Table 4.2.1: Fatty Acid Composition of Annona, Mango, and Papaya Seeds Oils (%w/w)

Fatty Acids in Oils	Annona seeds	Mango seeds	Papaya seeds
Lauric (C12:0)	ND	1.68	ND
Palmitic (C16:0)	17.68	22.01	15.20
Palmitoleic (C16:1)	0.30	ND	1.81
Stearic (C18:0)	6.93	14.62	2.42
Oleic (C18:1)	42.39	25.00	66.52
Linoleic (C18:2)	30.22	30.30	8.52
Other	2.48	6.39	5.53

The mango seed oil extracted presented similar levels of linoleic acid compared to annona seed oil (30.30% vs. 30.22%, respectively). Herein, the main fatty acids in mango seeds oil besides linoleic acid were oleic acid (25.00%), palmitic acid (22.01%), and stearic acid (14.62%). Awolu and Manohar (2019) extracted mango seed oil with different solvents (hexane, petroleum ether, and acetone) and the profiles they obtained for the oils were similar. The oils extracted had high levels of oleic acid in the range 44.7%-46.34% and stearic acid 39.83%-40.38%. Kittiphoom et al., (2013) reported 8.73% palmitic, 37.7% stearic, 44.75% oleic, and 5.67% linoleic acids from Thailand-origin fruit seeds. Erwa et al., (2019) reported 10.34% palmitic, 39.79% stearic, 36.77% oleic acids from Sudan-origin fruit seeds. In the present study, the fatty acid composition of the mango seed oil is different probably because of different stages of maturation or conditions of production of the fruits.

Table 4.2.2: Comparison of Results of Annona Seed Oil with Previous Studies.

Oil Yield and Major Fatty Acids in Annona Seed Oil	Qty (%) This Study	Qty (%) Chen et al., 2016	Qty (%) Mondel 2015	Qty (%) Kimbonguila et al., 2010
Oil Yield	28.90	22.70	36.80	40.00
SFAs				
Palmitic (C16:0)	17.68	9.92	12.40	20.41
Palmitoleic (C16:1)	0.30	ND	ND	1.44
Stearic (C18:0)	6.93	9.14	8.60	4.13
Other SFAs	ND	3.57	1.20	NFD
UFAs				
Oleic (C18:1)	42.39	56.50	52.40	41.29
Linoleic (C18:2)	30.22	20.49	19.60	30.85
Other UFAs	ND	0.38	5.80	1.88
SFA	24.91	22.63	22.20	25.98
UFA	72.61	77.37	77.80	74.02
Others	2.48	ND	ND	ND

Table 4.2.3: Comparison of Results of Mango Seed Oil with Previous Studies

Oil Yield and Major Fatty Acids in Mango Seed Oils	Qty (%) This study	Qty (%) Kittiphoom et al 2013	Qty (%) Erwa et al 2019	Qty (%) Awolu et al 2019
Oil Yield	5.00	8.04	10.80	8.77
SFAs				
Lauric (C12:0)	1.68	ND	ND	ND
Palmitic (C16:0)	22.01	8.73	10.34	6.25
Stearic (C18:0)	14.62	37.70	39.77	40.29
Other SFAs	ND	ND	5.87	1.64
UFAs				
Oleic (C18:1)	25.00	44.75	36.77	44.07
Linoleic (C18:2)	30.30	5.67	6.02	4.69
Other UFAs	ND	3.07	0.41	0.09
SFA	38.31	46.43	55.98	48.18
UFA	55.30	53.49	43.20	48.85
Others	6.39	0.08	0.82	2.97

Table 4.2.4: Comparison of Results of Papaya Seed Oil with Previous Studies

Oil Yield and Major Fatty Acids in Papaya Seed Oil	**Qty (%) This Study**	**Qty (%) Syed et al., 2012**	**Qty (%) Saha et al, 2018**	**Qty (%) Yanti et al, 2014**
Oil Yield	24.85	30.10	24.01	27.00
SFAs				
Palmitic (C16:0)	15.20	13.50	15.96	15.80
Palmitoleic (C1y6:1))	1.81	0.21	ND	0.40
Stearic (C18:0)	2.42	4.50	4.71	5.10
Other SFAs	ND	0.86	0.44	0.40
UFAs				
Oleic (C18:1)	66.52	72.50	78.88	73.50
Linoleic (C18:2)	8.52	2.90	ND	4.00
Other UFAs	ND	0.28	ND	0.80
SFA	19.43	19.07	21.11	21.70
UFA	75.04	75.68	78.88	78.30
Others	5.53	5.25	0.01	ND

