INSTITUTO POLITÉCNICO DE BRAGANÇA
Escola Superior Agrária

Ananas comosus L. bio-waste as a potential source of phenolic compounds with food application

Bruna Penedo de Carvalho Moreira

Dissertation submitted to Escola Superior Agrária de Bragança to obtain the Degree of Master in Biotechnological Engineering

Supervised by

Lillian Barros

Cristina Caleja

Bragança

2021

Master thesis conducted under the project "BIOMA – Bioeconomy integrated solutions for the mobilization of the Agro-food market" (POCI-01-0247-FEDER-046112), by "BIOMA" Consortium, and financed by European Regional Development Fund (ERDF), through the Incentive System to Research and Technological development, within the Portugal2020 Competitiveness and Internationalization Operational Program.

Financed by:

UNIÃO EUROPEIA
Fundo Europeu
de Desenvolvimento Regional

Acknowledgments

I am really grateful to everyone who has supported me throughout this journey.

First, I would like to thank God, for never let me lose my faith and for bring in my life, special people that helped me through all the difficulties.

Secondly, I would like to express my sincere gratitude and give my warmest thanks to my supervisor **Doctor Lillian Barros** and my co-supervisor **Doctor Cristina Caleja** who made this work possible. Both gave me the necessary tools as the guidance and advice, so I could through all the stages of writing my project. Thank you for the patient, for helping me in the moments I needed most, for sharing your experience and your huge knowledge at this work. I would also like to thank my lab colleagues for their kindness, generosity, and support in being able to carry out research activities.

I must thank my family and friends who contributed to this realization because without them, this thesis could never have been completed. I would like to thank my mother, **Edilena**, who has never allowed me to give up in the face of adversity, for all the love and for always giving me wings instead of cutting them even knowing the great distance between us. My enormous gratitude to my father, **José Moreira**, the person who made it possible with all his support that I was able to achieve my goals, for believing in my dream and for always being by my side.

My great thanks to my childhood friend, **Kissila Dal Col**, for the comfort of her friendly words, for the advice, by the fellowship even though far, for the support and always making of my achievements, as hers. Thank you for your friendship. My gratitude to my friend, **Ivanildo Oliveira**, who helped me in each step of this journey by donating his time with prayers, his friendship and support for everything that I needed so far, even when I lost faith in myself. Last, but not least, I must thank my boyfriend, **Jorge David**, for his constant encouragement, for understanding, caring and being my life partner.

INDEX

LIST OF ABBREVIATIONS

*a**	Red/green chromaticity
AGS	Gastric adenocarcinoma
AOAC	Association of Official Analytical Chemists
A-T0	"Súplicas" with *Ananas comosus* L. bio-residue in time 0 days
A-T3	"Súplicas" with *Ananas comosus* L. bio-residue in time 3 days
A-T7	"Súplicas" with *Ananas comosus* L. bio-residue in time 7 days
ATCC	American Type Culture Collection
ATCC 11632	*Staphylococcus aureus* culture collection
ATCC 11730	*Aspergillus versicolor* culture collection
ATCC 13311	*Salmonella typhimurium* culture collection
ATCC 25922	*Escherichia coli* culture collection
ATCC 35030	*Enterobacter cloacae* culture collection
ATCC 36839	*Penicillium funiculosum* culture collection
ATCC 6275	*Aspergillus niger* culture collection
ATCC 9197	*Aspergillus fumigatus* culture collection
*b**	Blue/yellow chromaticity
CA	Pineapple peel
CFU	Colony Forming Unit
CH_0	Optical haemolysis density completes at 0 min
C-T0	Control "súplicas" in time 0 days
C-T3	Control "súplicas" in time 3 days
C-T7	Control "súplicas" in time 7 days
DAD	Diode Array Detector

DM	Dry Matter
DMEM	Culture medium for animal cells
DPPH	2,2-diphenyl-1-picrylhydrazyl
DV	Daily Values
dw	dry weight
EMM	Estimated Marginal Means
EPRS	European Parliamentary Research Service
ESI/MS	Electrospray Ionization Mass elestroscopy
EtOH	Ethanol
FA	Pineapple leaves
FAO	Food and Agriculture Organization
FBS	Fetal bovine serum
FID	Flame ionization detector
fw	fresh weight
GAE	Gallic Acid Equivalent
GC-FID	Gas chromatography with flame ionization
GI_{50}	Concentration that inhibits 50% of cell growth
HaCaT	Human keratinocyte cell line
HBSS	Hank's saline solution
HPLC	High Performance Liquid Chromatography
Ht_{50}	50% haemolytic time (min)
I	Incorporation of the pineapple extract
IC_{50}	Extract concentration corresponding to 50% of the antioxidant activity
INT	*p*-iodonitrotetrazolium chloride

LPS	Lipopolysaccharides
MA	Malt agar
MCF-7	Human breast adenocarcinoma
MDA	Malonaldehyde
MFC	Minimum fungicidal concentration
MIC	Minimum inhibitory concentration
MHB	Mueller-Hinton agar
MUFA	Monounsaturated Fatty Acids
MS	Mass spectrometer
NCI-H460	Lung cancer cell line
NCTC	National collection of type cultures
NO	Nitric Oxide
OxHLIA	Oxidative Haemolysis Inhibition Assay
pH	Potential of hydrogen
PSW	Peel, Seed and Waste
PUFA	Polyunsaturated Fatty Acids
rpm	Revolutions per minute
RSA	Radical Scavenging Activity
SFA	Saturated Fatty Acids
SRB	Sulforhodamine B
ST	Storage time
TBARS	Thiobarbituric Acid Reactive Substances
TE	Trolox Equivalent
UV	Ultraviolet Radiation
v/v	Volume/volume

w/v	weight per volume

LIST OF FIGURES

LIST OF TABLES

LIST OF EQUATIONS

ABSTRACT

Pineapple *(Ananas comosus* [L.] Merr.) is one of the much-appreciated fruit and consumed worldwide not only for its nutritional properties, but also for being recognized for its beneficial characteristics with action on consumer health. However, a large percentage of this fruit is represented by the crown leaves and peel that are not consumed and, industrially, are not exploited. In addition, the fruit pulp processing industries generate tons of various waste, which has aroused great economic and environmental concern, since disposal solutions are quite limited. In this sense, the present study aimed to characterize the phenolic profile of the peel and the crown leaves as well as to evaluate its bioactive potential, aiming in the application of the most promising extract in a pastry product as a natural ingredient with potential application in the food industry.

The phenolic compounds of both matrices were extracted using a heat-assisted hydroethanolic extraction, and subsequently identified and quantified by high performance liquid chromatography coupled with diode detector and mass spectrometry detector (HPLC-DAD-ESI/MS). Twenty-five phenolic compounds were identified in both extracts, comprising phenolic acids and flavonoids. The main compounds detected were caffeic acid derivatives and glycoside flavones, such as apigenin 6,8-*C*-diglucoside.

The antioxidant activity of the extracts was tested by two *in vitro* tests: the lipid peroxidation inhibition assay (TBARS) and the oxidative hemolysis inhibition assay (OxHLIA). The antiproliferative activity of both extracts was evaluated in tumor (MCF-7, NCI-H460, AGS and CaCo-2) and non-tumor (VERO) cell lines using the sulforhodamine B method. Finally, the antimicrobial activity was evaluated, testing the potential of both extracts against a panel of six bacteria and six fungi. The results revealed that both extracts showed an excellent performance in terms of bioactive assays and demonstrated that none of the extracts showed toxicity up to the maximum concentration tested (GI_{50} > 400 μg/mL), proving its application potential as a natural ingredient. Despite the excellent results, the peel extract stood out, presenting an antioxidant and antimicrobial potential superior to that presented by the crown leaves extract, having therefore been selected for being incorporated in a typical Portuguese pastry product, called “súplicas".

The effects of incorporating the natural ingredient on the chemical and proximate composition of the "súplicas" were evaluated over different shelf life (0, 3 and 7 days

after incorporation) and compared with the traditionally produced product. The proximate composition (ash, fats, proteins, carbohydrates contents and energy value) was determined through official methodologies of food analysis (AOAC), while the individual chemical composition was obtained by quantifying the fatty acid content through Gas Chromatography coupled to a Flame Ionization Detector (GC-FID) and the sugar content by HPLC coupled to a refraction index. Also, the variation in colour parameters (*L**, *a** and *b**) was evaluated in all samples of all times. The results showed that the extract did not cause changes in proximate composition, sugar and fatty acid content and in colour parameters.

In turn, the evaluation of the antioxidant activity of the "súplicas" revealed that the incorporation of the extract increased the antioxidant potential of the food product compared to the traditional recipe. Thus, this study confirms the potential application of this pineapple bio-waste as a source of bioactive compounds, aiming at reusing a low-cost natural resource and directly contributing in the environmental and economic impact.

Keywords: Pineapple, bio-waste, bioactive compounds, phenolic compounds, "súplicas", nutritional value.

RESUMO

O ananás (*Ananas comosus* [L.] Merr.) é um fruto muito apreciado e consumido mundialmente não só pelas suas propriedades nutricionais, mas também por lhe serem reconhecidas características benéficas com ação na saúde do consumidor. No entanto, grande parte da constituição deste fruto é composto pela coroa de folhas e casca que não são consumidas e, industrialmente, não são exploradas. Além disso, as indústrias de processamento de polpas de frutas geram toneladas de diversos resíduos, o que tem despertado grande preocupação económica e ambiental, uma vez que as soluções de descarte são bastante limitadas. Nesse sentido, o presente estudo teve como objetivo caracterizar o perfil fenólico da casca e da coroa de folhas, bem como avaliar seu potencial bioativo, visando a aplicação do extrato mais promissor num produto de pastelaria como ingrediente natural com potencial aplicação na indústria alimentar.

Os compostos fenólicos de ambas as matrizes vegetais foram extraídos por extração hidroetanólica termicamente assistida e posteriormente identificados e quantificados por cromatografia líquida de alta eficiência acoplada a detector de diodo e detector de espectrometria de massa (HPLC-DAD-ESI / MS). Foram identificados vinte e cinco compostos fenólicos em ambos os extratos, distinguidos entre ácidos fenólicos e flavonóides. Os principais compostos detectados foram derivados do ácido caféico e flavonas glicosídicas, nomeadamente a apigenina 6,8-*C*-diglucosídeo.

A atividade antioxidante de ambos os extratos foi avaliada através de dois ensaios *in vitro*: o ensaio de inibição da peroxidação lipídica (TBARS) e o ensaio de inibição da hemólise oxidativa (OxHLIA). A atividade antiproliferativa de ambos os extratos foi avaliada em linhagens celulares tumorais (MCF-7, NCI-H460, AGS e CaCo-2) e não tumorais (VERO) utilizando o método sulforhodamina B. Por fim, a atividade antimicrobiana foi avaliada, testando o potencial de ambos os extratos contra um painel de seis bactérias e seis fungos. Os resultados revelaram que ambos os extratos apresentaram um excelente desempenho em termos de ensaios bioativos e demonstraram que nenhum dos extratos apresentou toxicidade até a concentração máxima testada (GI_{50}> 400 μg / mL), comprovando seu potencial de aplicação como ingrediente natural. Apesar dos excelentes resultados, o extrato da casca destacou-se, apresentando um potencial antioxidante e antimicrobiano superior ao apresentado pelo extrato da coroa de folhas, sendo consequentemente, selecionado para a incorporação num produto de pastelaria típico português, denominado "súplicas".

Os efeitos da incorporação do ingrediente natural na composição química e centesimal das "súplicas", foram avaliados ao longo de diferentes tempos de prateleira (0, 3 e 7 dias após a incorporação) e comparados com o produto tradicionalmente produzido. A composição centesimal (cinzas, gorduras, proteínas, hidratos de carbono e valor energético) foi determinada através das metodologias oficiais de análise de alimentos (AOAC), enquanto a composição química individual foi obtida pela quantificação do teor de ácidos gordos por Cromatografia Gasosa acoplada a um Detector de Ionização por Chama (GC-FID) e o teor de açúcar por HPLC acoplado a um índice de refração. Adicionalmente, a variação nos parâmetros de cor (*L**, *a** e *b**) foi avaliada em todas as amostras de todos os tempos. Os resultados demostraram que o extrato não causou alterações na composição centesimal, no teor de açúcares e ácidos gordos ou nos parâmetros de cor.

Por sua vez, a avaliação da atividade antioxidante das "súplicas" revelou que a incorporação do extrato aumentou o potencial antioxidante do produto alimentar relativamente à receita tradicional. Assim, este estudo confirma a potencial aplicação deste bio-resíduo do ananás, como uma fonte de compostos bioativos, visando o reaproveitando de um recurso natural de baixo custo e contribuindo diretamente no impacto ambiental e económico.

Palavras-Chave: Ananás, bio-resíduos, compostos bioativos, compostos fenólicos, "súplicas", valor nutricional.

1. INTRODUCTION

In recent years, it has attracted the interest of the researchers all over the world in relation to the beneficial effects generated by plant compounds. As a result, new food sources have begun to be studied and ancient foods were rediscovered (Battino et al., 2021). The benefit of these bioactive compounds in front of frequent food consumption or even with direct supplementation of these compounds, generated multiple health effects, such as anti-aging effects, protection against cardiovascular diseases, control and prevention of metabolic, neurodegenerative diseases and cancer (Martín Ortega & Segura Campos, 2019). Bioactive ingredients are found in different matrices such as fruits, cereals, vegetables and food processing residues, which preserve their characteristics even after extraction (Fernandes et al., 2019).

Within the group of bioactive compounds, phenolic compounds are one of the main groups within the phytochemicals existing in medicinal and aromatic plants (Manousi et al, 2019). These compounds are secondary metabolites, contributing with the colour, flavour, astringency, and harshness, which is the typical organoleptic characteristics of the foods (Rashmi & Negi, 2020). According to Cádiz-Gurrea et al. (2020) tropical fruits comprise a wide spectrum of bioactive compounds such as phenolic compounds among other phytochemicals, which are regarded to have functional activities in the prevention or amelioration of some diseases. Among the main tropical fruits that occupy the highest production volumes are pineapple, avocado, mango and papaya, with the exception of bananas (FAO, 2020a). As a consequence of the large industrial production, it entailed the generation of large amounts of residues or by-products of tropical fruits comprising mainly seeds, peels and leaves (Cádiz-Gurrea et al. ,2020).

Being the third most important tropical fruit in the world after banana and citrus, the popularity of pineapple is given by the fruit's extremely low average export unit values in world trade (Lobo & Paull, 2017; FAO, 2020a). Not only for its peculiar characteristics but also for the recognition of its remarkable nutritional qualities, pineapple (*Ananas comosus* (L.) Merr.) is a fruit appreciated in many countries of the world (Piedade & Canniatti-Brazaca, 2003). According to Alexandre et al. (2014) pineapple can be used for both fresh consumption and industrialization, being transformed into various forms. However, is estimated that only 60% of pineapple infructescence is edible, therefore, processing residues vary between 45 and 65% (Silva et at., 2013). During the processing of fruit pulp is generated many residues, among them the main ones are the peels, stalks and seeds, which have nutrients and low caloric content (Feitosa et al., 2019).

Studies have shown that fruits are rich in many nutrients and antioxidant compounds, and that these constituents are concentrated mainly in peels and seeds (Contreras-Calderón et al., 2011). Adding value to these products, it is an economic and environmental interest to the food industry, requiring scientific and technological research, which allows its efficient, economical and safe use (Schieber et al., 2001). In addition, the demand for products containing natural additives that promote health rather than synthetic substances has been increasingly growing over the past years both in the food industry and in the cosmetic and pharmaceutical industry (Karimi et al., 2021). Foods and their by-products, which are often intended for animal feed, could be used as alternative sources of micronutrients, improving the body's physiological processes, in addition to reducing waste, reducing environmental impact and adding value to by-products (Bergamaschi, 2010).

Concerns such as these have generated the development of various techniques by scientists and industries for the use of fruit and vegetable by-products as a cheap, abundant and high potential source of valuable bioactive compounds (Arjeh et al., 2020). Given the high amount of waste generated by pineapple in the industry, this work intends to enhance the bio-residue of *Ananas comosus* L., aiming at its application as a potential natural ingredient for the food industry. The complete use of pineapple, from fruit to waste generated, would result in a profitable good both in the food industries and in other sectors could provide a wide variety of food products and bio-residue processing with great economic importance (Ali et. al., 2020). Thus, promoting an improvement in human health, as well as a reduction of the impact on the environment.

