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Papaya (*Carica papaya* L.) by-products. Characterization and valorisation of bioactive and energetic potential.

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Abstract

Papaya (*Carica papaya* L.) is the third most cultivated tropical crop worldwide. The fruit processing industries generate a high volume of by-products, including seeds and peels. Considering the environmental issue, utilization of papaya wastes becomes a major challenge. These by-products hold nutrients and phytochemicals that can be used as value added ingredients essential to guide ways for their reuse and map industrial sectors interested in their application as food additives, or further as substrate for extracting of relevant compounds, such as antioxidants, for pharmaceutical and cosmetic purposes.

The aim of this study was to evaluate the nutritional composition and antioxidants content of seeds and peels of two papaya varieties marketed in Portugal (Aliança and Formosa). Macronutrients, fatty acids profile, vitamin E profile, total phenolic and flavonoid compounds, and antioxidant activity (DPPH$^{\cdot}$ inhibition and FRAP assays) were also determined in seeds and peels of these two fruit varieties.

Results showed that papaya seeds are a rich source of proteins, lipids, and inorganic matter. Sugars in the free form (glucose and fructose) were detected in low amounts, contrary to the fibre content observed. Regarding the fatty acid profile, seeds of both fruit varieties are rich in oleic acid (18:1) (72.60 % and 73.60 % for Aliança and Formosa, respectively), a monounsaturated fatty acid linked to health benefits. Regarding fruit peels, it also presents high content of proteins, low content of total fat but high mineral content. Like seeds, fruit peels also show considerable fibre content. The fatty acid profile in fruit peels was quite different from that described in seeds, being α-Linolenic acid (C:18:3n3 the most representative one. Vitamin E was significantly superior in fruit peels.

These high values for proximate composition make these by-products rich natural sources of nutrients. Therefore, papaya seeds and peels, are promising by-products as source of macro and micronutrients. Also, with the presence of bioactive compounds (phenolics and flavonoids), these by-products could be used as source of antimicrobial, antioxidants, colorants, flavouring and thickening agents. Although the individual profile of bioactive compounds should be considered in a future study, these results confirm the great potential for industrial recovery and related applications, such as formulation of new food ingredients.

Keywords: *Carica papaya* L., by-products, proximate composition, fatty acids, vitamin E, bioactive compounds, antioxidant activity.

Resumo

Papaia (*Carica papaya* L.) é a terceira fruta tropical mais cultivada em todo o mundo. As indústrias de processamento de fruta geram um elevado volume de resíduos, como sementes e cascas. Considerando a questão ambiental, a utilização de resíduos de papaia torna-se um grande desafio. Estes subprodutos contêm nutrientes e fitoquímicos que podem ser utilizados como ingredientes de valor acrescentado, essenciais para orientar a sua reutilização e mapear os sectores industriais interessados na sua aplicação como aditivos alimentares, ou ainda como substrato para extração de compostos relevantes, tais como antioxidantes, para fins farmacêuticos e cosméticos.

O objetivo deste estudo foi avaliar a composição nutricional e o teor de antioxidantes de sementes e cascas de duas variedades de papaia comercializadas em Portugal (Aliança e Formosa). Para isso, foram determinados os macronutrientes, o perfil de ácidos gordos, perfil de vitamina E, os compostos fenólicos e flavonoides totais e a atividade antioxidante (DPPH$^{\cdot}$ e FRAP), nas sementes e cascas das duas variedades.

Os resultados mostraram que as sementes de papaia são uma rica fonte de proteínas, lípidos e matéria inorgânica. Os açúcares na forma livre (glucose e frutose) foram detetados em quantidades baixas, ao contrário do teor de fibras observado. Quanto ao perfil de ácidos gordos, as sementes de ambas as variedades são ricas em ácido oleico (18:1) (72,60 % e 73,60 % para Aliança e Formosa, respetivamente), ácido gordo monoinsaturado ligado a benefícios para a saúde. Relativamente às cascas, estas apresentam também um elevado teor de proteínas, baixo teor de gordura total, mas elevado teor de minerais. Tal como as sementes, as cascas também apresentam um teor considerável de fibras. O perfil dos ácidos gordos nas cascas dos frutos foi bastante diferente do descrito nas sementes, sendo o ácido α-linolénico (C:18:3n3), o mais representativo. A vitamina E é significativamente superior nas cascas dos frutos. Estes valores elevados de composição proximal, tornam estes subprodutos ricas fontes naturais de nutrientes, sendo as sementes e cascas de papaia, subprodutos promissores como fonte de macro e micronutrientes. Também, com a presença de compostos bioativos (fenólicos e flavonoides), estes subprodutos poderiam ser utilizados como fonte de antimicrobianos, antioxidantes, corantes, aromatizantes e agentes espessantes. Embora o perfil individual dos compostos bioativos deva ser considerado num estudo futuro, estes resultados confirmam o grande potencial de recuperação industrial e aplicações relacionadas, tais como a formulação de novos ingredientes alimentares.

Palavras-chave: *Carica papaya* L., sub-produtos, composição centesimal, ácidos gordos, vitamina E, compostos bioativos, atividade antioxidante.

Publications and Communications

[1] Soares, S.C.B., Costa, A.S.G., Melo, D., Vinha, A.F., Oliveira, M.B.P.P. 2021. Nutritional and phytochemical composition of *Carica papaya* L. by-products: new strategies for food security and sustainability. Livro de Resumos do XV Encontro de Química dos Alimentos: Estratégias para a Excelência, Autenticidade, Segurança e Sustentabilidade Alimentar, p. 304. ISBN: 978-989-8805-68-3.

[2] Soares, C.S.B, Vinha, A.F., Oliveira, M.B.P.P. 2019. Papaya seeds: A sustainable by-product of excellence of natural compounds with beneficial effects for health. Book of Abstracts 12th Meeting of Young Researchers of University of Porto, P. 15147. ISBN: 978-989-746-203-0.

Table of contents

List of tables

List of figures

Abbreviations and acronyms

BHT – Butylated hydroxytoluene

CE – Catechin equivalents

DPPH· – 2,2-diphenyl-1-picrylhydrazyl radical

EFSA - European Food Safety Authority

FA – Fatty acids

FAO –Food and Agriculture Organization

FC – Folin-Ciocalteu reagent

FDA – Food and Drug Administration

FRAP – Ferric ion reducing antioxidant power

GA – Gallic acid

GAE – Gallic acid equivalents

GC-FID – Gas chromatography coupled with flame ionization detector

HPLC – High-performance liquid chromatography

HPLC-DAD-FLD – High-performance liquid chromatography coupled to diode array detector and fluorescence detector

KOH – Potassium hydroxide

n.d.– not detected

RNS – Reactive nitrogen species

ROS – Reactive oxygen species

Rpm – Revolutions per minute

RT – Room temperature

TE – Trolox equivalents

TFC – Total flavonoids content

TPC – Total phenolic content

I. Introduction

1. Sustainability and food security

Crops growing conditions have been affected, mostly due to increased salinization and aridity, consequences of global climate change. Climate change models predict that future precipitation patterns will involve less frequent but more vigorous rainfall events, increasing the duration of dry soil conditions. Consequently, crop productivity may be affected by long periods of drought, and this implies that farmers acquire new tools to adapt to these changes (1).

At the same time, the world population is increasing and will reach nearly 10 billion by 2050 (2). This represents an urgent concern since already today 870 million people are hungry in underdeveloped countries, and more than two billion people are undernourished because of inadequate diets (3).

To follow up population growth, the world's agricultural system must simultaneously increase food production, provide economic opportunities for the rural poor who depend on agriculture for their livelihoods and, consequently, reduce environmental impacts (4).

Food provision is the human activity with the single largest environmental impact, being a major proportion of surface water and a large proportion of energy appropriated by food supply (5). Food systems are at the core of global environmental, social, and economic challenges, such as resource scarcity, ecosystem degradation, and climate change. Poverty, hunger and malnutrition, inadequate diets, land degradation, water shortages, social inequalities, biodiversity loss, and climate change are inherently rooted in the way we produce, distribute, and consume food (4).

Therefore, sustainability is also a major concern, and the agri-food industry will have to respond to these demands by increasing the sustainability of processes and products. Sustainable diets should provide nutritious food at affordable costs, while having a low impact on the environment. Thereby, the growing demand for food must co-exist with the need to preserve arable land for agricultural food production, and genetic diversity to safeguard ecosystem resilience (3).

The report of the World Commission on Environment and Development (WCED), entitled "Our Common Future" and more commonly known as the Brundtland Report, defines sustainable development as development that meets the needs of the present without compromising the ability of future generations to meet their own needs (6).

The concept of sustainable development is multidisciplinary, referring to long-term balances between economy, ecology, and society, simultaneously benefiting from each other, known as the three pillars of sustainability, often represented diagrammatically as shown in figure 1 (7). Sustainable development is not a static state of harmony, but a process of change in which the exploitation of resources, the direction of investments, the orientation of technological development, and institutional change are made consistent with future as well as present needs (6).

Figure 1- The three pillars of sustainability, based on "sustainable development" (Taken from Tedeschi *et al.* (8)).

Food safety and security are two complementing elements of our sustainable future. A sustainable food system is one that supports food security, makes optimal use of natural and human resources, is culturally acceptable and accessible, environmentally friendly, and economically fair and viable, and provides the consumer with nutritious, safe, and affordable food for present and future generations (2).

Several approaches are possible for achieving sustainability and food security, such as limiting food losses and waste, eating more plant-based foods or recycling foodstuffs (2). The tools and strategies used to achieve food security must align with food safety, and public health as well as sustainability and, therefore, changes in both food consumption and food production are important to ensure more sustainable food systems and to achieve food and nutrition security (4).

1.1. Agro-industrial by-products

A United Nations Food and Agriculture Organization (FAO) study conducted by Gustavsson *et al.* (9) estimated that about one third of the food produced is lost or wasted along the production chain, corresponding to a worldwide production of food waste of almost 1.3 billion tons/year.

By eliminating global food waste and loss, one could feed more than one billion additional persons. Also, less food lost or wasted would lead to more efficient land use and better water resource management with positive impacts on climate change, livelihoods, and sustainability (10).