The papaya seed oil extracted with the Soxhlet method had a different fatty acid composition when compared with annona and mango seed oils, mainly in oleic (66.52%) and linoleic (8.52%) acids levels (Table 4.2.1.). These results are similar to other studies that analyzed this oil, namely Syed et al., (2012), Saha et al., (2018) and Yanti et al., 2014. Although, Chielle et al. (2016) obtained a fatty acid profile with high levels of oleic acid (89.33%) and low levels of linoleic acid (5.04%). And Saha et al., (2018) reported 15.96% palmitic, 78.88% oleic, and 4.71% stearic acids. Probably the difference in geographical origin of fruits may explain the fatty acid profile obtain in this study.

The most prominent fatty acids in annona and mango seed oils are omega-9 (oleic acid), and omega-6 (linoleic acid), and it is omega-9 that is prominent in papaya seed oil. Both fatty acids are nutritionally, functionally, and medicinally important, health-promoting unsaturated fatty acids (Chen et al., 2016; Erwa et al., 2019)) where oleic is a monounsaturated and linoleic is a polyunsaturated fatty acid. Due to the high yield of oil and the satisfactory fatty acid profile, papaya seeds provide a good base for commercial oil production. Even though, we found similar levels of omega-6 fatty acid (linoleic) in annona and mango seed oils, annona seeds are “preferred” for oil extraction over mango seeds due to the difference of oil yields (29 % and 5 %, respectively). Thus, annona seeds might be favored for commercial scaling up and mango seeds provide more opportunities to look for alternative better valorization methods.

Chapter 5

Conclusions and Future Research

Byproduct valorization is a universal concept and oil is a product with global demand that can be satiated by extracting palatable healthy oils from non-palatable fruit seeds. This study shows the possibility and value of oil extraction from the selected fruit seeds. Annona seed oil contained 42.4% omega-9 fatty acids and 30.2% omega-6 fatty acids thus prominent in UFAs over SFAs. Mango seed oil contained 25.0% of omega-9 fatty acids and 30.3% omega-6 fatty acids thus giving healthier UFAs to be prominent. In contrast, in this study, Similarly, omega-9, and omega-6 fatty acid content of papaya seed oil was 66.52% and 8.52% respectively providing well satisfactory level of UFAs.

The effect of the geographical origin of the fruits, and putative influence of soil and climatic conditions as well as the maturity states, the impact of preprocessing, and post-extraction conditions on the oil yield, fatty acids profile, and bioactive components should be studied further. Moreover, since the marketability, consumer acceptance, sustainability of the whole recovery, and practical challenges in the process have not been studied in detail, they should be addressed for the further conclusion and eventual commercialization. However, according to the Food and Agriculture organization, as the oil yield should be higher than 17% to consider in biodiesel production, mango seed kernel oil seems to be inappropriate for commercialization in the bio-fuel industry.

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APPENDIX

Table A.1: Oil Yield of Annona, Mango, and Papaya Seeds.

Fruit seeds	Sample	Sample Weight (g)	Initial Bulb Weight (g)	Bulb + Oil Weight (g)	Oil Weight (g)	Oil Yield (%w/w)	Mean	Std. Dev.
Annona	1	5.1830	127.1288	128.8144	1.6856	32.52	29.75	1.86
	2	5.1505	121.6355	123.1308	1.4953	29.03		
	3	5.5404	122.0335	123.6202	1.5867	28.64		
	4	5.1513	121.6773	123.1610	1.4837	28.80		
Mango	1	5.5795	132.5050	132.7820	0.2770	4.96	4.97	0.01
	2	5.6421	124.7806	125.0613	0.2807	4.98		
	3	5.2215	125.9624					
	4	15.5087	123.2263	123.9965	0.7702	4.97		
Papaya	1	4.8604	123.2329	124.4628	1.2299	25.30	24.48	1.12
	2	5.1991	124.6885					
	3	5.0276	133.1787	134.3443	1.1656	23.18		
	4	5.3220	130.8053	132.1280	1.3227	24.85		