2. LITERATURE REVIEW

2.1. Phenolic compounds: characterization and applications in the food industry

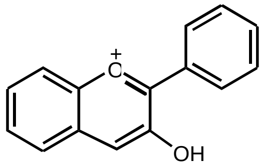
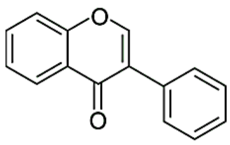
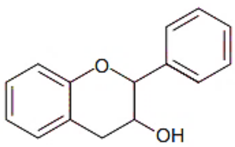
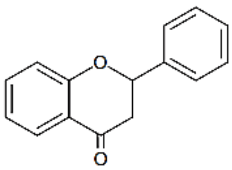
As a definition of plant phenolics or polyphenol, the term ‘phenol’ is a chemical term that defines a phenyl ring bearing one or more hydroxyl substituents, including their functional derivatives (e.g., esters, methyl ethers, glycosides) (Lattanzio, 2013). This term still refers to all secondary natural metabolites arising biogenetically from the shikimate phenylpropanoids-flavonoids pathways, producing monomeric and polymeric phenols and polyphenols (Oksana et al., 2012).

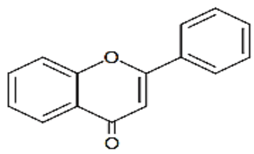
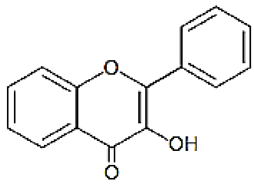
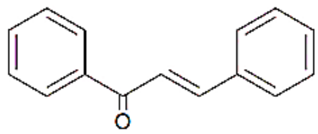
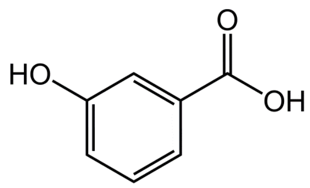
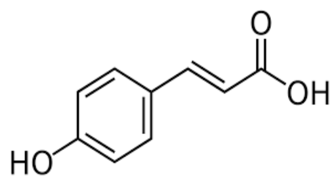
Phenolic compounds are secondary metabolites produced by higher plants, which play defence roles against pathogens in plant physiology and have potential healthy properties on human organism, mainly as antioxidants, anti-allergic, anti-inflammatory, anticancer, antihypertensive, and antimicrobial agents (Daglia, 2012). Due to this reason, research has been intensified with the objective of finding fruits, vegetables, plants, agricultural and agro-industrial waste as sources of bioactive compounds (Martins et al., 2011). More than 8000 phenolic compounds have been reported in different plants until now (Vuolo et al., 2019). These compounds are not generally detected in bacteria, fungi, and algae (Lattanzio, 2013). They are classified into two main groups: flavonoids and non-flavonoids (Libro, 2016) (**Table 1**). In the group of non-flavonoid compounds can be included phenolic acids, stilbenes, lignans and other polyphenols (Vauzour, 2014). While flavonoids are divided into six subgroups: flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins (Dai & Mumper,2010).

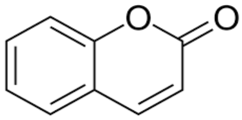
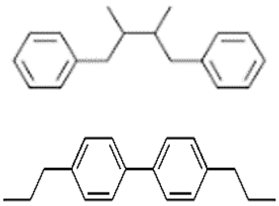
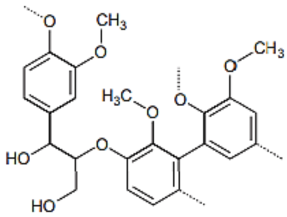
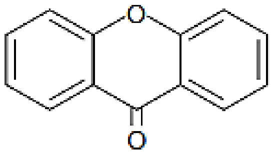
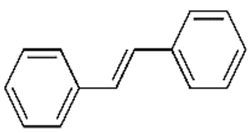
Being responsible for bitterness, astringency, flavour, colour and oxidative stability of fruits and vegetables, phenolic compounds have shown an effect in health protection, with not only antioxidant activity by scavenging free radicals, but also inhibition of hydrolytic and oxidative enzymes and anti-inflammatory functions in human cells (Naczk & Shahidi, 2004). Due to these health-beneficial properties, phenolic compounds are one of the main interested nutraceuticals in the food and pharmaceutical industries (Esfanjani et al.,2018). Regarding the pharmaceutical area, polyphenols as bioactive compounds are being used in producing anti-cancer drugs. The particular interest in these natural components occurred by the search for new chemo preventive agents more effective and less toxic than conventional therapies (Niedzwiecki et al., 2016).

According to Rahaiee et al. (2020), there are various studies suggesting different mechanisms for anticancer action of phenolic compounds or polyphenol-rich extracts, exhibiting cytotoxicity effects against numerous kinds of diseases, including colorectal, multiple myeloma, breast, pancreatic, prostate, oral, and lung cancers. Among these cancer preventions mechanisms, several have been identified as affected by polyphenols, including the prevention of oxidation, detoxification of xenobiotics, induction of apoptosis, as well as estrogenic/anti-estrogenic activity, stimulating effects on immune system function, anti-inflammatory properties and their effects on the cellular signalling system (Niedzwiecki et al., 2016).

Table 1: Main phenolic compound classes and chemical features.

Phenolic Class	**Description**	**Chemical Structure**	**Examples**
Flavonoids	Phenolic constituents with higher abundance in the plant kingdom. They are polyphenolic compounds derived from chalcones (formed by the junction of two aromatic ring with a three-carbon α, β unsaturated carbonyl system) with complex structures, a C6–C3–C6 skeleton that derived from the shikimate and acetate pathways. They possess three aromatic rings with fifteen carbons: a benzene ring (A), condensed with a six-member ring (C) and a phenyl benzene in the position 2 (B), as substituents. Flavonoids mostly occur in leaves and skin of fruits, displaying numerous benefits to the plants. They also occur joined with sugars, being commonly distinguished six different subclasses of flavonoids: anthocyanins, flavanols, flavanones, flavones, flavonols, and isoflavones.	 Anthocyanins	Cyanidin, delphinidin, malvidin, pelargonidin, petunidin, peonidin
		 Isoflavone	Genistein, daidzein, glycitein, formononetin, biochanin A, puerarin
		 Flavan-3-ols	(Epi)catechin, (epi) gallocatechin
		 Flavanones	Hesperidin, naringenin, taxifolin, eriodictyol, isosakuranetin

		 Flavones	Apigenin, luteolin, chrysin, scutellarein, diosmetin, chrysoeriol
		 Flavonols	Quercetin, kaempferol, myricetin, galangin, fisetin, morin
		 Chalcones	Phloretin, arbutin, butein, naringenin chalcone
Hydroxybenzoic acids	Phenolic constituents widely distributed in the plant kingdom, possessing seven-carbon molecules with a C6–C1 skeleton. Tannins are also part of this group, varying from small to large molecules. Two main classes of tannins may be distinguished, hydrolysable (possess a central core of polyhydric alcohol—glucose and hydroxyl groups—esterified or not by gallic acid) and nonhydrolyzable (more complex structures, resulting from the polymerization of some flavonoids) tannins.		*p*-Hydroxybenzoic acid, protocatechuic acid, vanillic acid, syringic acid, gallic acid, gentisic acid, salicylic acid
Hydroxycinnamic acids	Group of phenolic constituents derived from the cinnamic acid displays a C6–C3 skeleton constituted by trans-phenyl-3-propenoic acid and one or more hydroxyl groups, being some of them methylated.		Caffeic acid, coumaric acid, ferulic acid, sinapic acid, chlorogenic acid
Coumarins	Phenolic substances derived from phenolic acids formed by fusion of a benzene ring with an oxygen heterocycle. More than 1000 different		Scopoletin, umbelliferone, aesculetin

	coumarins occurs in higher plants, mainly in roots and seeds, but it can be also found in different organs of plants.		
Lignans	Phenolic compounds commonly found in several plant, being even quantified in all of the organs, resulting from the union of two cinnamic acids residues or its biogenic derivatives.		Secoisolariciresinol, matairesinol, sesamin, enterodiol, enterolactone
Lignins	Phenolic constituents with a complex structure, being composed by many branches of three simple alcohols (monolignols). Comprises the three most abundant polymer in the nature, also occurring in plant tissues.		
Xanthones	Phenolic constituents constituted by a C6–C1–C6 structure, mainly occurring in higher plants. Its heterocyclic structure is quite similar to flavonoids, being commonly divided into five different groups: simple oxygenated xanthones, xanthone glycosides, prenylated and related xanthones, xanthonolignoids, and miscellaneous.		Mangostin, mangiferin
Stilbenes	Phenolic constituents with a C6–C2–C6 structure widely distributed in several plants' species. Resveratrol constitutes the most important and widely known stilbene, being discovered renowned benefits.		Resveratrol

Adapted: Ferreira et al. (2017).

In recent years, attention has also been devoted to research in relation to the solubility properties of natural phenolic compounds to expand their applications, for example, such as dietary supplements or food and cosmetic stabilizers in non-aqueous media (Panzella, 2020). Polyphenols can improve the oxidative stability and storage stability of emulsions due to their antioxidant properties (Di Mattia et al., 2010). According to Zillich et al. (2015), phenolic extracts are found to inhibit the activity of proteinases, which catalyse the degradation of skin proteins, such as collagen and elastin. These properties can be beneficial to the skin since collagen in the dermis is responsible for firmness and elastin fibers lend elasticity. Studies have shown enormous potential of polyphenolic extracts as active ingredients in topical application products for the prevention and therapy of UV damage, skin aging and cancerous diseases (Zillich et al., 2015).

In the food industry these compounds have been used as natural colouring agents, nutritional additives, natural antioxidants, and chelating agents (Rahaiee et al., 2020). They have been widely studied for their application in food to improve the shelf life of perishable products, as they function as bio preservatives. Due to the current concern of the impact of food on the consumers lives, the use of phenolic compounds of natural origin in food has been an interesting opportunity for the application of their biological activities and still allows the production of foods without synthetic additives for consumers. Estimates indicate that of 500,000 plant species, 1-10% are used as food worldwide (Martillanes et al., 2017).

However, the greatest challenge of the use of food additives of natural origin involves the isolation, purification, stabilization, and incorporation of these compounds to foods, without this harming the sensory, nutritional characteristics and their health guarantee (Martillanes et al., 2017). Pineapple fruit contain diverse phenolic compounds such as *p*-coumaric, ferulic, caffeic, sinapic, *p*-coumaroylquinic, *p*-hydroxybenzoic, and *p*-hydroxy benzoic aldehyde and they are the major contributors to the antioxidant potential in the fruit besides ascorbic acid. The main phenolic compounds in pineapple peels found were catechin (58.51 mg/100g), epicatechin (50.00 mg/100g), gallic acid (31.76 mg/100g dry extracts) and ferulic acid (19.50 mg/100g) (Lobo & Paull, 2017).

2.1.1. Bioactive properties of phenolic compounds

Phenolic compounds, commonly known as polyphenols, refer to compounds produced by the substitution of hydrogen atoms on the benzene ring of aromatic hydrocarbons with hydroxyl groups, and can be divided into monophenols and polyphenols according to the number of hydroxyl groups contained in their molecules (Ge et al.,2020). These hydrogen atoms from the ring of phenolic compounds can be donated to reactive oxygen species, and then decreasing and neutralizing these free radicals, consequently, reducing the oxidative damage. Furthermore, they have the ability of metal chelation, particularly iron and copper, suppressing metal-catalyzed free radical formation (Vuolo et al., 2019; Goufo, & Trindade, 2015). There are a range of assays that can assess the abilities of the phenolic compounds to scavenge free radicals. The assays are carried out in such a way that a single electron transfer reaction or a hydrogen atom transfer reaction is evaluated.

Regarding the chemical structure, they comprise a wide range of molecules with polyphenol structure and are generally divided into flavonoids and non-flavonoids. The most abundant and bioactive group in fruits and vegetables are the flavonoids, which account for nearly two-thirds of dietary phenolic compounds. The structure contains a phenyl benzopyran skeleton: two phenyl rings (A and B) joined through a heterocyclic pyran ring (ring C; **Figure 1**). Flavonoids can be subdivided into many subclasses, depending on the oxidation state of the central pyran ring, that are flavonols, flavones, flavanones, anthocyanidins, flavanols (catechins) and also isoflavones. In each subclass, individual compounds differ in their pattern of hydroxylation and methylation of rings A and B (De la Rosa et al., 2019). Birt and Jeffery (2013) report that such variations are due to the substitutions which can be oxygenation, alkylation, glycosylation, sulfation, and acylation. In addition, C ring are mainly responsible for the varieties of flavonoid classes, being that variations in their substitution patterns provide the major flavonoid classes (Birt and Jeffery, 2013).

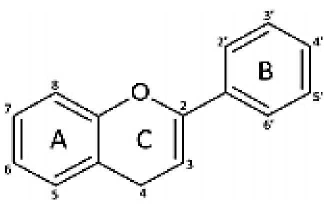


Figure 1: Phenylbenzopyrane skeleton, basic structure of flavonoids.
Source: De la Rosa et al. (2019).

According to Vuolo et al. (2019), the number of flavonoids present in fruits and vegetables may vary depending on species variety, edaphoclimatic conditions, part of the plant, cultivation, and degree of ripeness. In addition to being responsible for the colour in food, flavonoids also participate in taste, protection of lipid peroxidation, enzymes, and vitaminic compounds (Vuolo et al., 2019). Antioxidant properties of flavonoids are related to the hydroxyl phenolic groups bonded to cyclic structures. This property works reducing agents, hydrogen donors, singlet oxygen, and superoxide radical scavengers and even as metal ion-chelating agents. Besides, they are capable of activating antioxidant enzymes, reducing α-tocopherol radicals (tocopheroxyls), inhibiting oxidases, attenuating nitrosative stress, and increasing both uric acid and low molecular weight molecules (Atmani et al., 2009). In addition to antioxidant activity, flavonoids have anti-inflammatory properties and are found in fruits, vegetables, legumes, red wine, and green tea (Niedzwiecki et al., 2016).

The non-flavonoids can be subdivided into derivatives of benzoic acid, such as gallic acid and protocatechuic acid, and derivatives of cinnamic acid, that consist chiefly of coumaric, caffeic, and ferulic acid, second stilbenes, whose main representative is resveratrol, that exists in both cis and trans isomeric forms, and third lignans, produced by oxidative dimerization of two phenylpropane units (Daglia, 2012). Phenolic acids are considered the most important group of nonflavonoids in fruits and vegetables, containing a single phenyl group substituted by one carboxylic group and one or more OH groups. They can be further divided into hydroxybenzoic acids, hydroxycinnamic acids, and other hydroxyphenyl acids (acetic, propanoic, and pentanoic), differing among them in the length of the chain containing the carboxylic group. They can be found in berries, nuts, tea, chicory, and some spices (De la Rosa et al., 2019).

Jimenez-Lopez et al. (2020) emphasized that phenolic compounds have been reported to have several properties of interest for different applications. Studies have shown that phenolic compounds extracted from marine algae also demonstrated antiviral activity, specifically anti-HIV compounds. In the case of cancer, for example, plant polyphenols can prevent the development of it by modulating some of the signal transduction pathways related to the cancer process (Rahaiee et al., 2020). Isoflavones were reported by exhibit multifunctional actions against cancer, including inhibition of hormone-dependent cancers, altering the expression of different estrogen receptors, tumour suppressors, and transcription factors in cancer cells (Pool et al., 2018).