According to European Regulation (Directive 91/156/EEC and 91/689/EEC), food wastes correspond to highly organically loaded wastes, which are generally obtained during the transformation of raw materials into food products. In turn, by-products are a designation that refers to "food waste" as substrates for the recapture of functional compounds with viability in the development of new products with market value (11, 12).

In this way, the valorisation of agro-food by-products is currently not only a necessity, but an opportunity to obtain new added value products with great impact on the economy of industries. The strategy of implementing a feasible waste management, besides strongly valuing a by-product, considerably reduces the pollutant load resulting from agro-industrial activity and boosts the agricultural and economic system of a country.

As food waste is generally susceptible to microbiological degradation, when not properly treated, it can represent sources of environmental contamination and expensive operational costs. Thus, there is a time limit for its treatment and in addition, the cost of drying, storage and transportation are economically limiting factors.

One of the biggest current challenges, centres on the processing of agri-food by-products for the recovery of high-value compounds and production of relevant metabolites through chemical and biotechnological processes. Depending on the raw material and processing that originated them, by-products may contain many valuable substances, such as dietetic fibre, pigments, organic acids, flavours, and antibacterial or antifungal substances and may also include functional compounds of high differentiated value, such as vitamins, carotenoids, polyphenols, and peptides. Some of these by-products have been recognized by several organizations, concretely Food and Drug Administration (FDA) and European Food Safety Authority (EFSA) to possess health benefits (13, 14).

Industries benefit both from the reduction of waste disposal or treatment costs, and from converting these by-products into commercial products, either as raw materials for secondary processes (intermediate food ingredients) as operating supplies, or as ingredients of new products (13, 15).

However, with the introduction of Regulation (EC) No. 1924/2006 of the European Parliament and Council, related to nutrition and health claims made on foods, legislative restrictions, applied particularly in the food area by EFSA, may hinder the valorisation of some of the ingredients with bioactivity, for which health claims have not yet been approved, due to lack of sufficient scientific evidence (14).

Another major limitation for the economically sustained development of these processes is the availability of the required by-product quantities, that may vary with seasonality. In general, most by-products produced in the fruit and vegetable sector include peel and seeds of fruits and/or vegetables, stems, leaves or products that have physical or chemical damage.

Regarding tropical fruits, through the processing to obtain edible product, non-edible by-products such as skin and seeds are formed, in quantities close to/or even exceeding those of the product with economic value, affecting the economics of fruit cultivation (16).

1.2. Tropical fruits

Tropical fruits represent a group of fruits produced in the tropics, such as papaya, mango, pineapple, avocado, açaí and bananas.

Considering the global agricultural trade, tropical fruits represent only 3 % of world agricultural food products. However, being a treasured commodity, their high average export unit values place them as the third most valuable fruit group (17).

Most of the tropical fruit production comes from low-income developing countries, mainly in Latin America and Caribbean regions, but also from Asiatic and African areas. Also, most of tropical fruits are cultivated at small and medium-scale subsistence agriculture (17).

Nowadays, tropical fruit production has been increasing worldwide due to the evolution of agricultural techniques, transport, and marketing systems, in addition to the increase in consumer demand, not only for its organoleptic properties, but also for the growing recognition of its nutritional value related to health promoting activities (18-20).

Epidemiological studies have shown that a diet rich in fruits, including tropical fruits, leads to a lower risk of developing cancer (21, 22), cardiovascular diseases (23), cataracts, asthma, and bronchitis (24), being these beneficial effects associated with the presence of different nutrient and non-nutrient compounds that have biological properties.

Papaya, a tropical fruit known worldwide, has gained interest from scientific research not only due to its considerable size and consequent overproduction of by-products but also

because of its diverse bioactive compounds with recognized biological properties, among which phenolic compounds, carotenoids, tocopherols, and ascorbic acid stand out (25).

2. Papaya (*Carica papaya* L.)

Papaya (*Carica papaya* L.) belongs to the Caricaceae family and is widely grown in tropical and subtropical regions. Although the exact centre of origin is unknown, the papaya is believed to be native to tropical America, with its region of origin being southern Mexico and neighbouring Central America (26). Most likely due to the abundant and highly viable seeds, this fruit rapidly propagated through the tropics and adapted well to tropical climates, with fertile soils and abundant rainfall (14).

The Caricaceae family comprises 6 genera and 35 species. The genus *Cylicomorpha* (2 species) is represented by species originating from Africa and the genera *Horovitzia* (1 species), *Jarilla* (2 species), *Jacaratia* (7 species), *Vasconcellea* (21 species) and *Carica* (1 species) are represented by species originating from the American continent. *Carica papaya* L. is the most economically important fruit in the Caricaceae family (27).

Although this fruit is identified internationally by its botanical name (*Carica papaya* L.), it can adopt country-specific terminology (28). For instance, in India it is known as "*papita*", "*tree melon*" in the Netherlands, "*papaye"* in France, "*paw paw*" in Australia and, in Brazil and Portugal as "*mamão*" or "*papaya*" (28).

Papaya plant is a small, sparsely branched tree with huge palmate-shaped leaves (figure 2). These trees may reach 2 meters in height in only 5 to 8 months. At this stage they are already bearing the first fruits. The fruit, papaya, grows and hangs from the central stem (29).

Figure 2 - Papaya crops (anankkml, Getty Images adapted from Canva).

The papaya is a polygamous species and has different flowers depending on the sex of the plant. Some trees bear female (or pistillate) flowers, which are short, white, five-petaled flowers, while others bear male flowers, which are clustered on long clusters called staminate. Others even have hermaphrodite (bisexual) flowers or may have flowers of both sexes (30).

Climatic factors, such as drought and variable temperatures seem to trigger this tendency to change in sexual expression. Hermaphroditic or female plants are preferred because male trees are unfruitful (30).

Regardless of the species, the morphology of papaya fruits is always very identical being characterized as an ovoid, spherical or pyriform berry (Figure 3). They are normally composed of five carpels, joined to a central cavity (placenta) that contains the seeds. The skin is thin, with a variable colouring, predominating light yellow and orange when the fruit is ripe. The colour of the pulp is usually yellow, orange, or reddish, depending not only on its maturity index but also on the species.

Figure 3 - Papaya fruit and papaya cross section, specie Carica (chengyuzheng and chorboon_photo, Getty Images adapted from Canva).

Regarding *Carica papaya* L., we can find different varieties within the species, for example, the variety Formosa (figure 4) is a large, oblong variety with yellow and green spotted skin and a firm, salmon-coloured pulp and is the heavyweight among the papayas with the fruits weighing up to 3 kg. Other varieties, such as Golden, Sunrise and Aliança (figure 5) from “Solo” group are smaller sized and pear-shaped fruits. They weight around 0.5 kg and possess a yellow to red-orange pulp.

Figure 4 - Papaya Formosa (chiravan39, Getty Images adapted from Canva).

Figure 5 - Papaya Aliança (http://www.nortefrut.com.br/).

Nowadays, papaya fruit is of great economic importance and is consumed throughout the world for its organoleptic, nutritional, and therapeutic properties (31). This fruit has also achieved great popularity among producers since it can be intensively cultivated, its quick return and its increasing demand.

Commercial papaya cultivation is restricted to tropical and subtropical areas due to cold damage in low temperatures. However, there is growing interest for this crop in colder areas such as Spain, Israel, Argentina, Australia, and Japan (32).

Papaya is the third most cultivated tropical crop worldwide with 13,158,575 tonnes produced per year. India and Brazil stand out as the largest producers, together producing more than 50 % of world's total (33, 34).

Not only the increasing production of this fruit boosts the international economic market but, above all, potentiates its consumption, whose nutritional and therapeutic benefits are of great interest. Consequently, there is a greater production of by-products, making it interesting for scientific research to study their properties to provide them with commercial value.

2.1. Nutritional value and biological properties

Papaya is recognized for its phytochemical, biological, nutritional and medicinal properties (35, 36).

The compounds responsible for the benefits exhibited by this fruit are found in its different parts, including leaves, all fruit (peel, pulp, and seeds), latex, and roots (28).

At the unripe stage, the fruit is consumed as a cooked vegetable in some Asian countries (37). In Puerto Rico, unripe papaya fruits are canned in sugar syrup and sold either in local markets or exported. Also, green or unripe papaya must be cooked prior to consumption to denature the papain in the latex (26).

Ripe papaya fruit is consumed in many ways. The most common one is to eat it like a melon. It can be peeled, the seeds removed, cut into pieces, and served as a fresh fruit. Ripe papaya is also used in jam, jelly, marmalade, and other products containing added sugar. Other processed products include puree, nectar (a non-fermented beverage produced from fruit juice, sugar, and water (38)), juice, frozen slices or chunks, mixed beverages, papaya powder, concentrated and candied items (39).

Papaya is a low-calorie fruit and its edible part, the pulp, contains proteins (constituted by amino acids), lipids (myristic, palmitic, stearic, linoleic, linolenic and vaccenic acids), carbohydrates (approximately 60 % sugars and 30 % fibre), minerals (calcium, phosphorus, iron, potassium, and magnesium) and vitamins (A, E, K, C, thiamine, riboflavin, niacin, folic acid and pantothenic acid) (40-42).

Papaya contains α- and β-carotene, lutein, zeaxanthin, and lycopene, carotenoids that act as biological antioxidants, contributing to the defense of the organism against reactive oxygen species (ROS) and play a protective role in conditions, such as diabetes and CVD, impacting cellular signalling pathways and influencing the expression of certain genes, and inhibiting specific enzymes involved in the development of certain types of cancer (43-45).

However, its nutritional composition varies according to the degree of ripeness, soil and climate conditions and variety (44). For instance, fruits stored at 25 °C have higher carotenoids content than fruits stored at 1 °C, due to chilling damage (46).

Ripe fruit contains, per 100 g of edible product, approximately 39 kcal (163 kJ), 0.6 g protein, 0.1 g fat, 0.5 g minerals, 0.8 g fibre, 7.2 g carbohydrate, 888 μg β-carotene, 3 mg sodium, 0.10 g iron, 1094 IU vitamin A, 0.73 mg vitamin E, 3 mg niacin and 89 % water (28, 47).

The nutritional composition of the fruit is related to its biological properties. Papaya can be used for treatment of several diseases like warts, corns, sinuses, eczema, cutaneous tubercles, glandular tumours, blood pressure, dyspepsia, constipation, amenorrhoea, general debility, expel worms and stimulate reproductive organs and many and, as a result, *Carica papaya* L., can be regarded as a nutraceutical (48).

Papaya is a nutritious fruit that aids the weight loss process, as it is low in calories and has a high fibre content. In addition to its laxative power, which helps solve constipation problems, the soluble fibres help reduce cholesterol. The fruit has also anti-inflammatory, antioxidant, emollient, sedative, and diuretic properties due to its high vitamin content,

soluble fibre, and bioactive compounds, which include carotenoids and polyphenols (48-50).

The latex from unripe papaya fruit contains proteolytic enzymes like papain and chymopapain which have antiviral, antifungal, and antibacterial properties (28, 48, 51).

Proteolytic enzymes help break proteins down into smaller protein fragments called peptides and amino acids. Therefore, papain is a popular ingredient in meat tenderizer. Papain is a popular folk remedy to reduce pain, inflammation, and swelling. It has also been used to improve digestion and to treat infections, diarrhoea, and allergies. Moreover, it is being studied for potential use in cancer and other diseases (52).

If the papaya is ripe, it can be eaten raw. However, unripe papaya should always be cooked before eating, especially during pregnancy, as the unripe fruit is high in latex, which can stimulate contractions (53). During the ripening process of the fruit, changes occur in the profiles and contents of phenolic compounds, which contribute to the taste and to the disappearance of the latex, a natural indicator of the ripeness of the fruit (54, 55).

As it is widely known, the oxidative stress caused by an increased reactive oxygen and nitrogen species (ROS and RNS, respectively) formation is associated with deleterious effects in various cellular components and has been recognized to be involved in the pathology of various diseases such as neurodegenerative diseases, diabetes, cancer, and atherosclerosis (56).

As is generally known, phenolic compounds present in plants tissues are an essential part of the human diet and are of considerable interest due to their antioxidant activity, associated with several health benefits. Fruits, vegetables, and beverages are the major sources of phenolic compounds in the human diet (57).

Several epidemiological studies suggest that there is a correlation between the intake of phenolic compounds with a decrease in the incidence of oxidative stress-related diseases (58).

Papaya has powerful antioxidant effects, which may reduce oxidative stress and lower your risk of several diseases. Many researchers believe that excessive free radicals in the brain are an important factor in Alzheimer's disease. Barbagallo *et al.* (59) studied people with Alzheimer's by given a fermented papaya extract during six months experienced a 40% drop in a biomarker. Fermented papaya proved to be a nutraceutical supplement with beneficial effects on immunological, haematological, inflammatory, and oxidative stress parameters in chronic/degenerative diseases. This fruit ingestion is also linked to aging and cancer prevention (60).

Also, other studies asserted that fermented papaya could reduce oxidative stress in older adults and people with prediabetes, mild hypothyroidism, and liver diseases (61-64).

The reduction in oxidative stress is attributed to papaya's lycopene content and its ability to remove iron excess, which is responsible for the production of free radicals (65, 66). Also, another research suggested that lycopene can reduce cancer risk (65). Additionally, papaya may have some unique effects not shared by other fruits. Among 14 fruits and vegetables recognized for their antioxidant properties, only papaya showed anticancer activity against breast cancer cells (67).

Other studies have reported that fruits high contents of lycopene and vitamin C may prevent heart diseases (68-70).

In fact, papaya is a rich source of three powerful antioxidants: vitamin C, vitamin A and vitamin E (48). Vitamin A is a vitamin that is good for the eyesight and helps to support a healthy skin. Vitamin A is also good for the immune system, helping in create better immunity. Vitamin C also has many functions, for example, is good for immunity when engaging in physical exertion such as sport and for learning and focus capacity. This vitamin is also good for bone formation (71).

2.2. Papaya by-products

Besides the importance of the edible part of the fruit, the chemical composition of the by-products of this fruit is also important, not only for its richness in bioactive compounds, but also for the reuse of these raw materials for various industrial areas, including food, pharmaceutical and cosmetics (28).

One example is the proteolytic enzyme papain, extracted from the latex of papaya peel, which is of high commercial importance due to its wide use in the textile, pharmaceutical, cosmetic and food industries (72).

In the textile industry, its use stands out in silk degumming, wool softening, and leather processing (42). In the pharmaceutical industry papain is used for the manufacture of medicines and other chemical formulations to treat various diseases related to the digestive tract, in the formulation of vaccines for deworming cattle, wound treatment, and fever (42). In the food industry, it is used as a meat tenderizer acting on muscle fibres and connective tissue components and in the beverage industry it is used to hydrolyse high molecular weight proteins in beer clarification, avoiding turbidity of this product during prolonged storage and refrigeration (32). Other industrial uses include cosmetic products, such as shampoos, soaps and skin products that incorporate papain in their formulations (42).

The food and agricultural product processing industries generate substantial quantities of phenolic rich by-products, which could be valuable natural sources of

antioxidants (57). Given the high levels of food waste and losses the world produces, it is crucial to value by-products and benefit from their properties to promote a sustainable society, reducing the impact of waste on human health and the environment, as well as the costs of its disposal (73, 74). The recovery of papaya by-products could be economically attractive and a strategic approach to reduce food waste and promote sustainable development.

Although papaya can be eaten fresh, it is known to be commonly used by the food industry to make processed foods where only the pulp is used and the remaining parts of the fruit, peel, and seeds, are rejected.

Some authors report that 6.51 % of seeds, 8.47 % of peel and 32 % of non-edible pulp are produced during the industrial processing of papaya and others report that a single fruit can produce around 1000 seeds or more and that the seed content can be approximately 15 – 20 % of the wet weight of the fruit (75, 76).

According to some authors, the seeds (figure 6) contain 30% – 34% oil with nutritional and functional properties like olive oil, and they can be used as feedstocks for biodiesel synthesis (76-78).

Figure 6 - Papaya seeds (siwaporn999, Getty Images adapted from Canva).

A study by Yanty *et al.* (79), also highlighted the physicochemical characteristics of papaya oil seeds, reporting considerable amounts of oil (27.0 %). The oil contains ten detectable fatty acids, of which 78.33 % were unsaturated. Oleic acid (73.5 %) was the dominant fatty acid, followed by palmitic acid (15.8 %) and stearic acid (9.9 %).

Papaya seeds are also gaining importance due to its medicinal value. Seeds have recently been linked to curing sickle cell diseases, poisoning related renal disorder, and as anti-helminths (80).

The seeds' anthelmintic activity is attributed essentially to carpaine (an alkaloid) and carpasemine (a glycoside). While unripe, the seeds of papaya are rich in benzyl-isothiocyanate, a sulphur compound with germicidal and insecticidal properties. Some

studies also show that the seeds have bacteriostatic activity against gram-positive and gram-negative organisms and may be useful in the treatment of chronic ulcers (80).

In some countries, papaya seeds added with honey are administered to expel roundworms, so its consumption is presented as a preventive strategy against intestinal parasitosis, especially in tropical communities, and has been shown to be effective against human intestinal parasites without significant side effects (81).

Hydroxybenzoic acid and vanillic acid have been described as the predominant phenolic acids in papaya seeds, constituting up to 75 % of total phenolics. These compounds have been associated with various biological properties, including antibacterial, antimicrobial, anti-inflammatory, antioxidant and chemopreventive effects (82).

Besides hydroxybenzoic acid and vanillic acid, Kadiri *et al.* (25) described more phenolic compounds in the seeds, including caffeic and *p*-coumaric acids, with two major flavonoids (quercetin 3-galactoside and kaempferol 3-glucoside). Da Cunha *et al.* (83) observed that caffeic acid and its derivatives exhibit anti-inflammatory activity *in vitro* and *in vivo*, and this activity is due, in part, to the removal of nitric dioxide (NO) and its ability to modulate the expression of nitric oxide synthase enzyme. The *p*-coumaric acid is an intermediate in the synthesis of phenylpropanoids, presenting antioxidant action, reducing serum cholesterol levels and, consequently, increasing the defence mechanism against atherosclerosis (84).

Kawabata *et al.* (85) reported that ferulic acid, present in papaya seeds, exhibits antineoplastic activity against colon cancer correlating the antioxidant capacity of this phenolic acid with the ability to eliminate free radicals and stimulate the cytoprotective effect of several enzymes.

In a general context, all studies to date show that papaya seeds are natural sources of biologically active phytochemicals such as carotenoids, phenolic acids, flavonoids, saponins, tannins and alkaloids, related to various biological properties (73). Seeds are recognised as excellent sources of fatty acids, oleic acid being the predominant one, crude protein, crude fibre, carpaine (alkaloid), benzyl-isothiocyanate and benzyl-glucosinolate (glucosinolates), β-cytosterol (phytosterol), myrosine (enzyme) (26, 61-63).

It should be noted that the qualitative profile of the bioactive compounds present in papaya seeds is still very unclear. It is suggested that further studies are carried out in this area to enhance their application in the food, pharmaceutical and cosmetic areas.

II. Objective

This works aims to evaluate the nutritional properties and bioactive potential of papaya's by-products, namely seeds and peels obtained from two commercial varieties marketed in Portugal (Aliança and Formosa).

Macronutrients, fatty acids profile, vitamin E profile, total phenolic and flavonoid compounds, and antioxidant activity (DPPH· inhibition and FRAP assays) were also determined.

III. Materials and Methods

This chapter describes the methodologies used in the evaluation of the nutritional composition, determination of lipid compounds, bioactive compounds quantification, and antioxidant activity analysis.

3.1. Samples preparation

Mature papaya fruits (varieties Aliança and Formosa) with origin from Brazil, were acquired from two Portuguese supermarket chains, in 2020, and fruit peels and seeds were manually collected. Samples (seeds and peels) were freeze-dried (Telstar Cryodos-80 Terrassa, Barcelona) for preservation and then reduced to a fine and homogeneous powder in an electric grinder (Grindomix GM200 knife mill). Proximate analysis and phytochemical analysis were carried out on dried samples. All the determinations were carried out in triplicates.