2.1.2. Phenolic compounds extraction techniques

Extraction is one of the most important steps in the purification, identification, and obtainment of phenolic compounds. According to Sagar et al. (2018), extraction methods may vary with respect to the targeted bioactive compounds. Many compounds cohabit with different chemicals, which represent differences in the solubility of the compounds in the extractor and interactions between compounds and components. (Manchón et al.,2015). Many factors may affect in the extraction process such as plant part, pressure, time and temperature of extraction, as well as the solvent nature and the solvent-to-sample ratio (Hernandez et al.,2009; Da Silva et al., 2016a). There are several extraction techniques for the bioactive compounds from plant materials and they can be classified into 2 main categories: conventional and novel techniques (Sagar et al., 2018). The main classical techniques are: (1) Soxhlet extraction, (2) Maceration and (3) Hydro-distillation (Azmir et al., 2013). Most of them are based on the extraction power of different solvents used together with the application of heat and/or agitation (Ignat et al., 2011).

Soxhlet extraction, initially developed for lipid extraction, is a technique widely used in the extraction of bioactive compounds from different parts of plants (Sagar et al., 2018). This technique consists of a small amount of dry sample is placed in a thimble, which is placed in the distillation balloon containing the solvent of particular interest. The thimble-holder solution is aspirated by a siphon after reaching an overflow level. Siphon discharges the solution back into the distillation bottle. This solution transports the extracted solutes into the bulk liquid. The solute is kept in the distillation flask and the solvent returns to the solid bed of the plant then times until the extraction process is completed.

The maceration became a popular and inexpensive way to get essential oils and bioactive compounds for small scales extractions (Azmir et al., 2013). This technique is composed by several steps: (1) grinding of plant samples into tiny particles for the proper mixing with solvent; (2) appropriate solvent named as menstruum is added in a closed vessel; (3) the liquid is discarded, and a large amount of the prepared solution is achieved by pressing the solid residue of this extraction process (Sagar et al., 2018).

Hydro-distillation is also a classic technique as well as maceration, which extract important oils and various bioactive compounds from plant sources, and it is used before dehydrating a plant sample. There are three types of hydro-distillation: water and steam distillation, water distillation, and direct steam distillation (Vankar 2004). Depending on the high extraction temperature some volatile components may be lost. This factor limits the use of this technique for thermolabile compounds extraction (Azmir et al., 2013).

Alternative technologies are being used as substitutes or auxiliaries of traditional solid liquid extraction methodologies, called modern techniques are composed by enzyme-assisted extraction, ultrasound-assisted extraction, microwave-assisted extraction, subcritical fluid extraction, supercritical extraction, and high pressure-assisted extraction (Ventura et al., 2017; Bart, 2011). One of the advantages of these extraction methodologies is that it usually has much better efficiency, shorter extraction time, lower cost and higher purity of extracted compounds. However, there are several problems associated with both techniques, including solvent toxicity, thermal instability, polarity, solubility and low selectivity (Zainal-Abidin, et al., 2017). The comparative advantages and limitations of various extraction techniques are summarized in **Table 2**.

Table 2: Comparative advantages and limitations of various extraction methods for bioactive compounds.

Technique	Advantages	Limitations	Recommended compounds
Soxhlet	• Widely used as classical technique • Basic model technique for the comparison of other techniques.	• Time consuming • Not environmentally friendly and requires large quantities of solvents	Lipid/fat extraction
Hydro-distillation	• Oldest and simplest technique for extracting essential oils from plants • Best suited for small-scale industries • Provides different options according to choose, that is, hydro-distillation, steam and water distillation, direct steam distillation, hydro-diffusion, and so on.	• Not suitable for heat-labile compounds because they may be lost or degraded at high temperature • Time-consuming and slow process	Oil and bioactive compounds
Liquid–liquid extraction (LLE)	• Suitable for liquid samples • Standard, easy, and cheap method for determining phenol in water • Can be utilized at room temperature to avoid phenolics degradation	• Requires hazardous and expensive chemicals • Labour intensive • Requires long time for sample analysis and degradation rate is high due to internal and external factors	Best for phenolic compounds, liquid by-products from beverage industries are best samples for this technique.

Technique	Advantages	Limitations	Recommended compounds
Supercritical fluid extraction (SFE)	• Lower viscosity and higher diffusion coefficient than liquid solvent extraction, which gives better mass transfer • Time saving and environment friendly due to requirement of little amount of sample and organic solvent • Minimum wastage because reusing and recycling of supercritical fluid is possible • Suitable for volatile compounds because performed at room temperature	• Not suitable for most drug and pharmaceutical samples • Polar molecules cannot be dissolved • Costly system thermodynamics complicated	Best suited for volatile compounds
Microwave-assisted extraction (MAE)	• Better quality and high selectivity of desired extracts • High extraction yield and less extraction time • Cost-effective compared with solvent extraction technique • Simply operable and economically feasible in comparison with supercritical fluid extraction • Short extraction time compared with ultrasonic-assisted extraction.	• Apparatus and equipment are expensive • Operation is difficult compared to ultrasonic-assisted extraction • Less environment friendly due use of organic solvents • Poor extraction yield for nonpolar compounds • Unfit for heat-labile biomolecules	For the rapid extraction of bioactive compounds (especially polyphenols)
Ultrasound-assisted extraction (UAE)	• Less energy and power usage • Higher product yield • Short processing time and less chemical usage	• Proper optimization in ultrasound frequency, nominal power of the device, propagation of cycle, input power. • system geometry is required for maximum yield	Phenolic compounds, lipids, chlorophyll, carotenoids

Adapted: Sagar et al. (2018).

2.2. Characterization of the *Ananas comosus L.*

Ananas comosus L. is an exotic fruit and highly valued for its aroma, flavour and juiciness. Discovered in 1493 by Europeans in the Caribbean Island, the fruit became appreciated by the nutritional and healthy properties in addition to its pleasant flavour (Lobo & Paull, 2017). There are many varieties of pineapple with different colours, shapes, sizes and flavors (Ali et. al., 2020). In the world, there are over 100 varieties of pineapple however, only 6 to 8 varieties are commercially grown (Steingass et al., 2020). Among them, Smooth Cayenne, MD-2, Red Spanish, Queen, Pérola and Manzana, are the most important cultivars (Lobo & Paull, 2017) (**Figure 2**). Pineapple production is concentrated in the tropical and sub-tropical regions, due to the temperate climate and rainfall distribution (Ali et al., 2020). According to the FAO (2020b), Costa Rica is the world's largest producer and exporter of pineapples followed by Brazil that has significant domestic consumption of pineapples.

Figure 2: The five most common pre-Columbian pineapple cultivars: 'Perola' (A), 'Queen' (B), 'Manzana' (C), 'Red Spanish' (D) and 'Cayenne' (E).
Source: Sanewski (2011).

Pineapple is a juicy fruit, berry type, formed by the coalescence of multiple fruitlets which fuse spirally in the central axis and may present various forms namely conical, slightly conical, cylindrical, and rounded (Silva et. al, 2016a). The plant is a perennial herbaceous monocot, which can vary in height from 0.8–2m and has the roots, stem, buds, leaves, peduncle, compound multiple fruit and crown leaves as the main morphological structures (**Figure 3**). Upon maturation, the peel of the fruit changes colour from dark green to yellow, orange–yellow or reddish, depending on the cultivar. The weight of the fruit can vary between 0.8 to 15kg. The pulp is usually white to golden yellow in turn, the crown leaves may have thorns and the colour may be green, red or with a red stripe according to the variety. The fruit peel is composed of sepals, bract tissues, and the ovaries apexes (Lobo & Paull, 2017).

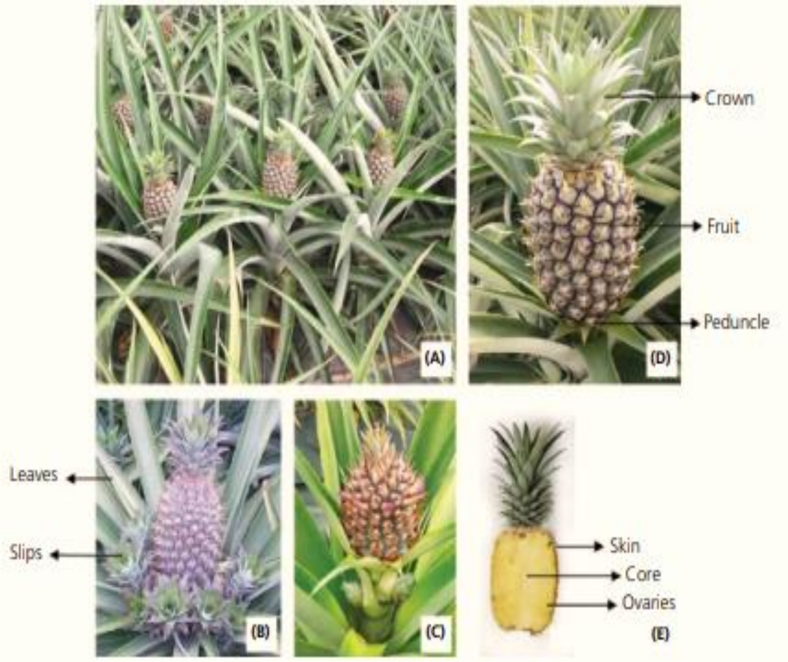


Figure 3: The main morphological structures of pineapple. Pineapple planted on double rows in a large plantation (A); Flower and fruit development (B, C, D); transversal cut of the fruit (E).
Source: Paull & Lobo (2012).

The fruit´s edible part consists of the fused ovaries, the bases of sepals and bracts, and the cortex of the central core. The pineapple is normally seedless so, the propagation is predominantly by asexual reproduction and is made for basically three types of vegetative propagules: slips, hapas and crown leaves (Zamperlini, 2010; Lobo & Paull, 2017). The classification pineapple is based on the degree of skin coloration, size (weight), absence of defects and disease, and uniformity of these characteristics before packaging. Characteristics such as maturity, firmness, good shape, flat eyes and well cured broken stem (peduncle) are also taken into account (Paull & Chen, 2020).

2.2.1. Etymology and applications

Currently, pineapple has aroused much interest both by the food industry and by its consumers, due to its interesting nutritional composition but also for its outstanding bioactive properties that refer to its potential use as a functional food and/or as a natural ingredient in the preparation of new food products (Ali et al., 2020).

Being an important tropical fruit, its consumption is done in many parts of the world as fresh fruit, juice, jam, jelly and dried product. (Hossain & Rahman, 2011). Also, the fruit *in natura* can be added in desserts, salads, pies, cakes, puddings, or made into sauces. Due to the proteolytic enzyme that pineapple contains, bromelain can be used in cooking as a meat tenderizer to break down the protein (Dhukani, 2013). There are other applications that can be used by bromelain such as, digestive aid, cleansing agent, antibiotic potentiating agent, wound debridement agent and therapeutic application (**Table 3**) (Lobo & Paull, 2017).

Regarding medicinal use, pineapple has been pointed out as an ally to increase appetite, as a diuretic and an aid in expulsion of intestinal worms. Additionally, the consumption of this fruit has been associated with the prevention of ulcers and increased fat elimination, which has been arousing a growing interest in the consumption of pineapples (Hossain, 2016). Likewise, pineapple processing residues have also been highlighted as ideal substrate for the production of cellulose derivatives with potential application in the polymer industry due to their high cellulose content (40.5% in peels and 43.5% in crown leaves; dry basis) (Pardo et al., 2014). Hydrogels are also produced from the cellulose content of pineapple peels, representing a very important class of synthetic biomaterials (Hu, Hu, Zeng, & Huang, 2010), in the manufacture of contact lenses, drug-delivery systems, dressing material for wounds, and a wide range of other therapeutic products (Caló & Khutoryanskiy, 2015).

Studies realized by Saravanan et al. (2013) demonstrated cellulase production (maximum cellulase activity of 8.61 IU/ml obtained under optimized conditions) from pineapple residues with *Trichoderma reesei*, and this enzyme is used in bioethanol production. Therefore, culture of microorganisms in biowaste can be considered a process of adding value capable of converting residues into industrially relevant metabolites, as in this case, an enzyme. The production of some organic acids is also reported in the literature, such as, the production of lactic acid, citric acid, succinic acid, and acetic acid from pineapple residues. Lactic acid can be produced from pineapple syrup using *Lactobacillus* lactis, having application in the food industry as a food preservative and is also used in baby food formulations. Acetic acid can also be produced by fermentation of liquid pineapple residues, being used in the food industry as a flavour enhancer and as a food preservative (Ueno et al., 2003; Banerjee et al., 2018).

Table 3: Different applications of bromelain.

Food Industrial	**Therapeutic**	**Textile Industry**	**Cosmetic Industry**
Baking industry • Improves dough relaxation •Produces hypoallergenic flour *Protein hydrolysate* • Hydrolyzing agent for meat, oyster, chicken and squid • Hydrolyzing fish protein • Estimating protein degradation in ruminant feed *Antibrowning agent* • Inhibits browning of fruits and phenol oxidation *Alcohol production* • Enhances protein stability of beers • Prevents haze Formation *Antimicrobial effects*	• Cardiovascular and circulation effects • Relieves osteoarthritis • Treatment of chronic inflammatory, malignant, and autoimmune diseases • Use in blood coagulation and fibrinolysis • Counteracts some of the effects of diarrhea • Debridement of necrotic tissue and acceleration of healing	• Minimizes softening time in cocoon cooking • Removes scale and impurities of wool and silk fibers • Enhances dyeing properties of protein fibers	• Removes stains, plaque and food debris on the outer surface of teeth • Treating acne, wrinkles and dry skin • Reduces post- injection bruising and swelling

Adapted: Lobo & Paull (2017).

2.2.2. Chemical and nutritional composition and bioactive properties

Ananas comosus L. is considered a tropical fruit that has attractive sensory characteristics, namely the colour, aroma (volatile compounds), taste (sweet, sour, salty and bitter sensations), but also nutritional composition rich in ascorbic acid content, minerals, fibers and the presence of antioxidants (Ramallo & Mascheroni, 2012). Pineapple can be used as supplementary nutritional fruit for good health with an excellent source of vitamins in addition to various minerals such as calcium, phosphorus, and iron (Hossain & Rahman, 2011). Pineapple pulp contains fructose, dietary fiber, vitamin B1, vitamin B6 and copper and a very good source of manganese (46% Daily Values, DV) and vitamin C (80% DV) (Paull & Lobo, 2012). Additionally, this fruit is considered a source of antioxidants, mainly due to its rich composition in flavonoids and pro-vitamin A. However, both the sensorial characteristics and the chemical composition of pineapples (sugars, organic acids, minerals, fiber, aromatic compounds, vitamins, amino acids, flavonoids, carotenoids, etc.) can change according to each variety (**Table 4**) (Lobo & Paull, 2017).

Table 4: Composition of pineapple fruit nutrients.

Nutrient	Unit	Smooth Cayenne	MD-2
Energy	kcal	45	51
Protein	g/100g (fw)	0.55	0.53
Total lipid	g/100g (fw)	0.13	0.11
Carbohydrate (by difference)	g/100g (fw)	11.82	13.50
Sugar (total)	g/100g (fw)	8.29	10.32
Sucrose	g/100g (fw)	4.59	6.47
Glucose	g/100g (fw)	1.76	1.70
Fructose	g/100g (fw)	1.94	2.15
Fiber (total dietary)	g/100g (fw)	1.4	1.4
Minerals			
Calcium, Ca	mg/100g (fw)	13	13
Iron, Fe	mg/100g (fw)	0.25	0.28
Magnesium, Mg	mg/100g (fw)	12	20
Phosphorus, P	mg/100g (fw)	9	13
Potassium, K	mg/100g (fw)	125	178
Sodium, Na	mg/100g (fw)	1	2
Zinc, Zn	mg/100g (fw)	0.08	0.20
Copper, Cu	mg/100g (fw)	0.081	0.113
Manganese, Mn	mg/100g (fw)	1.593	0.818
Selenium, Se	µg/100g (fw)	0.0	0.1
Vitamins			
Vitamin C (total ascorbic acid)	mg/100g (fw)	16.9	56.4
Thiamin	mg/100g (fw)	0.078	0.080
Riboflavin	mg/100g (fw)	0.029	0.033
Niacin	mg/100g (fw)	0.106	0.507
Vitamin B6	mg/100g (fw)	0.106	0.114
Folate, DFE	µg/100g (fw)		19
Vitamin A, RAE	µg/100g (fw)	3	3
Vitamin A, IU	IU/100g (fw)	52	57
Vitamin E (α-tocopherol)	mg/100g (fw)		0.02
Vitamin D (D2+D3)	µg/100g (fw)	0.0	0.0
Vitamin D	UI/100g (fw)	0.0	0.0 0
Vitamin K (phylooquinone)	µg/100g (fw)	0.7	0.7

Adapted: Lobo & Paull (2017).

Pineapple is essentially composed of water (80-85%) followed by sugars (12-15%), organic acids (0.6%), ash (0.5%), proteins (0.4%) and fats and vitamins (0.1%) (Salvi & Rajput, 1995). Organic acids present in this fruit stabilize the pH value of the cell sap, participate in photosynthesis, respiration, synthesis of phenols, amino acid metabolism, lipids metabolism and aromatic substance metabolism. The balance between sugar and acidity, as well as the high sugar content, aid in the taste of pineapple, giving an excellent flavour to it. Also, the variety and levels of sugars and acids are affected by genetic and environmental factors as well as fruit maturity (Sun et al., 2016). Different levels of maturation of pineapple result in changes in chemical composition and different aromatic profiles of the fruit, especially during storage (Ali et al., 2020). According Zhang et al. (2007), the major organic acids of pineapple fruit reported were citric acid (62%) and malic acid (14%). Others organic acids were found in less quantity as tartaric acid and acetic acid.

Others compounds that would be associated with the quality of the flavour of different types of pineapple varieties, would be the volatile compounds. More than 280 compounds have been found among the volatiles of pineapples so far. A few of them have been identified as contributing to pineapple flavour (Sun et al., 2016). In this field, esters represent the main group of volatile compounds by being found in abundant quantity (Zheng et al., 2012). According to Lasekan and Hussein (2018), it was possible to identify that this tropical fruit is rich in ester compounds including methyl-2-methylbutanoate, methyl hexanoate, methyl-3-(methylthiol)-propanoate, methyl octanoate, 2,5-dimethyl-4-methoxy-3(2H)-furanone, δ-octalactone, 2-methoxy-4-vinyl phenol, and δ-undecalactone. Other groups of compounds were detected in the different pineapple varieties such as ketones, alcohols, terpenes, lactones, and acids.

Pineapple can be considered as one of the most useful fruits for manufacturing value-added compounds, based on the physicochemical composition and nutritional values, such as antioxidants, organic acids, bromelain, and phenolic compounds (Ali et al., 2020). Bioactive compounds are extra nutritional constituents that are mainly attributed to their potent antioxidant properties and free radical scavenging ability. There are a lot of types of bioactive compounds including, carotenoids, flavonoids, phenolic acids, glucosinolates, dietary fiber, phytosterols, monoterpenes and very active molecules like ascorbic acid. The main bioactive compounds reported in pineapple pulp are antioxidant compounds such as ascorbic acid, phenolic compounds and carotenoids (**Table 5**) (Lobo & Paull, 2017). Phenolic compounds are present in high amounts in the rind, peel, and seeds of fruits and vegetables (Sagar et al., 2018). Studies realized by Sun et al. (2016), showed a predominance of catechin, epicatechin, and sinapinic acid in pineapples.

Table 5: Bioactive compounds in pineapple fruit (fw: fresh weight. dw: dry weight. GAE: gallic acid equivalent. PSW: peel, seed and waste. *mg/L as GAE).

Source	Total vitamin C (mg/100g)	Total phenolics (mg/100g of GAE fw)	Carotenoids (µg/100g of β-carotene dw)	Dietary fiber (%)	Phenolics bound to dietary fiber (mg/100g of GAE dw)
Flesh	47.8	40.4	42.8	2.8	-
Juice		358	-	-	-
Residue	-	26	-	-	-
Peel	-	2222	-	-	-
Shell	-	-	-	70.6	2270
PSW	-	269.8	156.10	-	-

Adapted: Lobo & Paull (2017).

2.3. Bio-waste as sources of bioactive compounds and potential application in the food

Food is an essential component for survival and existence of life. Thus, a great concern arises when this indispensable element, is poorly used and poorly administered at any stage of the food life cycle, leading to serious social, economic, and environmental consequences (Sharma et al., 2020). Food and Agriculture Organization (FAO) estimated that 1.3 billion tons of food is lost or wasted annually worldwide. That is a 1/3 of all food produced for human consumption. Of these wasted foods, 28% correspond to the production of agricultural area, using 1.4 hectares of fertile land in the world (Karthikeyan et al., 2018, Paritosh et al., 2017). Food loss and waste have negative impacts on the economic, social, and environmental sustainability of food systems (FAO, 2017). Around 690 million people today are hungry and three billion cannot afford a healthy diet. COVID-19 pandemic is increasing this problem of the food and nutrition security of up to an additional 132 million of people (FAO, 2020c). Economically, this loss represents the wasted investment that can reduce farmers' incomes and increase consumers' expenses. In relation to the environment, food loss and waste generate a range of impacts, including unnecessary greenhouse gas emissions and inefficiently used water and land, which in turn can lead to diminished natural ecosystems and the services they provide (Lipinski et al. 2013).

According to FAO, if food loss and waste were a country, it would be the third largest greenhouse gas emitter in the world, being responsible for 8% of the emissions (4.4 gigatonnes of CO_2/ year) (FAO, 2020c). Food loss and waste problem is really different around the world and related to the conditions of a single country, which are specific to time and space (Roda & Lambri, 2019). In developing countries, it is noted that losses are concentrated in the stages leading up to consumption: production, post-harvest, processing, distribution, and retail. In developed countries, food waste at the retail and consumer levels tends to be higher (Rodrigues, 2017; FAO, 2017). Europe even wastes 88 million tons of food, or 173 kg per capita per year. This loss ends up resulting in a problem in the emission of polluting gases, generating 170 million tonnes of CO_2 and consuming 261 million tonnes of resources (EPRS, 2016).

The growth in the demand for fruits and vegetables came from the increase of processed food, which resulted in vast generation of waste (Banerjee et al., 2018). This bio-waste is generated during different stages of the food supply chain, from farm to table, including production, processing, packaging, handling, storage and transportation (Ji et al., 2017). Thinking about this issue, the utilisation of bio-waste could prove to be a better alternative in the mitigation of environmental problems and also to propose a novel method to produce valuable chemicals with numerous applications (Banerjee et al., 2018). The fruit-processing wastes are generally constituted of peels, pomace and seed fractions, which could potentially be a good source for high added-value bioactive compounds such as mainly pectin, proteins, antioxidants and phenolic compounds. These by-products are also rich sources of complex polysaccharides, carbohydrates, fibre, and vitamins (Campos et al. 2020a).

Potentially marketable, this bio-waste can be used in several applications, namely in pharmaceutical, cosmetic, food, and non-food areas (Vasiljevic, 2020). Fruit and vegetable residues may be transformed into dietary fibre, sugars, oligosaccharides and/or antioxidants (Cheok et al., 2018), or being a source of bioactive components and functional ingredients (Upadhyay et al., 2010). This type of bio-waste can be converted to different forms of energy molecules or biofuels. The bioconversion of food waste can add values to products as in the production of biosurfactants, bioplastics and organic fertilizers (Sharma et al., 2020). Among all the fruits and vegetables produced for the industry, pineapple is considered one of the world’s major crops with a high production each year (Roda & Lambri, 2019). The consequence massive production and related by-products, has generated a significant amount of waste, consisting in peel, core, and crown leaves. This waste can be either used as animal feed or disposed to the soil as a waste that can cause environmental problems (Roda & Lambri, 2019; Roha et al., 2013).

According to Abdullah & Hanafi, (2008), pineapple fruit residue was noted to be an enriched raw material with insoluble fibres, pectin, simple sugars, and proteins as the main compounds, followed by a good level of micronutrients such as vitamins, minerals and phenolic compound. Pineapple waste offers a broad range of great opportunities to recover and produce high-value products, which has already been used by the industrial processes (Roda & Lambri, 2019). Due to the multifunctional nature of these components, the residues from the pineapple industry can be applied in several sectors (**Table 6**). This bio-waste has an attractive potential for large-scale utilization due to being readily available, practically free, and renewable (Vasiljevic, 2020). However, more studies are needed to evaluate the whole feasibility of the food technology processes with minimum environmental impact for effective transition to circular economy. (Roda & Lambri, 2019)

Table 6: Pineapple residues utilization.

Pineapple residues	
Peel	Antioxidant compounds (phenolic compound, ferulic acid, and vitamin A and C)
	Ethanol; vinegar
	Fibre-rich fraction as a potential ingredient
	Bromelain
	Source of dietary fibre with associated polyphenols
	Fluor
	→ cereal bars
	→carbon source during fermentation using bacteria with probiotic potential; source of bioactive compounds (fibre, antioxidants and prebiotic) for the development of lactic acid bacteria in cooked sausages
	Probiotic yogurt fortified with fibre-rich pineapple peel powder
	Phenolic antioxidant
Core	Ascorbic acid; proteolytic enzymes
	Ethanol; vinegar
Stem	Ascorbic acid; proteolytic enzymes
	Bromelain
	Starch
Crown leaves	Endopeptidases (proteolytic enzymes)
	Bromelain
	Citric acid
	Ethanol production
	Bioactive compounds and glycosides

Source: Roda & Lambri, (2019).

3. OBJECTIVES

The present work intended to enhance the bio-residue of *Ananas comosus* L., aiming at its application as a potential natural ingredient for the food industry (**Figure 4**).

The specific objectives of this work were:

• Identification and quantification of phenolic compounds present in pineapple peel and leaves;

• Evaluation of the bioactivities in the extracts (antioxidant, antimicrobial, antiproliferative and toxicity);

• Incorporation of the most promising extract in a pastry product and evaluation of its effects regarding colour, proximate composition, free sugars and fatty acid content, as well as the antioxidant activity evaluation along the 7 days of shelf-life in order to evaluate its preservative capacity.

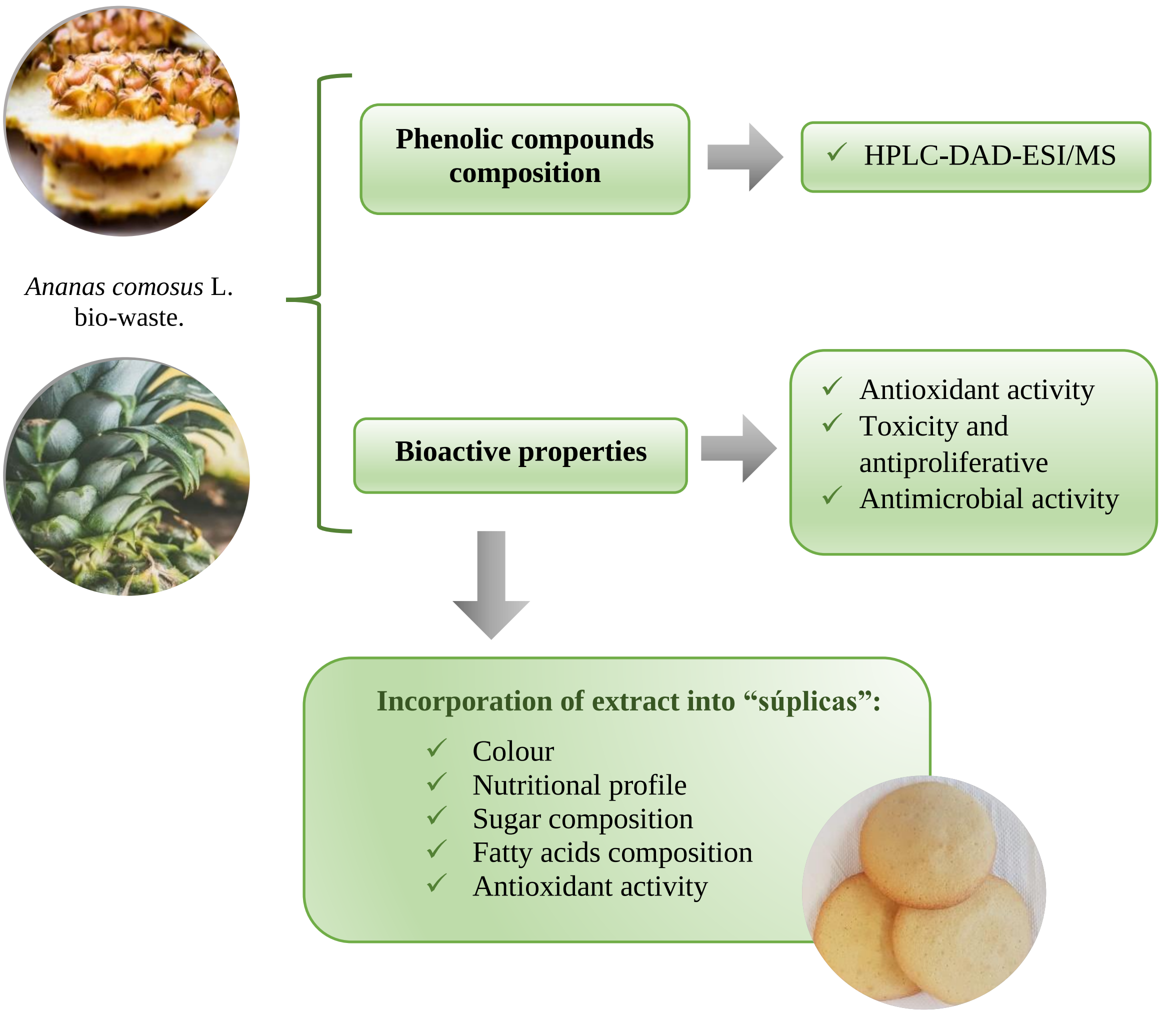


Figure 4: Explanatory scheme of the work. Source: Own authorship (2021).

4. MATERIALS AND METHODS

4.1 Preparation of samples

The *Ananas comosus* L. (peel and crown leaves) bioresidues was kindly provided by the company CAMPOTEC, S.A. (Torres Vedras, Portugal). Upon receipt in fresh form, both samples were frozen after freezing and then dehydrated by lyophilization (FreeZone 4.5, Labconco, Kansas City, MO, USA). guarantee the homogeneity of the samples. Finally, both samples were stored in a cool, dry place, protected from light, for further analysis as described in the **Figure 5.**

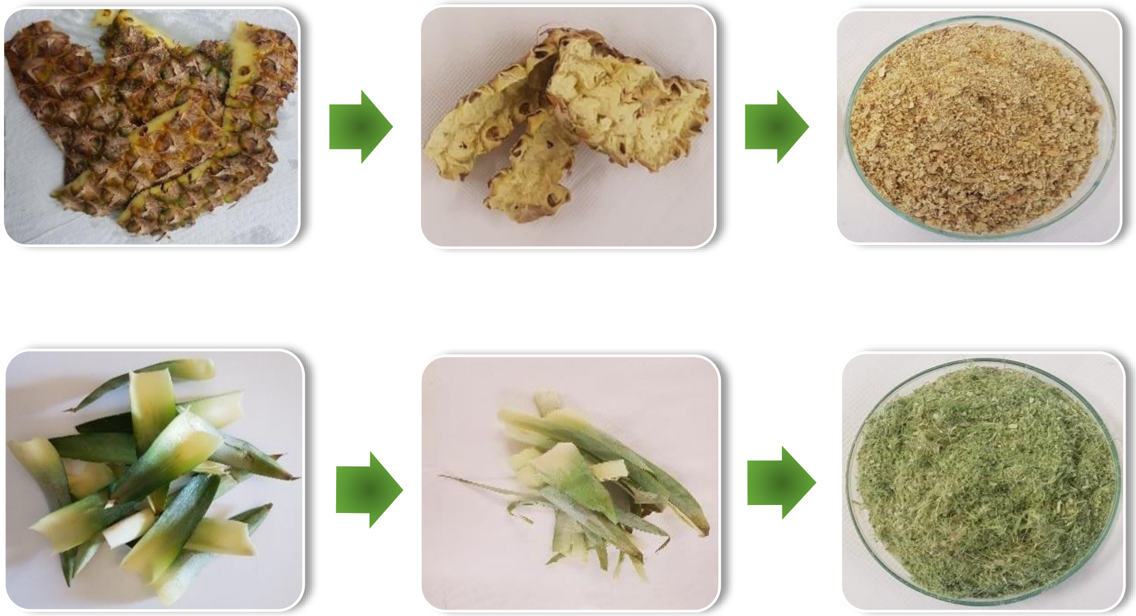


Figure 5: Samples of *Ananas comosus* L. in their fresh, lyophilized and crushed form.
Source: Own authorship (2021).