3.2. Proximate analysis

Moisture, protein, fat and ash content were determined accordingly to the AOAC procedures (86). Protein content was determined by Kjeldahl method and fat by Soxhlet extracting method, whereas ash content was determined by incineration. Free sugars determination was carried out in a High-Performance Liquid Chromatography (HPLC), and insoluble, soluble, and total fibre content was analysed through enzymatic-gravimetric method. Carbohydrates were calculated by difference. All proximate composition analyses were done in triplicate for each analysed sample.

3.2.1. Moisture

Moisture content was determined using a scale equipped with an infrared lamp (Model SMO Scaltec® 01, Scaltec Instruments, Germany). A portion of proximately 1 g was weighed and dried at 105 ± 2 °C till constant weight. The loss in weight was used to calculate the water content. Results were expressed % of dry weight.

3.2.2. Ash

The determination of total ash content was determined by direct incineration in a muffle furnace, following the official methods of AOAC (AOAC 920.153) (86). It was weighed approximately 1 g of sample that was placed in a muffle furnace (Thermolyne 48000, F48010-26, Electrothermal Engineering Ltd, Essex, UK), heated gradually (every 30 minutes, 50 °C) till 500-550 ± 15 °C and maintained for 12 hours, following the official method of AOAC (association of analytical communities) (86). The white ash content was determined by the weight difference before and after the incineration process. The analyses were performed in triplicate for each sample and the results expressed as g/100 g of sample (dry weight).

3.2.3. Fat

Fat content was determined by Soxhlet method (AOAC 991.36) (86). Soxhlet method is a traditional and the technique recognized by all organizations for the determination of the total fat content in foods. Briefly, anhydrous sodium sulphate (Na_2SO_4) was added to ≈5 g of each sample to retain any residual humidity and treated sand to prevent clogging the passage of solvent. The mixture was transferred to cellulose cartridges and placed into Soxhlet extraction ampoules. The extraction was carried out at 40-60 °C during 8 hours with petroleum ether. After the extraction, the solvent was removed, and the fat content was determined. For that, the fat extracted was put in an oven at 105 °C for periods of 30 minutes until constant weight. The analyses were performed in triplicate and results expressed in percentage of fat (dry weight).

3.2.4. Protein

The determination of protein content was performed by the Kjeldahl method by quantification of total nitrogen in samples (AOAC 984.13) (86). Enough sample (≈0.7 g) weighed in a nitrogen-free paper, was placed in a Kjeldahl tube, along with two Kjeldahl tablets (free of selenium and mercury) (Merck, Darmstadt, Germany), and 20 mL of sulfuric acid (H_2SO_4, 98 %). The Digestion Automat k-424 (Bücchi Labortechnik AG, Switzerland) was connected to the Scrubber B-414 (Bücchi Labortechnik AG, Switzerland) for absorbing the resulting exhaust fumes during digestion and the digestion program was carried for 60 minutes at 420 °C. When the liquid was clean and green the samples were let cooling down at room temperature. Then, after the alkalinization with 90 mL of sodium hydroxide (NaOH, 32 %), it is released ammonia. The ammonia was collected in the distillation K-360 automatic distillation unit (Bücchi®, Bücchi Labortechnik AG, Switzerland) in 60 mL of boric acid (H_3BO_4 4 %; pH 4.65). The result of digestion was titrated with H_2SO_4 (0.2 M) using as an indicator methyl red. Analyses were performed in triplicate for each sample and results were calculated using the conversion factor 6.25, expressed in % dry weight.

3.2.5. Free sugars

Previous preparation of the samples was performed by weighting enough sample (≈500 mg seed and ≈250 mg peel) to a falcon tube completed to 10 mL with deionized water. Tubes were homogenized for 30 minutes, and then centrifuged (5000 rpm, 15 minutes). Afterward, samples were filtered into injection vials using syringe filters. Free sugars determination was carried out in a High-Performance Liquid Chromatography (HPLC) system (Jasco, Tokyo, Japan) equipped with an AS-4050 auto-sampler (Jasco, Tokyo, Japan) and a pump (PU-4180, Jasco, Tokyo, Japan).

The chromatographic separation of the compounds was achieved using a normal phase Supelcosil™ LC-SI column (75 mm × 3.0 mm, 3.0 µm) (Supelco, Bellefonte, USA). The mobile phase was acetonitrile/deionized water, 75:25 (v/v).

Sugars were identified by comparing the relative retention times of each sample peaks with standards. Data were analysed using ChromNav Software (Jasco, Tokyo, Japan). Results were expressed in g/100 g of dry weight.

3.2.6. Carbohydrates

Carbohydrates are presented in five forms: total carbohydrates available, total carbohydrates expressed as monosaccharides, mono + disaccharides, starch, and oligosaccharides. These are expressed in grams and evaluated to the decigram.

Total carbohydrates includes monosaccharides or simple sugars (glucose, fructose, and galactose), disaccharides (sucrose, lactose, and maltose), oligosaccharides (raffinose, stachyose and verbascose) and polysaccharides (starch, glycogen and dextrin), and dietary fibre is not included.

The total carbohydrates content was obtained indirectly by calculation according to the following equation:

T*otal carbohydrates (%) = 100 - (% moisture + % protein + % fat +% ash)*

3.2.7. Fibre

Dietary fibre are substances of plant origin that are resistant to digestion and absorption in the small intestine, with complete or partial fermentation in the large intestine. They include soluble and insoluble non-starch polysaccharides (cellulose, pectin, and hydrocolloids), lignin and resistant starch.

Insoluble, soluble, and total fibre content was analysed through enzymatic-gravimetric method using the Sigma-Aldrich Total Dietary Fibre Assay Kit (TDF-100A kit).

Dietary fibre was considered separately and is not included in the total HC. It was evaluated to the decigram level. Analysis was performed in quadruplicate, and dietary fibre contents were corrected for ash and residual protein.

A sufficient amount of sample (≈0.5 g peel; ≈0.3 g defatted seed) was suspended with 50 mL of 0.08 M sodium phosphate buffer (pH 6.0) and incubated with 100 µL of thermostable α-amylase at 95 °C for 20 minutes under constant stirring (GDE Heated Circulating Bath, VELP® Scientifica, Italy). After adjusting the pH to 7.5 ± 0.2 with sodium hydroxide (NaOH 0.275 M), 100 µL of protease solution (30 mg protease + 600 µL sodium phosphate buffer) was added, and the mixture was incubated for 35 minutes at 60 °C. The pH was adjusted to 4-4.6 with hydrochloric acid (HCl), and 100 µL of amyloglucosidase was added. The suspension was incubated for 35 minutes at 60 °C. To the samples for soluble fibre determination, it was added a last step of adding 200 mL ethanol (96 % v/v). It was left overnight at room temperature. Samples were transferred to crucibles in a filtration ramp (CSF 6 Filtration System, VELP® Scientifica, Italy).

Soluble fibre was obtained by washing the sample under vacuum, with three volumes of 200 mL of ethanol (78 % v/v), two volumes of 10 mL of ethanol (96 % v/v), and two

volumes of 10 mL of acetone. Insoluble fibre was obtained by washing the sample under vacuum, with three volumes of 200 mL of water, two volumes of 10 mL of ethanol (96 % v/v), and two volumes of 10 mL of acetone. After filtration, crucibles were stored in the oven at 105 °C, overnight.

Half of the samples was used for ash determination by incineration (5 hours at 525 °C) and the other half was used for protein determination by titration after Kjeldahl digestion (nitrogen conversion factor: 6.25). Total dietary fibre (TDF) content was obtained according to the following equation:

TDF *(%) = [(sample residue - protein - ash - blank) / sample mass] × 100*

3.3. Lipidic fraction extraction

The lipid fraction of each sample was extracted according to the conditions previously described by Alves *et al.* (87), with minor modifications.

An appropriate amount of sample (≈150 mg) with a 15 % total fat content, were added 75 µL of BHT 0.1 % (m/v), 50 µL of tocol (internal standard, 0.1 mg/mL) and 1 mL of absolute ethanol. The solution was mechanically homogenized for 30 minutes in an orbital vortex mixer (VV3, VWR Int.) and afterwards was added 2 mL of n-hexane (HPLC grade). The solution was homogenized for 30 minutes and then was added 1 mL of NaCl 1 % (m/V). The solution was vortexed and centrifuged (5000 rpm for 5 minutes) (Heraeus Labofuge A, Hanau, Germany) and the supernatant was collected to a falcon tube and stored.

The residue obtained was re-extracted with 2 mL of n-hexane, homogenized for 30 minutes, and then centrifuged (5000 rpm for 5 minutes). The supernatant was collected, and the organic phases of both extractions were mixed. It was added a sufficient amount of anhydrous sodium sulphate (Na_2SO_4) to the solution. The mixture was vortexed and centrifuged (5000 rpm for 5 minutes) to collect the n-hexane layer.

The supernatant was collected and evaporated in nitrogen stream until 1 mL final volume. From this solution, 500 µL were promptly injected in a HPLC-DAD-FLD (high-performance liquid chromatography coupled to diode array detector and fluorescence detector) system for determination of the vitamin E profile. The other 500 µL were used to the determination of the fatty acids (FA) profile by GC-FID (gas chromatography coupled with flame ionization detector). All extractions were performed in amber glassware in order to be protected from light.

3.3.1. Vitamin E determination

The vitamin E profile by HPLC-DAD-FLD (of the lipidic fraction of the samples), was determined according to the conditions previously described by Alves *et al.* (87), with minor modifications.

The chromatographic analysis was carried out in a HPLC system (Jasco, Tokyo, Japan) equipped with a MD-2015 multiwavelength diode array detector (DAD) coupled to a FP-2020 fluorescence detector (Jasco, Tokyo, Japan), programmed for excitation at 290 nm and emission at 330 nm. The chromatographic separation of the compounds was achieved using a normal phase Supelcosil™ LC-SI column (75 mm × 3.0 mm, 3.0 µm) (Supelco, Bellefonte, USA). The eluent used was 1.2 % 1,4-dioxane in n-hexane (HPLC grade), at a flow rate of 0.600 mL/min.

Vitamin E vitamers were identified by comparison of their retention times with those of standards (α, β, γ, δ-tocopherols and α, β, γ, δ-tocotrienols), based on their UV spectra. Quantification was performed based on the fluorescence signals, converted to concentration units through calibration curves obtained from commercial standards of each compound, using the internal standard method. The results were expressed as mg/100 g of sample (dry weight).