4.2 Reagents

The solvents n-hexane 95%, acetonitrile 99% and ethyl acetate 99.98% of HPLC grade were acquired at Fisher Scientific (Lisbon, Portugal) and the analytical grade methanol solvent was acquired at Paralab (Lisbon, Portugal). The other solvents used, also of analytical grade were the following: ethyl ether (Lab-Scan); toluene and sulfuric acid (Sigma Chemical Co.; St. Louis, MO, USA). The water was treated prior to its use by the Milli-Q-Water purification system (TGI Pure Water Systems, Greenville, SC, USA). The standard mixture with 37 fatty acid methyl esters (FAME) (C4-C24; standard 47885-U) was acquired at Sigma (St. Louis, MO, USA), as well as other individual fatty acid isomers, sugar standards ((D (-)-fructose D (+)-sucrose, D (+)-glucose, D (+)-

trehalose and D (+)-raffinose pentahydrate). Regarding the phenolic compounds standards used: caffeic acid, catechin, epicatechin, and quercetin-3-*O*-glucoside were acquired in Sigma-Aldrich and Extrasynthese.

Fetal bovine serum (FBS), L-glutamine, Hank's saline solution (HBSS), penicillin/streptomycin solution (100 U/mL and 100 mg/mL, respectively) and DMEM medium (Dulbecco Modified Eagle) were purchased at Hyclone (Logan, Utah, USA). Acetic acid, ellipticine, sulforhodamine B (SRB), trichloroacetic acid (TCA) and Tris were supplied by Sigma Aldrich (St. Louis, MO, USA). Mueller-Hinton agar (MHB) was obtained from Biolab® (Hungary). The solution trypan blue 0.4% were acquired by Gibcon and trypsin- EDTA 1:250 by BioConcept. The compound p-Iodonitrotetrazolium chloride (INT) was acquired from Panreac Applichem (Barcelona, Spain). Sodium sulphite (E221) and potassium metabisulphite (E224) were applied as a food additive for positive controls.

4.3 Preparation of hydroethanolic extracts

Both lyophilized samples (2g) were submitted to a maceration extraction with an ethanol/water solution (80:20, v/v, 50 mL) at room temperature, with magnetic agitation (150 rpm) during 1 hour. Subsequently, it was filtered through a filter paper (Whatman No. 4) and the process was repeated, and the sample was re-extracted with 50 mL of the same hydroethanol solution. Finally, the ethanolic fraction of the obtained extracts was evaporated under reduced pressure (100 rpm, 40°C) (rotary evaporator, Heidolph, Schwabach, Germany) and the aqueous phase was frozen and lyophilized (Labconco Freeze Zone 6, USA), for further analysis (**Figure 6**).

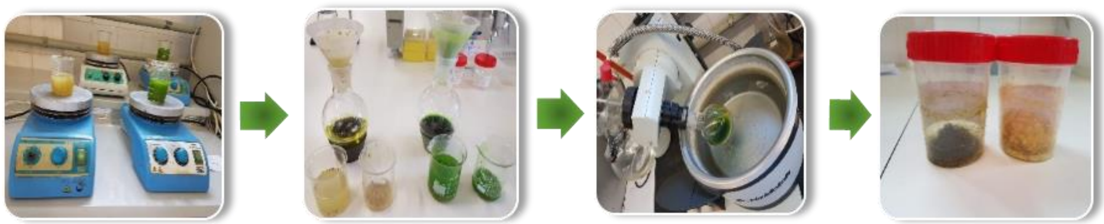


Figure 6: Stages of hydroethanolic extract preparation.
Source: Own authorship (2021).

4.4 Determination of phenolic compounds

For the analysis of phenolic compounds, a methodology previously described by the authors Bessada et al (2016) and Gonçalves et al. (2017) was followed.

The extracts described above were redissolved in 2 mL of the EtOH/H_2O solution (20:80, v/v) and filtered. The chromatographic analysis was performed in a Dionex Ultimate 3000 UPLC (Thermo Scientific, San Jose, CA, USA) system equipped with a diode array detector coupled to an electrospray ionization mass detector (LC-DAD-ESI/MSn), a quaternary pump, an auto-sampler (kept at 5 ºC), a degasser and an automated thermostat column compartment. Chromatographic separation was achieved with a Waters Spherisorb S3 ODS-2 C18 (3 µm, 4.6 mm × 150 mm, Waters, Milford, MA, USA) column thermostat at 35 °C. The solvents used were: (A) 0.1% formic acid in water, (B) acetonitrile. The elution gradient established was isocratic 15% B (5 min), 15% B to 20% B (5 min), 20-25% B (10 min), 25-35% B (10 min), 35-50% B (10 min), and re-equilibration of the column, using a flow rate of 0.5 mL/min.

A double online detection was carried out in the DAD using 280, 330 and 370 nm as preferred wavelengths and in a mass spectrometer (MS) connected to HPLC system via the DAD cell outlet. MS detection was performed in negative mode, using a Linear Ion Trap LTQ XL mass spectrometer (Thermo Finnigan, San Jose, CA, USA) equipped with an ESI source. Nitrogen served as the sheath gas (50 psi); the system was operated with a spray voltage of 5 kV, a source temperature of 325 °C, a capillary voltage of -20 V. The tube lens offset was kept at a voltage of -66 V. The full scan covered the mass range from m/z 100 to 1500. The collision energy used was 35 (arbitrary units). Data acquisition was carried out with Xcalibur® data system (Thermo Finnigan, San Jose, CA, USA). The phenolic compounds were identified by comparing their retention times, UV-vis and mass spectra with those obtained from standard compounds, when available. Otherwise, compounds were tentatively identified comparing the obtained information with available data reported in the literature. For quantitative analysis, a calibration curve for each available phenolic standard was constructed based on the UV signal. For the identified phenolic compounds for which a commercial standard was not available, the quantification was performed through the calibration curve of the most similar available standard. The results were expressed as mg/g of extract.

4.5 Evaluation of the bioactive properties of *Ananas comosus* L. peel and crown leaves

4.5.1 Antioxidant activity

4.5.1.1 Inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS)

Lipid peroxidation inhibition in porcine (*Sus scrofa*) brain homogenates was evaluated by the decrease in thiobarbituric acid reactive substances (TBARS), to which tris-HCl buffer was added (20 mM, pH=7.4), homogenized and centrifuged by 10 min (3500 rpm). Extracts of different concentrations (10, 5, 2.5, 1.25, 0.625, 0.325mg/mL) contained in the test tubes (200 µL) were added along with 100 µL of ascorbic acid, 100 µL of iron sulfate and 100 µL of brain suspension supernatant. The tubes were placed in a bath at 37 °C for 1 hour, at the end of which 500 µL of trichloroacetic acid (28%) and 380 µL thiobarbituric acid (2%). The tubes were then incubated at 80 °C for 20 minutes. After centrifugation at 3000rpm for 5 min for protein removal, the colour intensity of the malonaldehyde complex (MDA) -TBA of the supernatant was measured by absorbing it at 532 nm. The percentage of lipid peroxidation inhibition (%) was calculated using the following formula: [(A - B)/A] 100%, where A and B were the absorbance of the control and the sample solution, respectively (Barros et al., 2013a). The results were presented in IC_{50} values that represent the sample concentration that provides 50% of antioxidant activity.

4.5.1.2 Oxidative haemolysis inhibition assay (OxHLIA)

The antihaemolytic activity of the extracts was evaluated by the oxidative haemolysis inhibition assay (OxHLIA) described previously by Takebayashi et al. (2012) with some modifications by Lockowandt et al. (2019). Sheep blood samples were collected from healthy animals and centrifuged at 1000 g for 5 min at 10 °C. Plasma and buffy coats were discarded and erythrocytes were first washed once with NaCl (150 mM) and three times with phosphate-buffered saline (PBS, pH 7.4) (Evans et al., 2013). The erythrocyte pellet was then resuspended in PBS at 2.8% (v/v). Using a flat bottom 48-well microplate, 200 µL of erythrocyte solution was mixed with 400 µL of either PBS solution (control), antioxidant sample dissolved in PBS, or water (for complete haemolysis). Trolox was used as positive control (7.81–250 µg/mL PBS). After pre-incubation at 37 °C for 10 min with shaking, 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH, 160 mM in PBS, 200 μL) was added and the optical density was measured at 690 nm (**Figure 7**).

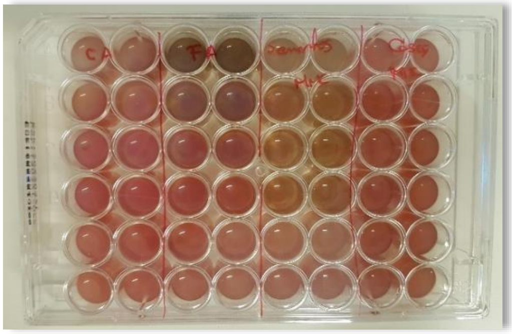


Figure 7: Microplate after incubation.
Source: Own authorship (2021).

After that, the microplate was incubated under the same conditions and the optical density was measured every 10 min at the same wavelength for approximately 400 min (Takebayashi et al., 2012). The percentage of the erythrocyte population that remained intact (P) was calculated according to Eq. (1):

$$P\ (\%) = (S_t - CH_0 / S_0 - CH_0) \times 100$$

Equation 1. Percentage of the erythrocyte (PE) population in the OxHLIA assay

where S_t and S_0 correspond to the optical density of the sample at t and 0 min, respectively, and CH_0 is the optical density of the complete haemolysis at 0 min. The results were expressed as delayed time of haemolysis (Δt), which was calculated according to Eq. (2):

$$\Delta t\ (\text{min}) = Ht_{50}\ (\text{sample}) - Ht_{50}\ (\text{control})$$

Equation 2. Haemolysis delay time.

where Ht_{50} is the 50% hemolytic time (min) graphically obtained from the haemolysis curve of each antioxidant sample concentration. The Δt values were then correlated to antioxidant sample concentrations (Takebayashi et al., 2012) and, from the correlation obtained, the extract concentration able to promote a Δt haemolysis delay was calculated. The results were given as IC_{50} values (μg/mL) at Δt 60 and 120 min, i.e., extract concentration required to keep 50% of the erythrocyte population intact for 60 and 120 min.

4.5.2 Evaluation of toxicity and antiproliferative activity

The extracts effects on the growth of human tumor cell lines were evaluated by sulforhodamine B (SRB) assay in order to determine cell growth inhibition. For this, 4 tumor cell lines were used: MCF-7 (breast carcinoma), NCI-H460 (lung carcinoma), AGS (gastric adenocarcinoma) and CaCo-2 (colon adenocarcinoma).

The cells were maintained as adherent cultures in RPMI-1640 medium containing 10% fetal bovine serum (FBS) (MCF-7, NCI-H460, AGS and CaCo-2) or in DMEM supplemented with 10% FBS, 2 mM glutamine, 100 U/mL penicillin and 100 mg/mL streptomycin, at 37 °C in an incubator with humidified air and 5% CO_2. Each cell line was prepared at the appropriate density (1.0×10^4 cells/well) in 96 well plates and left to adhere for 24 h. After this time the cells were tested for 48h with various concentrations of extracts. After the incubation period, the adherent cells were fixed by adding 10% cold trichloroacetic acid (TCA, 100 µL) and left to stand for 60 min at 4 ºC. The plates were then washed with deionized and dried water.

To each well 100 µL of the SRB solution (0.1% in 1% of acetic acid) were added and the plates were allowed to incubate at room temperature for 30 min. Excess RBS was removed by washing the plates with 1% acetic acid and were left to air dry. Then the SRB on was solubilized with 10 mM Tris (200 µL) and the absorbance was measured at 540 nm in a microplate reader (Biotek Elx800). The dose-response curves were obtained for each extract and cell line tested. The GI_{50} value was also calculated, corresponding to the concentration of extract that inhibits 50% of cell growth (Vichai & Kirtikara, 2006; Abreu et al., 2011). Ellipticine was used as a positive control.

For non-tumor cells, a cell culture was prepared from the kidney of an African green monkey, which was called VERO. The kidney tissue was washed with Hank's balanced salt solution containing 100 U/mL of penicillin, 100 µg/mL of streptomycin and divided into 3 explants of 1×1 mm. Some of these explants were placed in tissue vials of 25 cm^2, with DMEM medium supplemented with 10% bovine fetal serum, 2 mM of non-essential amino acids and 100 U/mL of penicillin, 100 mg/mL of streptomycin and incubated at 37 ºC with humidified atmosphere and 5% CO_2. The medium was changed every 2 days. Cell culture was continued with direct monitoring every 2 or 3 days using a phase contrast microscope. Subsequently, a cell subculture was performed, and these were placed in plates of 96 wells with the density of 1.0 $x10^4$ cells/well and cultivated in DMEM medium with FBS 10%, 100 U/mL of penicillin and 100 mg/mL of streptomycin (Abreu et al., 2011).

4.5.3 Antimicrobial activity

4.5.3.1. Antibacterial activity

The methodology previously described by Carocho et al., (2015) was followed and the following Gram-positive bacteria were tested: *Staphylococcus aureus* (ATCC (American type culture collection) 11632), *Bacillus cereus* (food isolate) *Listeria monocytogenes* (NCTC (National collection of type cultures) 7973) and Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 35030) and *Salmonella typhimurium* (ATCC 13311). All the microorganisms are deposited at Mycological laboratory, Department of Plant Physiology, Institute for Biological research "Sinisa Stanković", University of Belgrade, Serbia. The antibacterial assay was done by microdilution method utilizing 96-well microtiter plates to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The bacterial suspensions were adjusted with sterile saline solution until the concentration of 1.0×10^8 CFU/mL. The extracts were dissolved in 30% methanol and added to Tryptic Soy Broth medium and after inoculated with bacteria in final concentration 1×10^6 CFU/well. The microplates were incubated for 24 h, at 37°C. The lowest concentrations without visible growth of bacteria under the optical microscope were defined as the minimum inhibitory concentrations (MICs).

Furthermore, the MIC of the samples was detected following the addition of 40 µL of iodonitrotetrazolium chloride (INT) (0.2 mg/mL) and incubation at 37 °C for 30 min. The lowest concentration that produced a significant inhibition (around 50%) of the growth of the bacteria in comparison with the positive control was identified as the MIC. MBC were determined by serial sub-cultivation of 2 µL into microplates containing 100 µL of TSB. The lowest concentration that showed no growth after this sub-culturing was read as the MBC. Sodium sulphite (E221) and potassium metabisulphite (E224) food additives were used as positive controls. The results were expressed in mg/mL.

4.5.3.2. Antifungal activity

For the evaluation of antifungal activity, the procedure described previously by Carocho et al (2015) was followed and the following micromycetes were tested:

Aspergillus fumigatus (ATCC 9197), *Aspergillus versicolor* (ATCC 11730), *Aspergillus niger* (ATCC 6275), *Penicillium funiculosum* (ATCC 36839), *Penicillium aurantiogriseum* (food isolate), *Trichoderma viride* (IAM (Culture Collection, Centre for Cellular and Molecular Research, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Japan) 5061). These organisms were acquired in the Mycology Laboratory of the Department of Plant Physiology of the Institute of Biological Research "Siniša Stanković" at the University of Belgrade in Serbia. The micromycetes were kept in malt agar (MA) and the crops were stored at 4 °C and undergrown once a month. Fungal spores were washed from the surface of agar plates with sterile saline solution at 0.85% containing Tween 80 to 0.1 % (v/v). The spore suspension was adjusted with a sterile saline solution to a concentration of approximately 1.0×10^5 at a final volume of 100 µL per well. The inoculums were stored at 4°C.

The dilutions of the inoculums were cultivated in solid MA to verify the absence of contamination and the validity of the inoculum. MIC was determined by successive dilution technique in 96-well microplates. The sample was added to the malt medium with the fungal inoculum and the microplates were incubated for 72 hours at 28 °C. The lowest concentrations without visible growth (using a binocular microscope) were defined as MIC. The minimum fungicidal concentrations (MFCs) were determined by a series subculture of 2 µL of each well that showed no colour change, in microplates containing 100 µL of malt broth per well and subsequent incubated for 72h to 28 °C. The lowest concentration without visible biomass concentration was defined as MFC indicating the death of 99.5% of the original. Sodium sulphite (E221) and potassium metabisulphite (E224) food additives were used as positive controls. The results of MIC and MFC were expressed in mg per mL of hydroethanolic extract.

4.6 Incorporating the extract into a pastry product

To evaluate the potential of the hydroethanolic extract obtained from the *Ananas comosus* L. peel, as a potential natural ingredient, it was incorporated into a typical pastry product from the Bragança region called "súplicas". The effects of this incorporation on different parameters over its shelf life was evaluated.

4.6.1 Preparation of samples

"Súplicas" are a pastry product typical of Bragança (Portugal) and were prepared following a traditional recipe. For this, a dough was prepared by mixing only 3 ingredients: 8 eggs, 1 yolk, 600 g of wheat flour and 500 g of sugar (**Figure 8**). The dough was then divided into two groups, one without addition of the extract (C) and other with the hydroethanolic extract obtained from pineapple peel (A). The amount of lyophilized extract for addition to the mass was determined from the tests previously performed to achieve 50% of the bioactivity effectiveness of bio-residues. Thus, 2.8 g of lyophilized extract were added to one of the masses to perform the analyses. After preparation, both doughs were divided separately into small spheres and led to cooking in an oven at 180 ºC for 12 minutes. All samples were lyophilized, finely crushed and analysed, in triplicate, immediately after preparation (T0) and after three (T3) and seven (T7) days of storage (at room temperature and packed in a sealed plastic bags covered with aluminium paper).

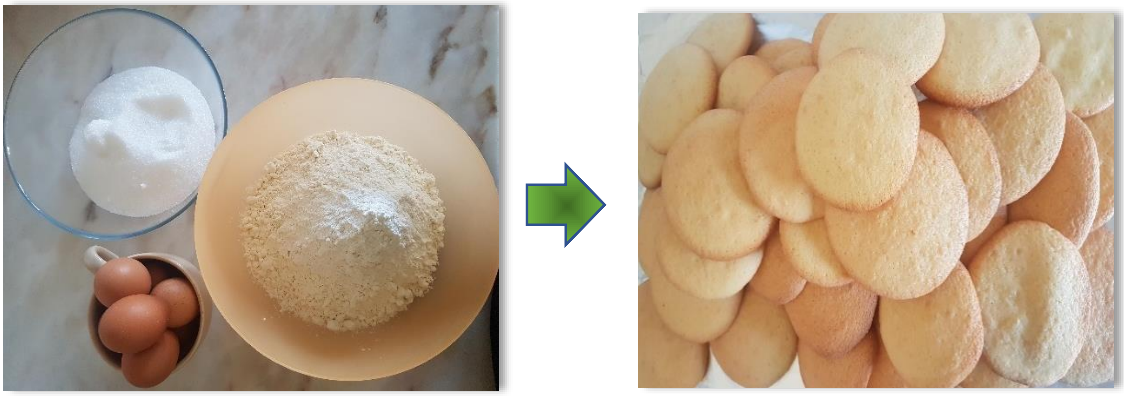


Figure 8: Preparation of "súplicas".

Source: Own authorship (2021).

4.6.1.1 Colour evaluation over time

The colour of the samples was measured in triplicate by each group of samples, at three different points using a colorimeter (model CR-400, Konica Minolta Sensing Inc., Tokyo, Japan). The Illuminate C was used and an 8 mm diaphragm aperture was previously calibrated against a standard white tile. CIE *L** (luminosity), *a** (green/red), *b** (blue/yellow) colour values were recorded using the data software "Spectra Magic Nx" (Fernandes et al., 2012).

4.6.2 Determination of the proximate composition of "súplicas"

The nutritional composition (protein, fat, carbohydrates, ash, humidity, and the total energy value) of the "súplicas" was determined according to official food analysis methodologies (AOAC, 2016).

For the determination of the humidity present in the sample, a known amount of sample (1 g) was placed in the oven (100 °C) until a constant mass is obtained.

The total protein content (N×5.70) was calculated as nitrogen content by the Kjeldahl method (AOAC 991.02). For this, concentrated sulfuric acid (H_2SO_4) was added to the sample (0.5 g) thus occurring the digestion of organic matter (**Figure 9**) and consequent formation of an inorganic salt, ammonium sulfate $(NH_4)_2SO_4$, in which nitrogen is retained. Then, the solution is alkalized by adding sodium hydroxide (NaOH), which enhances the release of nitrogen in the form of ammonia (NH_3). Ammonia is then distilled and collected in an H_2SO_4 solution (0.1 M). Finally, a titration with NaOH (0.1 M) is performed using a red methyl indicator, according to (AOAC 991.02).

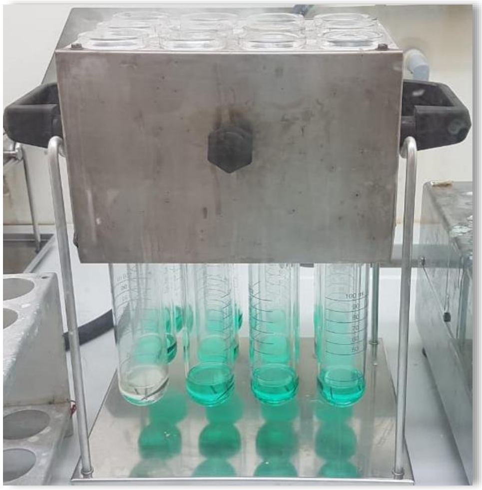


Figure 9: Block digester for determination of organic nitrogen by the Kjeldahl method.
Source: Own authorship (2021).

Lipids were determined by extracting a known sample mass (1.5 g) in a Soxhlet extractor for which the petroleum ether extraction solvent was used as extraction solvent at a temperature of approximately 120 °C for 7 h (AOAC 989.05), then the solution was evaporated as showed in the **Figure 10**.

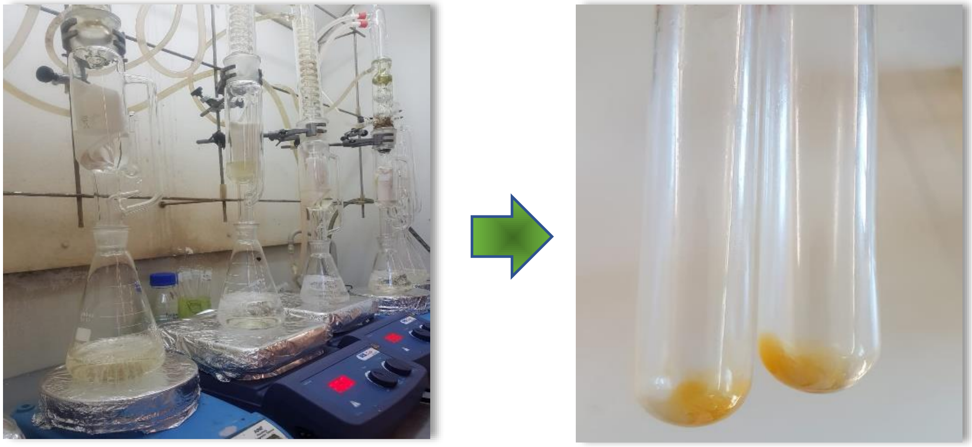


Figure 10: Lipids extraction with a Soxhlet extractor.
Source: Own authorship (2021).

While the ash content was determined from the incineration of 250 mg of sample at 550 ± 15 °C for 12 h (AOAC 935.42).

Carbohydrates were calculated by difference (**Equation 3**), while total energy was determined according to **Equation 4**, presented below.

$$\text{Carbohydrates} = 100 - (g_{proteins} + g_{fat} + g_{ash})$$

Equation 3. Equation for determination of carbohydrates.

$$\text{Energy (Kcal)} = 4 \times (g_{proteins} + g_{carbohydrates}) + (9 \times g_{fat})$$

Equation 4. Equation for determination of total energy.

4.7 Determination of the sugars and fatty acids content of "súplicas"

4.7.1 Sugars

Free sugars were determined by high-efficiency liquid chromatography coupled to a refraction index detector (HPLC-RI), as previously described by Barros et al., (2013b). The sample (1 g) was enriched with melezitose (used as an internal standard, 25mg/mL) and extracted with 40 mL of ethanol (80/20, v/v), in a bath at 80 °C (Julabo, SW22; Seelbach, Germany), for 1 hour and 30 minutes, with agitation every 15 minutes. Subsequently, the obtained centrifuge was centrifuged (Refrigerated Centrifuge K24OR, Centurion, West Sussex, United Kingdom) at 15,000 g for 10 minutes and transferred to a glass flask to evaporate the ethane fraction, using the rotary evaporator (Rotary evaporator Büchi R-210, Flawil, Switzerland) (60 ºC, reduced pressure). The aqueous phase was washed 3 times with diethyl ether (10 mL), and the remains of it were then evaporated (**Figure 11**). To the dry residue obtained, water was added until it made a final volume of 5 mL and 1.5 mL of it was filtered (nylon filters - 0.2 μm, Whatman) for a vial, for further analysis of the profile in sugars in the HPLC system.

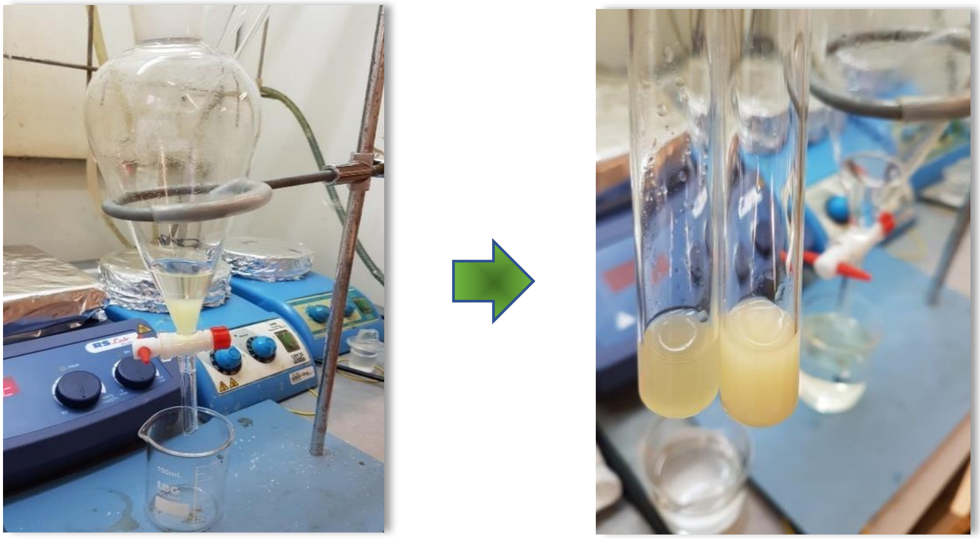


Figure 11: Aqueous phase wash with diethyl ether to remove impurities.
Source: Own authorship (2021).

The HPLC system is equipped with a pump (Knauer, Smartline System 1000, Berlin, Germany), a degassing system (Smartline manager 5000), an automatic sampler (AS-2057 Jasco, Easton, Maryland, USA) and a refraction index detector (Knauer Smartline 2300). Chromatographic separation was obtained through a Eurospher 100-5 NH2 column (4.6 x 250 mm, 5 μm, Knauer), which operated at a temperature of 30 ºC (7971 R Grace). As a mobile phase, acetonitrile/deionized water (70:30; v/v) was used with a flow rate of 1 mL/min. For the identification of the compounds, the Clarity 2.4 Software (DataApex) was used, from which the relative retention times of the sample peaks were compared with known patterns. The results were obtained by the internal pattern method and expressed in gram of composed of 100 g of fresh mass.

4.7.2 Fatty acids

Fatty acids were determined by gas chromatography with flame ionization (GC-FID) design, as previously described by Pereira et al., (2012). To the lipid extract previously obtained by extraction in Soxhlet, 5 mL of a solution of methanol/sulfuric acid/toluene was added, in the ratio of 2:1:1 (v/v/v) and the mixture remained in a bath (Julabo, SW22; Seelbach, Germany) at 50 °C (with agitation of 160 rpm) for approximately 12 h. After removing the bath tubes and, in order to enhance the phase separation, deionized water (3 mL) was added to the mixture and, later, for recovery of the methyl esters of fatty acids (FAME) added diethyl ether (3 mL), both steps with vortex agitation. After separation of the phases (**Figure 12**), the supernatant was transferred to a vial, in which anhydrous sodium sulfate was previously added in order to dehydrate the supernatant.

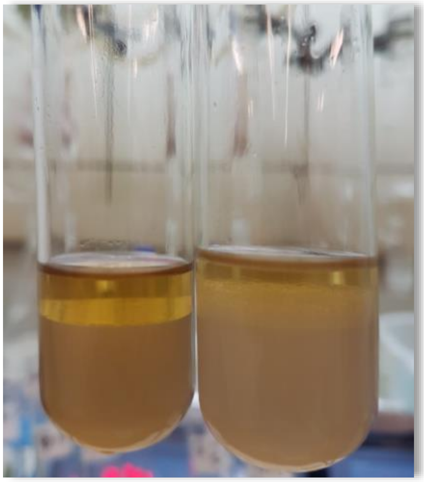


Figure 12: Separation of samples' phases.
Source: Own authorship (2021).

Finally, it was filtered through nylon filters (0.2 µm; Whatman) for a vial, for further analysis in CG. The fatty acid profile was obtained through a GC system (YOUNG IN Crhomass 6500 GC System) equipped with a split/splitless injector set at 250 ºC with a split ratio of 1:80, a flame ionization detector (FID) set at 260 ºC and a Zebron-Fame column (20 m × 0.18 mm ID × 0.15 µm df, Phenomenex, Lisbon, Portugal).

The following oven temperature program was used: initial temperature of 80 ºC, held for 1.5 min, increase 40 ºC/min to 160 ºC, followed by a 5 ºC/min ramp to 185 ºC, 30 ºC/min ramp to 260 ºC and held for 4 min. The carrier gas (hydrogen) flow-rate was 0.6 mL/min, measured at 250 ºC. Fatty acids identification and quantification was performed by comparing the relative retention times of FAME peaks from samples with standards (standard mixture 47885-U, Sigma, St. Louis, USA) and results were recorded and processed using the Software Clarity DataApex 4.0 Software (Prague, Czech Republic) and expressed in relative percentage of each fatty acid.

4.8 Determination of the antioxidant activity of "súplicas"

The antioxidant properties of the samples were evaluated through 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and reducing power to analyze whether the "súplicas" containing extract showed an antioxidant activity.

4.8.1 DPPH radical - scavenging activity

The DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical scavenger activity was evaluated according to a methodology described by Barros et al. (2008). The samples (30 μL) of different concentrations (200, 100, 50, 25, 12.5, 6.75 and 3.375mg/mL) of the extract solutions were added to the wells of a 96 well microplate with the methanolic solution (270 μL) containing DPPH radicals (6×10^{-5} mol/L). The mixture was left to stand in the dark for 30 minutes, and the absorbance was measured at 515 nm by using an ELX800 microplate reader (Bio-Tek Instruments, Inc; Winooski, USA). Free radical scavenging activity (RSA) was calculated using **Equation 5**:

$$\% \text{ RSA} = [(A_{DPPH}-A_S) / A_{DPPH}] \times 100$$

Equation 5. Equation for determination of DPPH radical scavenging activity

where, A_s is the absorbance of the solution in the presence of extract at a given concentration and A_{DPPH}, is the absorbance of the DPPH solution.