3.3.2. Fatty acids profile

The fatty acids profile was obtained using a gas chromatograph coupled with flame ionization detector (GC-FID) after derivatization to FA methyl esters according to ISO 12966-2017 (88).

The extract obtained in the previous lipid fraction extraction was resuspended in 1 mL dichloromethane and transferred to glass tubes and then added and mixed 1.5 mL of KOH (in methanol, 0.5 M). The tubes were placed in a heating block (SBH130D/3, Stuart, Stafford, UK) at 100 °C, for 10 minutes. After, the tubes were left at room temperature (RT) for 1 minute and then placed in ice for 5 minutes. Then, was added and mixed 1.5 mL of BF_3 (in methanol, 14 %).

The tubes were placed in the heating block at 100 °C, for 30 minutes. After, the tubes were left at RT for 1 minute and then placed in ice for 5 minutes. Then, were added and mixed 1.5 mL of deionized water and 3 mL of n-hexane (HPLC grade). The tubes were centrifuged at 3000 rpm, for 5 minutes (Heraeus Labofuge A, Hanau, Germany) and the superior phase was collected. It was added anhydrous sodium sulphate, the mixture was centrifuged (3000 rpm for 5 minutes) and the supernatant was transferred to an injection vial for GC analysis.

For separation, the equipment used was a GC-2010 Plus gas chromatograph (Shimadzu, Tokyo, Japan), according to Nunes *et al.* with minor modifications (89).

The work conditions were an automatic sampler and a split/splitless auto injector (AOC-20i Shimadzu) operating with a 50:1 split ratio at 250 °C (injection), a CP-Sil 88 silica capillary column, 50.0 m x 0.25 mm inner diameter and 0.20 μm film thickness from Varian (Middelburg, Netherlands) and a Flame Ionization Detector (Shimadzu, Tokyo, Japan) at 270 °C.

The injection volume was 1.0 μL, the carrier gas used was helium (3.0 mL/min) and the analyses were performed applying the following temperature programme: 120 °C held for 5 minutes, 2 °C/min to 160 °C held for 15 minutes and 2 °C/min to 220 °C held for 10 minutes.

The FA methyl esters were identified by comparison with a standard mixture (FAME 37, Supelco, Bellefonte, PA, USA) and data were analysed based on relative peak areas. Results of FA were expressed in relative percentage of total FA.

3.4. Bioactive compounds and antioxidant activity

For the preparation of papaya peel and seeds extracts, the powdered samples (250 mg peel and 500 mg seed) were extracted in triplicate with 50 mL of an ethanol:water solution (50:50 v/v) under stirring at 40 °C for 1 hour. Afterwards, the solution was filtered through Whatman n.° 4 filter paper. 1 mL of the filtered solution was collected to 10 mL tubes and the obtained extracts were stored at -20 °C.

The extracts were used to determine the total phenolics, total flavonoids, and antioxidant activity by DPPH free radical scavenging and ferric antioxidant power (FRAP) assays. All determinations were performed in triplicate.

3.4.1. Total phenolics content

Total phenolics content (TP) was spectrophotometrically determined by Folin-Ciocalteu (FC) method that is based on a transfer of an electron, in alkaline conditions, from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes from the FC reagent. The electron transfer reaction shifts the colour of the reaction medium to intense blue colour, increasing absorbance values measured at 765 nm (90, 91).

The TP content was determined according to the conditions previously described by Alves *et al.* (92), with minor modifications.

Using a 96-well clear bottom microplate for absorbance measuring, the standards, reactants, and samples were added. Briefly, 30 µL of each extract were mixed with 150 µL of FC reagent (1:10) and 120 µL of Na_2CO_3 aqueous solution (7.5 % m/v).

The mixture was first incubated at 45 °C, for 15 minutes, followed by 30 minutes incubation in the absence of light, at room temperature before absorbance readings at 765 nm were performed using a Synergy HT Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA).

Total phenolics content was calculated from a calibration curve prepared with gallic acid (5 - 100 mg/L; $R^2 = 0.999$) and expressed as mg of gallic acid equivalents (GAE)/g dry weight sample.

3.4.2. Total flavonoids content

Total flavonoids content (TFC) was quantified by a colorimetric assay performed according to the conditions previously described by Costa *et al.* (93), with minor modifications. This colorimetric method is based on the formation of coloured complexes upon the reaction between Al (III) and the carbonyl and hydroxyl groups of flavonoids, preceded by a nitridation of the flavonoids, which absorbance can be measured at 510 nm (94).

Using a 96-well clear bottom microplate for absorbance measuring, the standards, reactants, and samples were added. Briefly, aliquots of 1 mL of each sample extract were mixed with 4 mL of distilled water and 300 µL of 5 % sodium nitrite ($NaNO_2$). After 5 minutes at room temperature, 300 µL of 10 % aluminium chloride ($AlCl_3$) were added, and after 1 minute, were added 2 mL of sodium hydroxide (NaOH) (1 M) and 2.4 mL of distilled water.

Epicatechin was used to plot a standard curve (2.5 - 400 mg/L; $R^2 = 0.999$) and absorbance read at 510 nm with a Synergy HT Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA). Results were expressed as mg of catechin equivalents (CE)/g of sample.

3.4.3. Antioxidant activity

Regarding antioxidant activity, there is not a specific analytical method and for it determination in food samples, since foods are complex matrices, with several bioactive compounds with specific chemical characteristics.

The DPPH free radical scavenging and ferric antioxidant power assays were selected to evaluate the antioxidant activity of papaya seeds and peels extracts, since these methods are simple, relatively fast, act by two complementary mechanisms of action and can provide valuable information about the type of antioxidants present in the samples, including their mechanism of action.

3.4.3.1. DPPH· scavenging assay

The radical scavenging ability of extracts was analysed according to the method described by Costa *et al.* (93), with minor modifications.

In the day of the assay the Trolox standard (562 ppm (mg/L)) and a 6.1×10^{-5} M DPPH· stock solutions were prepared in absolute ethanol and kept in 4 °C, protected from the light. The DPPH· solution was adjusted with absolute ethanol to an absorbance of 0.650 - 0.660 at 525 nm in a 1 cm cuvette at 25 °C.

Using a 96-well clear bottom microplate for absorbance measuring, the standards, reactants, and samples were added. Briefly, 30 µL of Trolox standard (562 mg/L) / blank / diluted extract (1:10) were mixed with 270 µL of the DPPH· solution previously prepared. The decrease of the DPPH· was measured with a Synergy HT Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA), in equal time intervals of 10 minutes by monitoring the decrease of absorption at 525 nm, to observe the kinetics reaction. The reaction endpoint was attained in 20 minutes.

A calibration curve was prepared with Trolox (5.62 - 175,34 mg/L; $R^2 = 0.999$) and DPPH· scavenging activity was expressed in mg of Trolox equivalents (TE)/g of sample.

The DPPH· scavenging effect of the sample extracts was calculated according to the following formula:

$$Scavenging\ effect\ (\%) = [(absorbance_{t=0min} - absorbance_{t=20min}) / absorbance_{t=0min}] \times 100$$

3.4.3.2. Ferric reducing antioxidant power (FRAP) assay

Upon the reduction from Fe^{3+} to Fe^{2+}, because of the antioxidant action, the Fe^{2+}-TPTZ (ferrous-tripyridyltriazine) complex develops an intense blue colour. The absorbance can be measured to determine the amount of iron reduced from its ferric to its ferrous form and correlate that information with the antioxidant activity (95).

The FRAP assay was performed according to Costa *et al.* (93), with minor modifications. Using a 96-well clear bottom microplate for absorbance measuring, the standards, reactants, and samples were added. Briefly, 35 µL of ferrous sulphate standard

(5 - 600 μ*M*)/ blank/ diluted extract (1:10) were mixed with 265 μL of the FRAP reagent (containing 0.3 M acetate buffer, 10 mM TPTZ solution, and 20 mM of ferric chloride).

The mixture was kept for 30 minutes at 37 °C protected from light and then, the absorbance was measured at 595 nm using a Synergy HT Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA).

A calibration curve was prepared with ferrous sulphate (5 - 600 μM; $R^2 = 0.999$) and ferric reducing antioxidant power was expressed as μmol of ferrous sulphate equivalents (FSE)/g of sample.

IV. Results and discussion

Suitable nutrition is one of the pillars of public health. The estimation of nutrition intake from food consumption requires dependable data in food composition, which is essential of food based dietary guidelines for healthy nutrition, containing the necessary data regarding specific nutrients in different food sources.

Moreover, Information on food composition is also of great importance for scientists working in the field of sustainability and by-products reused. Proximate composition and starch nutritional properties of two papaya by-products fruit varieties were assessed to identify unique varieties that could be used in value added foods. Results of proximate analyses of the seeds and peels are shown in Table 1.

Table 1- Proximate composition in papaya fruit by-products (peels and seeds). Results are expressed in % of dry weight.

	PC	**MC**	**PS**	**MS**
Moisture	5.31 ± 0.35	6.65 ± 0.22	8.18 ± 0.32	8.14 ± 0.40
Total Fat	2.9 ± 0.30	3.5 ± 0.39	25.3 ± 0.23	25.3 ± 0.34
Protein	26.57 ± 0.03	19.86 ± 0.13	25.58 ± 0.21	23.67 ± 0.31
Ashes	15.82 ± 0.02	13.83 ± 0.06	8.62 ± 0.02	9.50 ± 0.08
Total carbohydrates	54.75 ± 0.33	62.84 ± 0.32	40.51 ± 0.02	41.57 ± 0.62
Total dietary fibre	34.76 ± 0.05	31.69 ± 0.05	45.22 ± 0.06	50.57 ± 0.11

PC - Aliança peels; MC - Formosa peels; PS – Aliança seeds; MS - Formosa seeds.

Proximate analysis of the papaya seeds of Formosa variety showed that it contained 8.14 ± 0.40 % moisture, 25.3 ± 0.34 % fat, 23.67 ± 0.31 % protein, 50.57 ± 0.11 % total dietary fibre, 9.50 ± 0.08 % ashes, and 41.57 ± 0.62 % remaining carbohydrates. Seeds of Aliança fruit showed similar values, 8.18 ± 0.32 % moisture, 25.3 ± 0.23 % fat, 25.58 ± 0.21 % protein, 45.22 ± 0.06 % total dietary fibre, 8.62 ± 0.02 % ashes, and 40.51 ± 0.02 % remaining carbohydrates. These values were comparable to those of the other varieties reported previously (79).

Total protein contents were similar in peels and seeds of both varieties, ranging between 19.86 % and 26.57 %.

Data recorded for carbohydrate ranged between 40.51 % to 62.84 %, and the highest percentage was found in the peel. Our results were lower to those described by USDA (96)

(64.5 - 87.8 %) and Saxholt *et al.* (97) but higher to the results described by Chukwuka *et al.* (41) for ripe and very ripe papaya (14.63 - 29.03 % and 9.95 - 27.50 %, respectively).

Despite the differences found with the results described by other authors, it should be noted that many factors affect the nutritional composition of fruits, such as cropping system, maturation index, among others.

However, experimental results showed that papaya seeds are a potential source of proteins, lipids, and inorganic matter while fruit peels, also present high content of proteins and minerals, but low content of fat. Fruit peels also revealed considerable fibre content.

Sugars in the free form (glucose and fructose) were detected in low amounts, although present in higher quantity on fruit peels (figure 7).

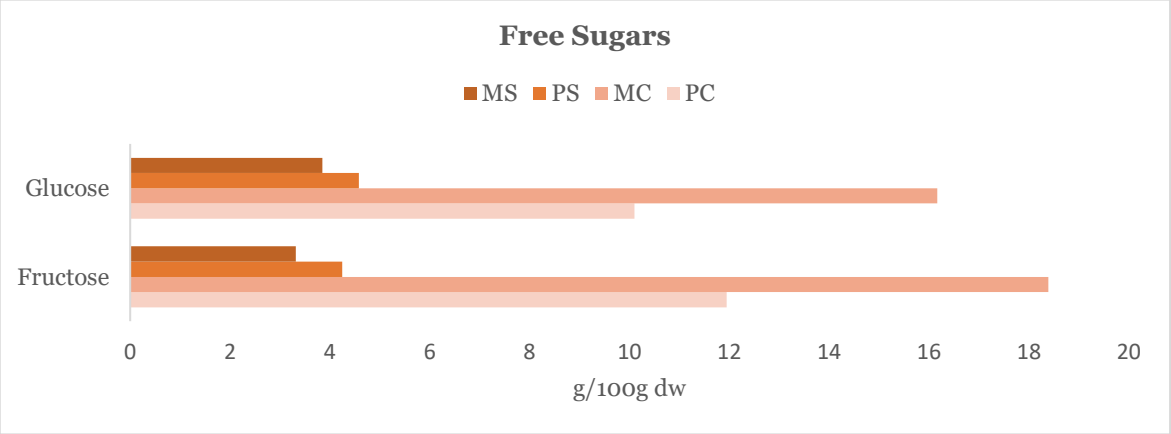

Figure 7 - Free sugar determination in papaya peels and seeds. Results are expressed in g/100 g of dry weight. (PC - Aliança peels; MC - Formosa peels; PS – Aliança seeds; MS - Formosa seeds).

As expected, free sugar content was higher in fruit peels than in fruit seeds. Formosa peels displays the highest content of free sugars (18.39 g/100g and 16.17 g/100g for fructose and glucose, respectively). Like seeds, the two fruit varieties peels also show considerable fibre content.

It is well known that sugar consumption, especially added sugars, is under attack. Several government and health authorities have suggested new sugar recommendations and guidelines as low as 5 % of total calories from free sugars. Therefore, water soluble sugars from papaya fruit peels could be a natural alternative to replace synthetic sweeteners.

As far as natural antioxidants are concerned, vitamin E content was also analysed (table 2). Vitamin E is known as a natural antioxidant providing strong immunity and healthy skin and eyes. In recent years, vitamin E supplements have become popular as antioxidants. Several studies have proven the presence of a wide range of bioactive compounds, including vitamin E, in several fruit industrial by-products which are essentially pomace, peels and seed fractions. Generally, seeds are rich in bioactive lipids whereas peels are considered as a rich source of dietary fibres.

Table 2 - Vitamin E content (mg/100 g) in seeds and peels of papaya Aliança and papaya Formosa.

Vitamer*	**PC**	**MC**	**PS**	**MS**
α-tocopherol	29.27±1.09	33.60±0.51	3.10±0.02	2.67±0.08
β-tocopherol	0.61±0.03	2.29±0.02	n.d.	n.d.
γ-tocopherol	23.13±0.82	36.57±0.36	0.63±0.00	0.59±0.01
γ-tocotrienol	6.48±0.26	10.48±0.03	n.d.	n.d.
δ-tocopherol	2.10±0.12	10.98±0.16	n.d.	n.d.
Vitamin E	61.59±2.29	93.93±1.06	3.73±0.02	3.25±0.09

* Results are expressed in mg/100 g of dry weight. PC - Aliança peels, MC - Formosa peels, PS – Aliança seeds, MS - Formosa seeds. n.d. - not detected.

Vitamin E is known as the most effective natural lipid-soluble antioxidant, protecting cell membranes from peroxyl radicals and mutagenic nitrogen oxide species.

Vitamin E is constituted by tocopherols (α, β, γ and δ) and the respective tocotrienols (that only differ from tocopherols in the saturation degree of their hydrophobic phenyl side chain) (98).

Regarding experimental results, vitamin E (mainly α-tocopherol and γ-tocopherol) ranged between 61.59 - 93.93 mg/100 g for Aliança and Formosa peels, respectively, and 3.25 - 3.73 mg/100 g for Formosa and Aliança seeds, respectively.

The individual fatty acid composition in papaya seeds and peels and the contents of saturated, monounsaturated, and polyunsaturated fatty acid are shown in Table 3.

Table 3 - Fatty acids composition (%) of seeds and peels of papaya Aliança and papaya Formosa.

Compound acid		**PC**	**MC**	**PS**	**MS**
lauric	C12:0	1.37± 0.11	2.34±0.21	n.d.	n.d.
myristic	C14:0	4.43±0.09	5.03±0.05	0.22±0.03	0.20±0.02
palmitic	C16:0	24.57±0.20	26.66±0.20	16.39±0.10	16.38±0.07
palmitoleic	C16:1	5.43±0.12	7.69±0.09	0.43±0.06	0.36±0.03
heptadecanoic	C17:0	0.80±0.08	0.53±0.09	n.d.	n.d.
stearic	C18:0	3.64±0.27	3.11±0.08	4.52±0.04	4.72±0.02
oleic	C18:1n9c	9.92±0.42	8.74±0.25	72.60±0.20	73.60±0.05
linoleic	C18:2n6c	14.45±0.41	8.41±0.29	4.81±0.22	3.56±0.05
arachidic	C20:0	1.02±0.10	1.13±0.08	0.40±0.03	0.37±0.04
α-linolenic	C18:3n3	28.14±0.48	30.28±0.20	0.16±0.02	0.20±0.04
cis-11-eicosanoic	C20:1n9	n.d.	n.d.	0.28±0.02	0.34±0.02
behenic	C22:0	1.83±0.07	1.79±0.05	0.18±0.03	0.25±0.01
tricosanoic	C23:0	0.83±0.13	0.83±0.03	n.d.	n.d.
lignoceric	C24:0	3.57±0.03	3.46±0.25	n.d.	n.d.
SFA		42.05±0.09	44.88±0.46	21.71±0.02	21.93±0.03
MUFA		15.35±0.31	16.43±0.25	73.32±0.23	74.31±0.04
PUFA		42.60±0.28	38.69±0.47	4.98±0.24	3.76±0.02
n6/n3		0.51±0.02	0.28±0.01	29.40±1.76	17.86±3.54
n9/n6		0.69±0.04	1.04±0.05	15.11±0.75	20.70±0.30

PC - Aliança peels, MC - Formosa peels, PS – Aliança seeds, MS - Formosa seeds. SFA - saturated fatty acids, MUFA - monounsaturated fatty acids, PUFA - polyunsaturated fatty acids. n.d. - not detected.

According to our results, fruit peels contain thirteen detectable fatty acids with predominance of polyunsaturated and saturated acids. The peel of both varieties of papaya, consists mainly of unsaturated fatty acids, due to the high contents of α-linolenic acid (C18:3n3), the most dominant fatty acid (30.28 % in Formosa and 28.14 % in Aliança variety), followed by palmitic acid (C16:0) (26.66 % and 24.57 % in Formosa and Aliança peels, respectively). According to Coimbra and Jorge (99) the presence of unsaturated fatty acids, mainly the essentials omega-3 and omega-6, are very important for human health. Despite the few existing studies on papaya by-products, our results are in agreement with data reported in literature revealing a very similar fatty acid profile and content in the other fruit by-products, where unsaturated fatty acids prevail in a defined amount of 70 to 85% and where palmitic acid and α-linoleic acid were the major saturated and unsaturated fatty acid, respectively (100).

The fatty acid profile in fruit seeds was quite different from that described in peels, being oleic acid the most representative one, a monounsaturated fatty acid linked to health benefits. The seeds contained ten detectable fatty acids, of which 74.31 % in Formosa and 73.32 % in Aliança variety, were monounsaturated. Oleic acid (C18:1n9c) was the dominant

fatty acid (73.6 % and 72.6 % in Formosa and Aliança, respectively), followed by palmitic acid (C16:0) (16.38 % and 16.39 % in Formosa and Aliança, respectively) and stearic acid (C18:0) (4.72 % and 4.52 % in Formosa and Aliança, respectively).

The fatty acid composition of Aliança and Formosa varieties is compared with those of other varieties reported in the literature. For example, Yanty *et al.* (79) described 78.33 % of unsaturated fatty acids in seeds of Hong Kong/Sekaki papaya variety, with oleic being the dominant fatty acid (73.47 %). Also, Malacrida *et al.* (101) evaluated lipid profile of papaya seeds and reported that 18:1n-9 (71.30 %), 16:0 (16.16 %), 18:2n-6 (6.06 %) and 18:0 (4.73 %) were the major fatty acids. Martin *et al.* (102) recommended that PUFA:SFA values should be higher than 0.45. Only papaya peels in both fruit varieties presented an healthy food value: 1 and 0.86 for Aliança and Formosa, respectively. These results are also comparable to the commercial high-oleic acid vegetable oils derived from sunflower (>80 %), safflower (77 %) and canola (75 %) (102).