The extract concentration corresponding to 50% of radical scavenging activity (EC_{50}) was calculated from the graphical representation of the RSA percentage as a function of the extract concentration. The control used in this trial was trolox (Barros et al., 2010)

4.8.2 Reducing power

Reduction power was evaluated according to a methodology described by Barros et al. (2011). This methodology was performed using the Microplate Reader described above and measuring the absorbance at 690 nm. The different concentrations of the extracts (0.5 mL) were mixed with sodium phosphate buffer (200 mmol L^{-1}, pH 6.6, 0.5 mL) and potassium ferricyanide (1% w/v, 0.5 mL) and the mixture was incubated at 50 ºC for 20 min, and trichloroacetic acid (10% w/v, 0.5 mL) was added. The mixture (0.6 mL) was poured in the 48-well plate, as also ferric chloride (0.1% w/v, 120 μL) and deionized water (0.8 mL) (**Figure 13**). The reducing capacity was expressed in percentage (%) and calculated as follows:

$$\text{Reducing capacity (\%)} = 100 - \left(\frac{A_0 - A_s}{A_0} \times 100\right)$$

Equation 6. Equation for determination of reducing capacity

where A_0 corresponds the absorbance of a 66-μM Prussian blue solution measured in the same reaction medium free of ascorbate and A_s is the sample absorbance.

Figure 13: Control and sample after addition of ferric chloride.
Source: Own authorship (2021).

The extract concentration that provides 0.5 absorbance (EC_{50} - 50% of the maximum absorbance value at which the Lambert-Beer Law applies) was calculated from the graphical representation of the absorbance at 690 nm as a function of the extract concentration. In this assay, trolox was used as a control (Barros et al., 2010).

4.9 Statistical analysis

Throughout the whole document, all data was expressed as mean ± standard deviation. Furthermore, to better understand the effects of the incorporation of the pineapple extract (I) and the storage time (ST), a two-way ANOVA with type III sums of squares using the SPSS Software, version 25 was used for the analysis. This multivariate general linear model treats the two factors, I and ST as independent, thus allowing the effect of each one to be analyzed independently, providing more insight on their contribution towards the changes. If a significant interaction (p<0.05) was recorded among the two factors (I×ST), these were evaluated simultaneously, and some general conclusions and tendencies were extracted from the estimated marginal means (EMM) when possible. If there was no significant interaction ($p>0.05$), each factor was evaluated independently using a student's t-test. All analyses were carried out using a *p*-value of 0.05.

5. RESULTS AND DISCUSSION

5.1 Phenolic compounds present in the *Ananas comosus* L. bio-residues

Fruit residues are of great importance due to provide nutraceutical and biological properties such as anticancer, antimutagenicity, antiallergy and anti-aging activity due to the presence of phenolic compounds, which have been reported both for natural and synthetic antioxidants (Varzakas et al., 2016). According to the literature, pineapple residues have considerable levels of phenolic compounds, among them the gallic, ferulic and caffeic acids, as well as high antioxidant activity and high fiber content, presenting potential to be applied as a food ingredient in the development of new affordable products (Farias, 2021).

In this way, it is necessary to search for bioactive molecules, such as phenolic compounds that are present in many foods born residues, such as pineapple. In general, twenty-five phenolic compounds were identified in peels and crown leaves extracts, among them, phenolic acids and glycosidic flavonoids. The main detected compounds were caffeic acid derivatives, namely caffeic acid hexosides and flavones such as apigenin 6,8-*C*-diglucoside (**Figure 14**). **Table 7** shows the phenolic compounds profile details regarding the retention time, maximum absorption wavelength in the UV-Vis region (λmax), pseudomolecular ion ($[M-H]^-$) and molecular ion fragmentation (MS^2), as well as their quantification. Based on their mass spectra, the peel presented thirteen molecules, nine phenolic acids (peaks 1, 2, 3, 5, 11, 15, 17, 19 and 21), one phenylpropane monoglyceride (peak 12), three flavonoids, among them, one flavan-3-ol (peak 18) and two flavones (peak 8 and 9). Peaks **1**, **2**, **3** and **5** ($[M-H]^-$ at *m/z* 341), were identified as caffeic acid hexoside, these compounds were described by Steingass et al. (2015) in *Ananas comosus* [L.] Merr. In another study performed by Lourenço et al. (2021), demonstrated that the intensity of all the peaks were higher in the ethanolic extract instead of the water extract. Peak **9** showed a pseudomolecular ion $[[M-H]^-$ at *m/z* 593, being identified as apigenin 6,8-*C*-diglucoside (vicenin-2), the same was also shown by Steingass et al. (2015) in the crown leaves and peel of *A. comosus.* Peak **8** was identified as diosmetin 8-*C*-glucoside ($[M-H]^-$ at *m/z* 461), taking into account its fragmentation pattern. This compound was also reported in sugarcane leaves (Colombo et al., 2013) and in mandarin juice (*Citrus reticulata* Blanco) (Testai et al,2021).

Compound **11** ($[M-H]^-$ at *m/z* 597) was identified as yunnaneic acid F that is biogenetically derivative of yunnaneic acid C and normally detected in *Salvia* *yunnanensis* (Tanaka et al., 1997). This compound was also found in lemon balm extract (*Melissa officinalis* L.) by Pérez-Sánchez et al., 2016. Yunnaneic acid is also considered a phenolic compound derived from rosmarinic acid (RA) as lithospermic acid, salvianolic acid, and melitric acid, which have all a diverse biological function (Kim et al., 2015). Peak **12** ($[M-H]^-$ at *m/z* 399) was identified as *p*-coumaroyl-caffeoylglycerol, this compound was previously found 'Phulae' Pineapple peel (Sari et al., 2017). *p*-coumaroylglycerol and caffeoylglycerol have been described in the pineapples stems and leaves, indicating that they were the primary forms of phenolic glycerides (Takata, et al. 1976; Wang, et al. 2006).

Compound **15** ($[M-H]^-$ at *m/z* 771) was identified as 3,5-di-*O*-caffeoyl-4-*O*- (3-hydroxy, 3-methyl) glutaroylquinic acid, this compound was also identified in *Gardenia jasminoides* Ellis fruits by Wang et al. (2016). Compound **17** ($[M-H]^-$ at *m/z* 337) was identified as *p*-coumaroylisocitrate, based on their UV and mass characteristics as reported by Steingass et al. (2017) and also by Difonzo et al. (2019) in the pulp and peel of *A. comosus*.

Peak **18** ($[M-H]^-$ at *m/z* 305) was identified as (epi)gallocatechin, a flavan-3-ol that has been previously identified in cabbage stalk flour (Brito et al., 2021). Peak **19** ($[M-H]^-$ at *m/z* 537), was identified as vanilloyl dihexoside (formic acid adduct) being previously identified in pineapple pulp by Steingass et al. (2015). Compound **21** ($[M-H]^-$ at *m/z* 577) was identified *N*-L-γ-Glutamyl-*S*-sinapyl-L-cysteine, a sinapyl derivative revealing a standard fragmentation in *m/z* 249, this assumption was made taking into account the study performed by Wen et al. (1999), which also reported the presence of this molecule in *A. comosus* juice.

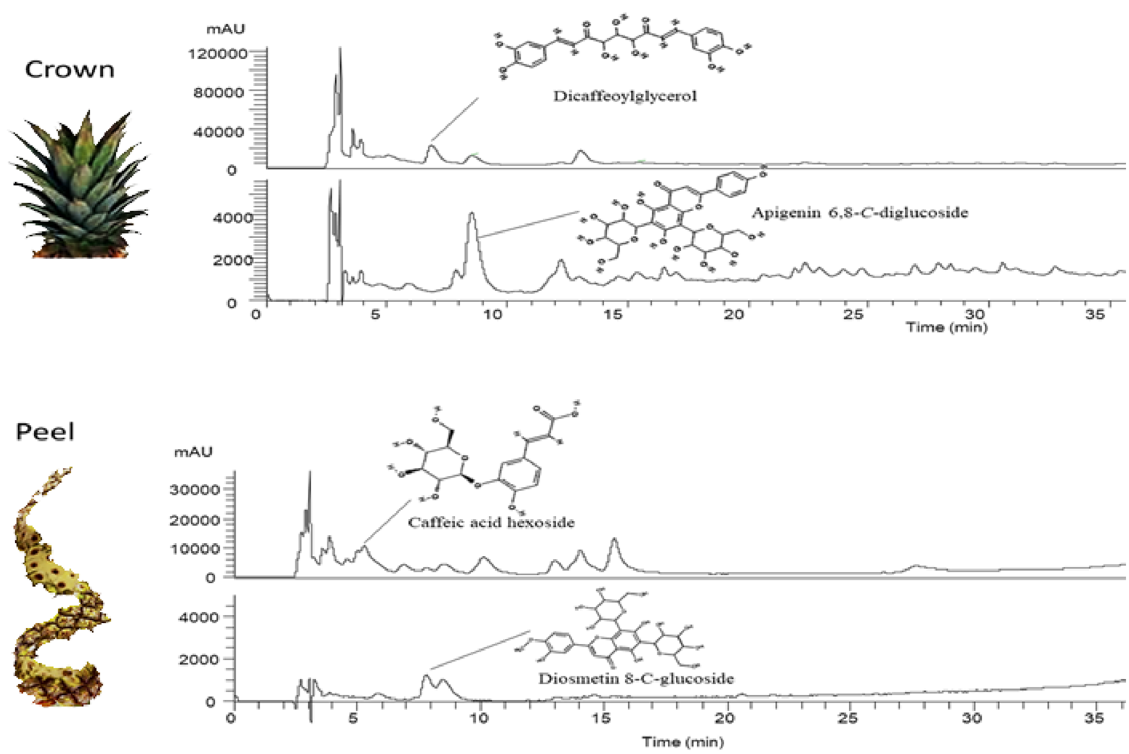


Figure 14: Phenolic compounds profile of the hydroethanolic extract of pineapple peel and crown leaves recorded at 280 nm and 370 nm as wavelength. Peak numbers correspond to the phenolic compounds identified in **Table 7**.

Regarding the pineapple leaves, twelve phenolic compounds were identified, compromising nine flavonoids and two phenolic acids. Among them two flavonols (peaks number **23** and **25**), one phenylpropane diglyceride, (**4**), two hydroxycinnamic acids (**7** and **13**) and seven flavones (**6**, **9**, **14**, **16**, **20**, **22** and **24**). Peak **4** ($[M-H]^-$ at *m/z* 451) was tentatively identified as dicaffeoylglycerol, a glycerol ester, this compound was previously identified in the peels, crown leaves and pulp of pineapple by Steingass et al. (2015). Compound **6** ($[M-H]^-$ at *m/z* 517) was tentatively identified as an apigenin-*O*-malonyl-hexoside, this flavone was identified taking into account the findings reported in a study regarding cardoon (*Cynara cardunculus* var. *altilis*) bracts describe preciously by Mandim et al. (2021). Peaks **7** and **13 (**$[M-H]^-$ at *m/z* 385**)** were identified as a typical pineapple glycosylated polyphenol, called feruloyl aldarate, found in the peel and pineapple crown leaves reported by Steingass et al. (2015) and Campos et al. (2020b). Compounds **6, 9, 14, 20** and **24** were identified as apigenin derivatives, linked to different sugars and typical *C*-glycosyl fragments. All the mentioned compounds were tentatively identified as apigenin-malonyl-hexoside (peak **6**; λmax, 321 nm; $[M-H]^-$ at *m/z* 517), apigenin-6,8-*C*-diglucoside (peak **9** and **14**; λmax, 328 nm; $[M-H]^-$ at *m/z* 593), apigenin-

6-*C*-hexoside-8-*C*-pentoside (peak **20**; λmax, 322 nm; $[M-H]^-$ at *m/z* 563), and apigenin-*C*-dipentoside (peak **24**; λmax, 327 nm; $[M-H]^-$ at *m/z* 533), being all previously found in *Ananas* sp (pineapple) by Frank et al. (2004) and Harnly et al. (2006). Compound **16** was identified as an eriodictyol-4'-*O*-neohesperidoside-7-*O*-glucoside ($[M-H]^-$ ion at *m/z* 757 and MS^2 ions at *m/z* 595 (M-glucose), 449 (M-308, neohesperidose), and 287 (M-glucose and neohesperidose). Compound **22** ($[M-H]^-$ ion at *m/z* 537) revealed a single fragment ion at *m/z* 375, derivate from the loss of a dehydrated hexose, and was tentatively identified as ananaflavoside B, sub classified as a metoxylated flavone, that can be synthetized from subsequent hydroxylations, methylations and glycosylation of luteolin (**Figure 15**) (Stefani, 2020; Ma, et al. 2007). Ananaflavoside B was reported previously by Steingass et al. (2015) in crown leaves and pineapple peel.

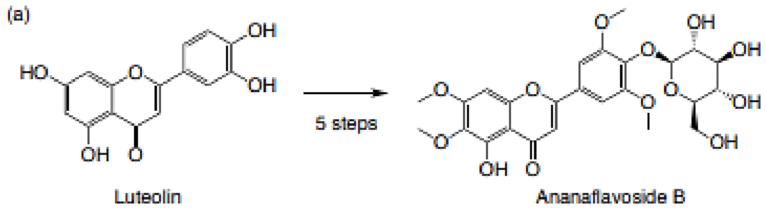


Figure 15: Biosynthetic pathway schematization of ananaflavoside B.
Source: Stefani, 2020.

Peak **23** was identified as quercetin-3-*O*-rutinoside (rutin) taking into account the chromatographic characteristics in comparison to the commercial standard. Two derivatives of quercetin were reported by Steingass et al. (2015) in the crown leaves of pineapple, being quercetin dihexoside ($[M-H]^-$ at *m/z* 625) and quercetin hexoside ($[M-H]^-$ at *m/z* 463). Compound 25 ($[M-H]^-$ at *m/z* 623) was identified as isorhamnetin-*O*-rhamosyl-hexoside also reported by Steingass et al. (2015) in pineapple peel and in the palm fruit peel (*Phoenix dactylifera* L.) by Farag et al. (2016). Comparing the total phenolic composition in the crown leaves and peel's the major predominance was in the crown leaves with 4.7±0.1 mg/g of extract, with the highest content in flavonoids. In contrast, the peel showed only 2.48±0.03 mg/g of extract, being the amounts of phenolic acids and flavonoids very similar. The main compounds in the pineapple peel were the (epi)gallocatechin (0.59±0.02 mg/g) followed by apigenin-6,8-C-diglucoside (0.46±0.01 mg/g). While in the crown leaves, apigenin-*O*-malonyl-hexoside and apigenin-6,8-*C*-diglucoside (1.1±0.1 mg/g and 0.56±0.01 mg/g, respectively) were predominant.

In this sense, the present work presented some phenolic compounds that differ slightly from those described in the literature. This is because fruits and vegetables have different pre-harvest and post-harvest treatments that can change the phenolic compounds content, which may induce the accumulation or degradation of these compounds (De la Rosa et al., 2019). Conditions such as temperature, soil properties, light irradiation, irrigation, fertilizers, harvesting stage, wounding, modified atmosphere, and elicitor treatments, are known to regulate the content of PCs in fruits and vegetables (**Figure 16**).

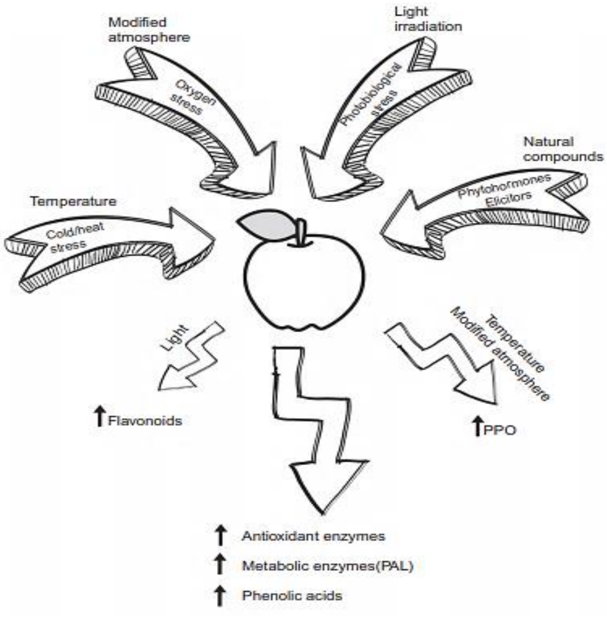


Figure 16: Increase in PCs by stress-inducing postharvest treatments in fruits and vegetables. Source: De la Rosa et al., 2019.