The proximate composition, and fatty acids composition of the evaluated fruit parts (peel and seeds) are different. Peel and seeds of these two papaya fruits varieties have different amounts of evaluated nutrients. The findings of this study highlight the potential of all parts of papaya as a valuable source of nutrients, including fatty acids.

4.1. *In vitro* bioactivity evaluation

Bioactive compounds from fruit by-products find several applications in the different fields of food processing industry. For instance, they can be included as natural additives in food products, thus promoting the preservation and the enhancement of the quality, as well as the prevention of food oxidation and pathogenic microorganisms' growth.

The total phenolic and total flavonoids content of the hydroalcoholic peel and seeds extracts obtained from both papaya varieties are presented in table 4. Also, antioxidant activity is presented in table 4.

Table 4 - Total phenolics, total flavonoids and antioxidant activities, obtained from seeds and peels hydroalcoholic extracts of papaya Aliança and papaya Formosa.

	Total Phenolics (mg GAE/g)*	**Total Flavonoids** (mg CE/g)*	**FRAP** (µmol FSE/g)*	**DPPH·** (% Scavenging effect)*
PC	8.92 ± 0.37	2.54 ± 0.08	89.39 ± 6.41	26.81 ± 1.7
MC	7.99 ± 0.45	2.27 ± 0.08	74.30 ± 4.40	26.16 ± 1.2
PS	4.67 ± 0.15	1.27 ± 0.05	79.16 ± 2.27	30.30 ± 1.6
MS	4.31 ± 0.15	1.11 ± 0.05	67.62 ± 1.79	28.46 ± 1.5

*Results are expressed in dry weight. PC - Aliança peels, MC - Formosa peels, PS – Aliança seeds, MS - Formosa seeds.'

The highest total phenolic content was found in Aliança peels (8.92 mg GAE/ g). Moreover, the total phenolic content in both fruit peels varieties, were significantly superior to those found in fruits seeds. This conclusion has been verified by other authors, in identical works, but with other fruits. In fact, all were unanimous in stating that the polyphenol content is always higher in the fruit peels than in its seeds (103, 104).

Flavonoids were, also, superior in fruit peels than in seeds. Papaya seeds, regardless of the fruit variety, revealed lower levels of both total phenolics and flavonoids, in an identical proportion, half the amount obtained in the fruit peels. Nevertheless, the total phenolic and flavonoids contents, in Formosa fruit variety were the lowest detected, either in seed or peel. High content of phenols (85.67 mg/100 g) in unripe papaya fruit was described by Joymak *et al.* (105). It appears that the increase in fruit maturation increases the content of bioactive compounds, not only in the edible part of the fruit, but also in its peels. Our results are in accordance with those reported by Santos *et al.* (106), in a similar study but with other papaya cultivars (Calimosa and Hawaii) and by Castro-Vargas *et al.* (107) in a valuation study about the recovery of phenolic antioxidants by supercritical fluid extraction. However, our results are inconsistent with the values described by Sancho *et al.* (84), who described lower values of total phenolics in Maradol papaya peel (1.9 mg/g).

As far as we know, total phenolics and flavonoids contents and antioxidant activity of plants are influenced by several factors including varieties, maturity, and solvent extractor. Also, the amount of flavonoids varies greatly depending on several factors such as disease, insect/pest attack, climate change, ultraviolet radiation, and others. Other factors include cultivars, location of growth, agricultural practices, harvest, and storage conditions as well as processing and preparation methods. Moreover, it is likely that the high level of total phenolics and flavonoids in the peel fruits is due to the protective activities of antioxidants, which are essential to protect the fruit and its seed from harmful environmental facts (75, 108, 109).

The DPPH free radical scavenging assay measures the ability of antioxidant substances, present in samples, to neutralise the free radical DPPH· (2,2-diphenyl-1-picrylhydrazyl). In solution, DPPH· presents a strong purple colour, with absorbance peaks between 515 and 520 nm. When it encounters certain antioxidant compounds in the sample, they will neutralise the radical by donating a hydrogen atom (or an electron) and converting into a yellow-coloured compound. The loss of purple colour can be monitored over time by spectrophotometry and correlates with the anti-radical capacity of the tested sample (90, 91, 110).

Despite the difference in the total content of bioactive compounds, the antioxidant activity was identical in both seeds and peels' fruit varieties. The values obtained by the DPPH· assay ranged between 26.16 % - 30.30 %, showing no significant variations between

the two sub-products (peels and seeds) and between fruit varieties. Through FRAP antioxidant activity assay, results shows that fruit variety may exert greater effect on antioxidant potential, since Aliança variety presents higher antioxidant activity in peels (89.39 μmol FSE/g) and seeds (79.16 μmol FSE/g) when compared to Formosa variety peels and seeds (74.30 μmol FSE/g and 67.62 μmol FSE/g, respectively). According to Halliwell (111), the natural antioxidant defense in fruits and vegetables is associated with three main groups: ascorbic acid and phenolic compounds as hydrophilic antioxidants, and carotenoids as lipophilic antioxidants. However, the antioxidant activity of fruits is affected by several factors, including environmental aspects, ripening, fruit variety, type of extraction solvent, and extraction conditions.

The results showed that the bioactive compounds contributing to the antioxidant capacity varied according to the samples and analytical methods. This is due to the presence of other compounds, as well as the chemical composition of each sample, once the antioxidant effects may be a result of the sum of individual components exhibiting synergistic or antagonistic effects.

V. Conclusion and future work

Millions of tons of fruit by-products are discarded globally every day by food processing industries, which represents a considerable loss in terms of nutrients. Moreover, peels and seeds from fruits are annually discarded in large quantities in an industrial production that only uses fruit pulps. The use of these by-products has been little explored. However, some reports have been attracting interest describing them as a good source of macro and micronutrients, dietary fibre, and bioactive compounds.

Papaya by-products (seeds and peels) are promising sources of macro and micronutrients. Also, with the presence of bioactive compounds (phenolics and flavonoids), these by-products could be used as source of antimicrobial, antioxidants, colorants, flavouring and thickening agents.

Although the individual profile of bioactive compounds should be considered in a future study, the results obtained confirm the great potential for industrial recovery and related applications, such as formulation of new food ingredients.

IV. References

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Supplementary materials

1. HPLC-DAD-FLD chromatograms of Vitamin E

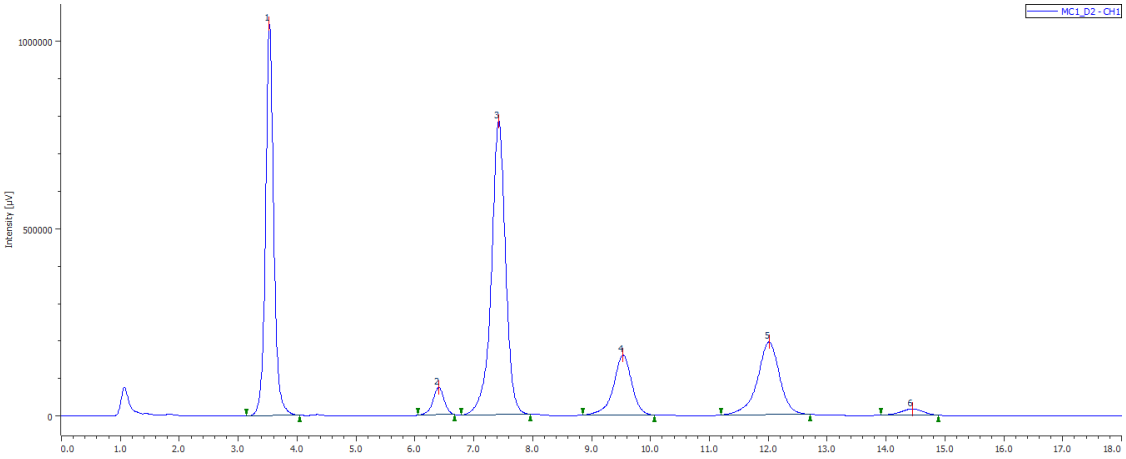

Figure 8- Vitamin E profile of papaya Formosa peel.

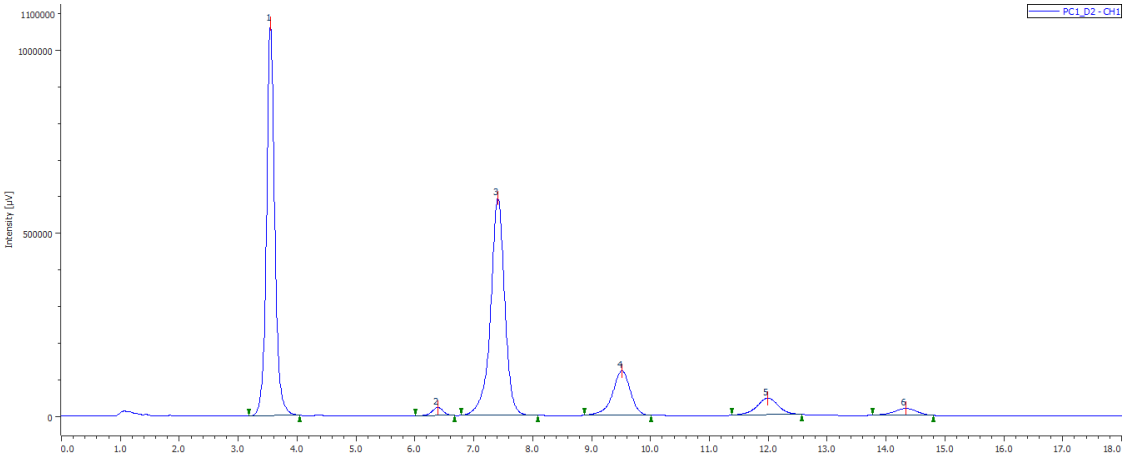

Figure 9- Vitamin E profile of papaya Aliança peel.

1- α-tocoferol, **2-** β-tocoferol, **3-** γ-tocoferol, **4-** γ-tocotrienol, **5-** δ-tocoferol

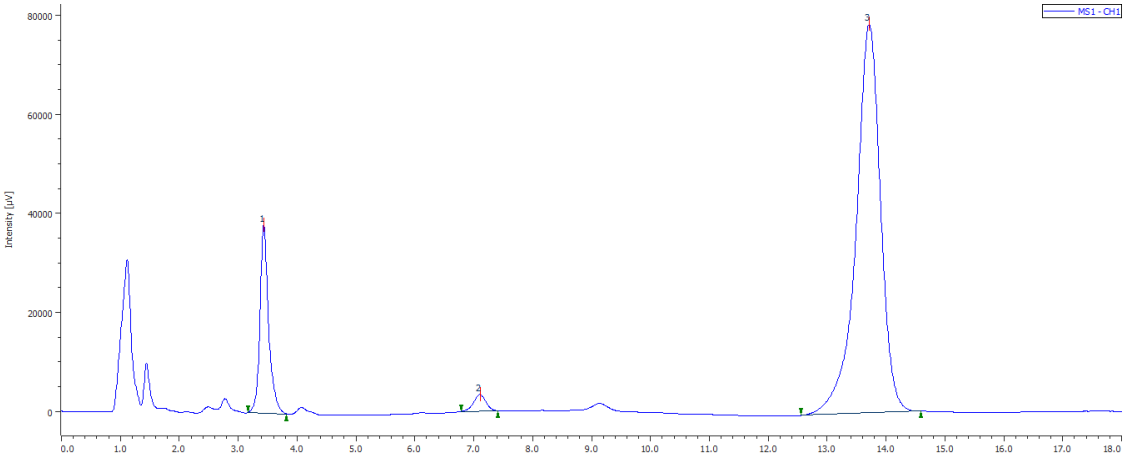

Figure 10- Vitamin E profile of papaya Formosa seed.

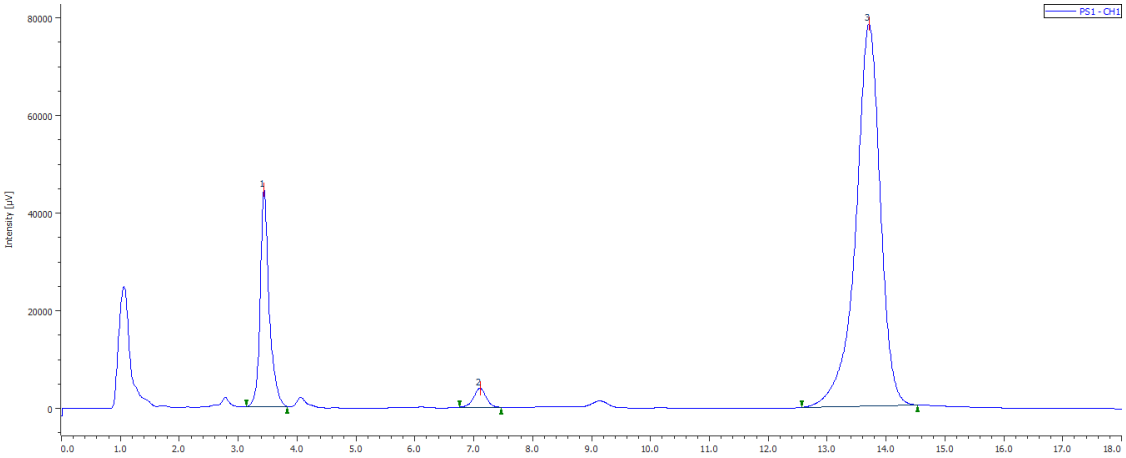

Figure 11- Vitamin E profile of papaya Aliança seed.

1- α-tocoferol, **2-** γ-tocotrienol

2. GC-FID chromatograms of fatty acids

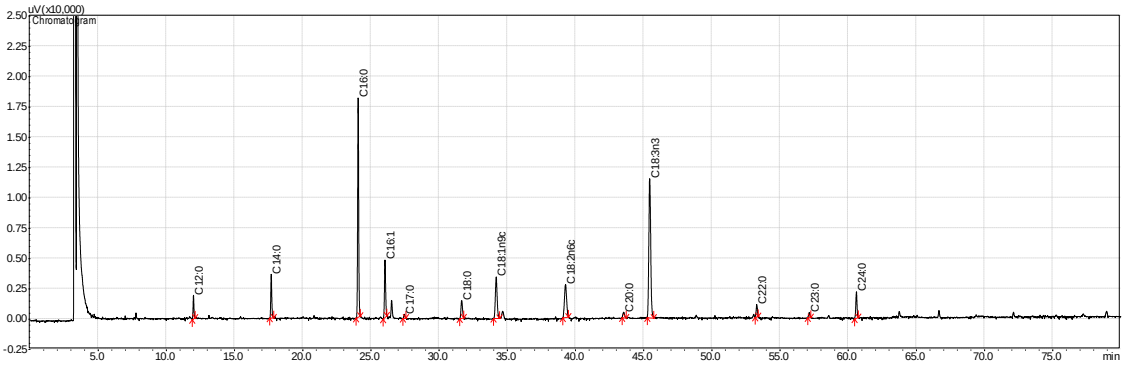

Figure 12- Fatty acids chromatogram of papaya Formosa peel.

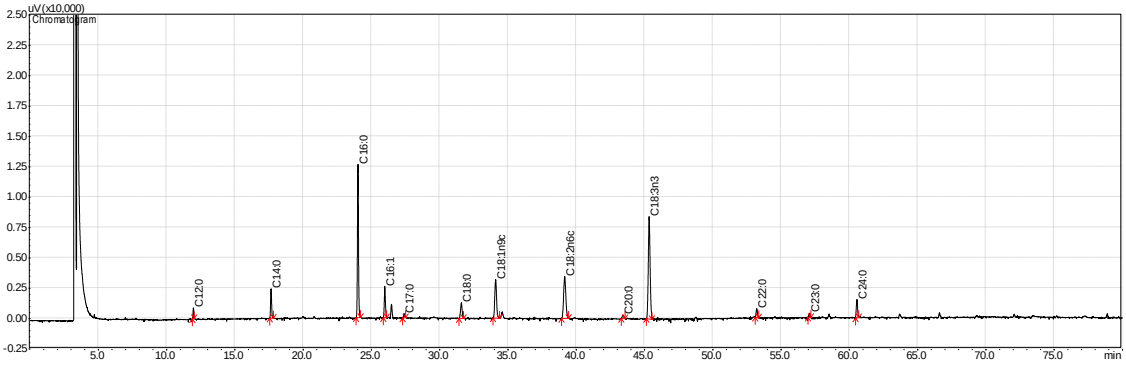

Figure 13- Fatty acids chromatogram of papaya Aliança peel.

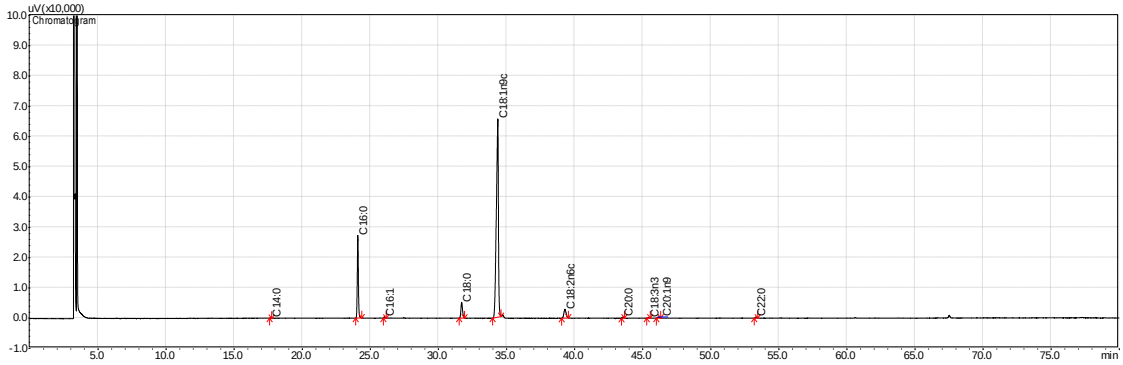

Figure 14- Fatty acids chromatogram of papaya Formosa seed.

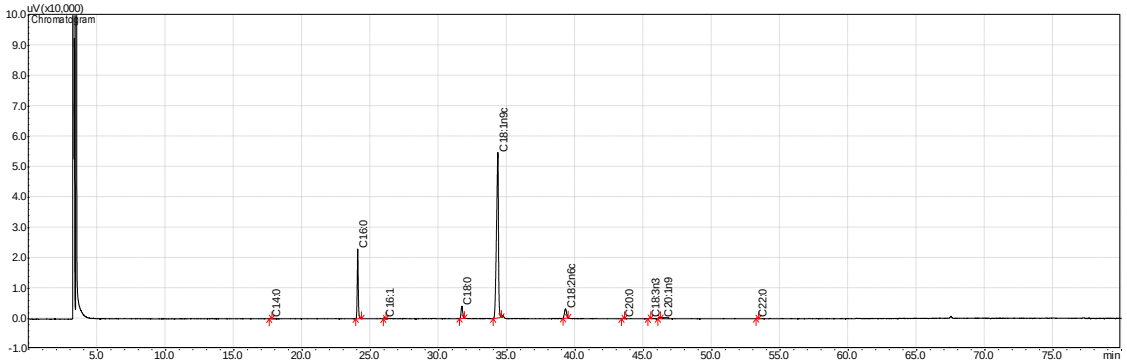

Figure 15- Fatty acids chromatogram of papaya Aliança seed.

C12:0 - lauric, **C14:0** - myristic, **C16:0** - palmitic, **C16:1** - palmitoleic, **C17:0** - margaric or heptadecanoic, **C18:0** - stearic, **C18:1n9c** - oleic, **C18:2n6c** - linoleic, **C20:0** - arachidic, **C18:3n3** - α-linolenic, **C20:1n9** - cis-11-eicosanoic, **C22:0** - behenic, **C23:0** - tricosanoic, **C24:0** - lignoceric