According to De la Rosa et al. (2019), these technologies induce oxidative stress (formation of reactive oxygen species (ROS) in fruits and vegetables), triggering the plants defence system, which involves the synthesis of antioxidant secondary metabolites such as PCs, and the activation of antioxidant enzymes.

Table 7: Retention time (Rt), wavelengths of maximum absorption in the visible region (λmax), mass spectral data, identification and quantification of phenolic compounds in peel extracts and crown leaves of *Ananas comosus* L

Peak	**Rt (min)**	**$\lambda_{max\ (nm)}$**	**[M-H]⁻ (*m/z*)**	**MS^2 (*m/z*)**	**Identification**	**Quantification**	
						Peel	**Crown leaves**
1	4.48	275	341	179(100)	Caffeic acid hexoside[1]	0.0009±0.0001	nd
2	4.93	275	341	179(100)	Caffeic acid hexoside[1]	0.0034±0.0002	nd
3	5.30	277	341	179(100)	Caffeic acid hexoside[1]	0.0294±0.0002	nd
4	5.90	292	451	253(100), 179(3), 161(20), 135(11)	Dicaffeoylglycerol[1]	nd	0.011±0.001
5	6.79	277	341	179(100)	Caffeic acid hexoside[1]	0.006±0.0002	nd
6	6.86	321	517	473(100), 269(18)	Apigenin-*O*-malonyl-hexoside[2]	nd	1.1±0.1
7	7.86	326	385	209(8), 191(100), 147(5), 129(11)	Feruloyl aldarate[3]	nd	0.035±0.001
8	8.03	332	461	347(100), 299(25)	Diosmetin-8-*C*-glucoside[4]	0.1175±0.0002	nd
9	8.45	328	593	473(100)	Apigenin-6,8-*C*-diglucoside[5]	0.46±0.01	0.56±0.01
11	9.29	274sh332	597	359(17), 295(20), 197(62), 179(56), 135(95)	Yunnaneic acid F[6]	0.41±0.005	nd
12	10.15	275,327	399	253(100), 235(23), 163(17), 145(9)	*p*-Coumaroyl-caffeoylglycerol[7]	0.25±0.01	nd
13	10.70	326	385	209(6), 191(100), 147(7), 129(15)	Feruloyl aldarate[3]	nd	0.031±0.001
14	12.19	324	593	431(100)	Apigenin-6,8-*C*-diglucoside[5]	nd	0.483±0.005
15	13.05	365	771	609(95), 301(32), 179(12)	3,5-*O*-Dicaffeoyl-4-*O*-(3-hydroxy, 3-methyl) glutaroylquinic acid[7]	0.18±0.01	nd
16	13.05	315	757	595(21), 449(46), 287(90)	Eriodictyol-4'-*O*-neohesperidoside-7-*O*-glucoside[8]	nd	0.058±0.003
17	13.29	277sh301	337	191(85), 173(100), 155(4)	*p*-Coumaroylisocitrate[7]	0.132±0.005	nd
18	14.06	225sh277	305	261(12), 221(47), 219(56), 179(100), 165(28), 137(30), 125(40)	(Epi)gallocatechin[9]	0.59±0.02	nd
19	14.40	315	537	491(72), 329(100), 209(2), 167(24), 152(4)	Vanilloyl dihexoside[10]	0.118±0.001	nd
20	14.63	322	563	473(100), 443(69), 383(22), 353(28)	Apigenin-6-*C*-hexoside-8-*C*-pentoside[5]	nd	0.462±0.002
21	15.42	225sh274	441	249(100)	N-L-γ-Glutamyl-S-sinapyl-L-cysteine[11]	0.17±0.02	nd
22	15.60	327	537	375 (100)	Ananaflavoside B[12]	nd	0.468±0.001
23	16.51	350	609	301(100)	Quercetin-3-*O*-rutinoside[12]	nd	0.471±0.001
24	17.09	327	533	311(100)	Apigenin-*C*-dipentoside[5]	nd	0.467±0.002
25	20.55	327	623	315(100)	Isorhametin-*O*-rhamosyl-hexoside[12]	nd	0.469±0.001
					TPA	1.31±0.05	0.078±0.002
					TF	1.17±0.02	4.6±0.1
					TPC	2.48±0.03	4.7±0.1

The results are expressed in mg/g of extract. nd: not detected. Calibration curves used: 1. caffeic acid (y = 388345x + 406369; R^2 = 0.994; LOD=0.78 μg/mL; LOQ=1.97 μg/mL); 2. apigenin-7-*O*-glucoside (y = 10683x - 45794; R^2 = 0.999; LOD = 0.10 μg/mL; LOQ = 0.53 μg/mL); 3. ferulic acid (y=633,126x−185,462; R^2=0.999; LOD=0.20 μg/ml; 1.01 μg/ml); 4. taxifolin (y=478.06x +657.33; R^2 =0.999; LOD = 0.67 μg/mL; LOQ = 2.01 μg/mL); 5. apigenin 6-C-glucoside (y = 107025x - 61531; R2 = 0.9989; LOD = 0.19 μg/mL; LOQ = 0.63 μg/mL); 6. romarinic acid (y = 191291x – 652903; R^2 = 0.999; LOD = 0.15 μg/mL; LOQ = 0.68 μg/mL); 7. clorogenic acid (y = 168823x - 161172; R^2 = 0.9999; LOD = 0.20 μg/mL; LOQ = 0.68 μg/mL); 8. naringenin (y = 18433x + 78903, R² = 0.9998; LOD = 0.20 μg/mL; LOQ = 0.64 μg/mL); 9. epicatechin (y = 10314x + 147331, R²= 0.9994; LOD = 0.15 μg/mL; LOQ = 0.78 μg/mL); 10. Vanillic acid (y=197,337x+30036; R^2=0.999; LOD=0.17 μg/ml; LOQ=1.22 μg/ml); 11. sinapic acid (y=197,337x+30036; R^2=0.999; LOD=0.17 μg/ml; LOQ=1.22 μg/ml); 12. quercetin-3-*O*-glucoside (y = 34843x – 160173; R^2 = 0.9998; LOD = 0.21 μg/mL; LOQ = 0.71 μg/mL).

5.2 Bioactive properties of *Ananas comosus* L. peel and crown leaves

5.2.1 Antioxidant activity

The antioxidant activity of the hydroethanolic extracts obtained from pineapple peel and crown leaves was evaluated through the inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS) and Inhibition of Oxidative Hemolysis (OxHLIA). The results were expressed in terms of IC_{50}, being represented in **Table 8**. Considering that the lower value of IC_{50} indicates higher antioxidant activity, in both assays the extract that revealed a better activity was pineapple peel, with an IC_{50} of 4.3±0.1 µg/mL for the TBARS assay and 190±7 µg/mL for the OxHLIA assay in a Δt 60 min. In relation of TBARS assay, the IC_{50} obtained by the peel extracts is lower than that presented by the standard used (Trolox), making these extracts a great source of compounds with antioxidant potential.

Table 8: Results of lipid peroxidation inhibition assay (TBARS) and inhibition of oxidative haemolysis (OxHLIA) of peel and crown leaves of *Ananas comosus* L. (mean ± SD).

Antioxidant Activity (IC_{50} values, µg/mL)				
		CA	**FA**	**Trolox**
TBARS		4.3±0.1*	6.6±0.3*	5.4±0.3
OxHLIA	Δ*t* 60 min	190±7*	395±19*	21.8±0.3
	Δ*t* 120 min	333±9*	714±33*	43.5±0.8

IC_{50} values for the antioxidant activity correspond to the concentration of extract responsible for inhibiting 50% of oxidation. * *t-student test p-value <0.001.*

According to Le (2014), lipid peroxidation is considered a biosensor for the evaluation of oxidative stress where free radicals such as radical hydroxyl, being the most reactive form of reactive oxygen species (ROS), can initiate lipid peroxidation by attacking polyunsaturated fatty acids. In the work conducted by Selani et al. (2016), the treatment with by-product of pineapple delayed lipid oxidation in raw hamburgers and cooked in relation to other treatments. This fact is probably due the phenolic compounds composition in the pineapple peel (3.78 mg gallic acid equivalent/g pineapple by-product), with antioxidant activity (DPPH: 5.76 µmol Trolox / g of pineapple by-product; ABTS: 13.46 µmol of Trolox/g pineapple - product) that could have helped protect hamburgers from lipid oxidation.

Reported by Gómez & Pablos (2016), pineapple residue extract reduced secondary oxidation formation by 45.92%, showing that polyphenols in extracts reduced the formation of secondary oxidation products, despite the presence of high concentration of primary oxidation products. The significant antioxidant capacity of pineapple extracts has been proven by Jovanović et al. (2018), where the highest antioxidant activity was detected in pericarp extract prepared with absolute methanol (IC_{50} = 1.74 ± 0.05 mg/mL) against the lowest, detected in pineapple juice (IC_{50} = 88.00 ± 2.09 mg/mL). Regarding to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay, the highest percentage of inhibition presented by pineapple bio residues was the crown leaves extracted with 100% ethanol ratio (75.57%), followed by the peel (72.67%) and core (49.14%) extracted with 50% ethanol ratio. Based on the results obtained by this study, it shows that the antioxidant activities showed greater inhibition capacity were in samples with a higher percentage of ethanol, concluding that the solubility of metabolites was lower in water compared to ethanol (Azizan et al., 2020).

5.2.2 Toxicity and antiproliferative activity

The results obtained for the antiproliferative activity testing four human tumor cell lines and for the primary culture of non-tumor cells are also found in **Table 9** and are expressed in values of the concentration of extract responsible for inhibiting cell proliferation by 50% (GI_{50}), values in μg/mL. Being lower GI_{50} values represent a higher cytotoxic potential of the tested samples, the extract that most demonstrated no cytotoxicity was the pineapple crown leaves with the GI_{50} values higher than 400 μg/mL for all tested cancer cell lines. Both extracts did not express cytotoxicity against non-tumor cell culture, VERO (GI_{50} > 400 μg/mL). Sah et al. (2016) reported other antiproliferative activity against HT29 colon cancer cells, where probiotic yogurt with pineapple peel powder significantly was higher than in nonsupplemented probiotic yogurt. The PPP-supplemented probiotic yogurt presented a stronger antiproliferative activity of 56.36% against the nonsupplemented control probiotic yogurt with 40.52% and plain yogurt with 35.71% after 28 days of refrigerated storage.

Table 9: Results of toxicity and antiproliferative activity activity of peel and crown leaves extracts of *Ananas comosus* L. (mean ± SD).

Antiproliferative (GI_{50} values; μM)				
		CA	**FA**	**Ellipticine (μM)**
Tumoral cell lines	AGS	>400	>400	0.9±0.1
	CaCo-2	378±7	>400	0.8±0.1
	MCF_7	322±3	>400	1.020 ± 0.004
	NCI-H460	>400	>400	1.01±0.01
Non-tumoral culture	VERO	>400	>400	0.6±0.1

GI_{50} values correspond to the concentration that causes 50% inhibition of cell proliferation; AGS - human gastric adenocarcinoma; CaCo-2 - human colon adenocarcinoma MCF-7 - human breast adenocarcinoma; NCI-H460 - human lung carcinoma. *t-student test *p*-value <0.001.

5.2.3. Antimicrobial activity

5.2.3.1 Antibacterial activity

The antibacterial activity of hydroethanolic extracts obtained from pineapple peel and crown leaves was tested against a panel of six bacteria, including three Gram-positive bacteria *(Staphylococcus aureus, Bacillus cereus* and *Listeria monocytogenes)* and three Gram-negative bacteria (*Escherichia coli, Salmonella typhimurium and Enterobacter cloacae*). **Table 10** presents the results obtained for each extract in minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

The results presented showed that the bacteria *E. coli* was the most sensitive bacteria for both tested extracts with lower MIC value, followed by *S. aureus* and *E. cloacae*. Both analyzed extracts showed similar antibacterial performance for all strains tested, however, it is important to highlight the better performance when compared to controls (E211 and E224).

Table 10: Antibacterial activity of *Ananas comosus* L. peel and crown leaves extract (MIC and MBC in mg/mL).

Samples		*S. aureus*	*B.cereus*	*L. monocytogenes*	*E. coli*	*S. typhimurium*	*E. cloacae*
FA	**MIC**	1.00	1.00	2.00	0.50	1.00	1.00
	MBC	1.00	2.00	4.00	1.00	2.00	1.00
CA	**MIC**	1.00	1.00	2.00	0.50	1.00	1.00
	MBC	2.00	2.00	2.00	1.00	2.00	1.00
E211	**MIC**	4.00	0.50	1.00	1.00	1.00	2.00
	MBC	4.00	0.50	2.00	2.00	2.00	4.00
E224	**MIC**	1.00	2.00	0.50	0.50	1.00	0.50
	MBC	1.00	4.00	1.00	1.00	1.00	0.50

S. aureus: Staphylococcus aureus; B. cereus: Bacillus cereus; L. monocytogenes: Listeria monocytogenes; E. coli: Escherichia coli; S. typhimurium: Salmonella typhimurium; E. cloacae: Enterobacter cloacae. MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration. Sodium sulphite (E221) and potassium metabisulphite (E224) food additives were used as positive controls.

Regarding MBC values, it is possible to highlight *E. coli* and *E.cloacae* as the most sensitive bacteria for both pineapple extracts. In this case, it is interesting to note that the performance of the extracts was more efficient than the E211 control and similar to that presented by the E224 control. These positive controls are used as a food preservative in food industry, applied in most of the fruits and vegetables to extend the shelf life by shielding them against deterioration caused by microorganisms, being effective bleaching agents, antimicrobials, oxygen scavengers, reducing agents, and enzyme inhibitors (Garcia-Fuentes et al., 2015; Silva et al., 2016b; Mandal, 2019). The work presented by Wijayati et al. (2016) showed good results for pineapple peel extract against *Escherichia coli* and *Staphylococcus aureus*, proving to be effective as an antibacterial agent. According to Wijayati et al. (2016) the hand sanitizer formulation with pineapple peel extract at concentrations 0.5%, 1% and 1.5% extract produced inhibition zone against *Escherichia coli* that is 9 mm, 13 mm and 15 mm, while against *Staphylococcus aureus* produced inhibition zone of 10 mm, 15 mm and 15.5 mm. In conclusion, the higher the concentration of extract added, the greater the zone will be.

In a study developed by Goudarzi et al. (2019), pineapple peel extract presented the strongest antibacterial activity with highest inhibition zones (30 mm and 28 mm) and the lowest MIC (1.56 mg/L and 6.26 mg/mL) against *S. sanguis* and *S. Mutans*, respectively. The hydroethanolic extracts of pineapple peel showed better results for the minimum bactericidal concentration for both bacteria (3.12 mg/mL for the *S. Sanguis;* 12.5 mg/mL for the *S. Mutans*) against, 25 mg/mL (*S. Sanguis)* and 100 mg/mL (*S. Mutans*) for the pulp.

This also was confirmed by Ogwu et al. (2019) and Okoh et al. (2019), where the peel extracts revealed stronger antibacterial activity than the pulp against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus faecalis* and *Pseudomonas aeruginosa*.

In the work conducted by Punbusayakul (2018), the antibacterial activity of the pineapple peel was tested against four foodborne pathogens, and the results revealed that *B. cereus* exhibited the most sensitive strain with minimum inhibition concentration (MIC) of 0.0675 g/mL, followed by *S. aureus* and *E. coli* (0.1349 g/mL) and *S. typhimurium* (0.2699 g/mL), respectively. Regarding pineapple crown leaves, Brito et al., (2021) reported that the essential oil of pineapple-crown flour showed to be active against *B. cereus, E. coli* and *L. monocytogenes*. The same result obtained by this study, except for *L. monocytogenes* which demonstrated higher or similar indices of MIC and MBC in relation to positive control. In the research presented by Dutta & Bhattacharyya (2013), proved that the crown leaves extract possessed bacteriostatic and fungistatic components, exhibiting 70-95% inhibition of microbial growth with minimum inhibitory concentration range of 1.65-4.95 mg/mL against *Saccharomyces cereviseae*, *Escherichia coli*, *Bacillus subtilis*, *Candida albicans* and *Staphylococcus aureus*.

According to Zharfan & Mustika (2017), previous studies suggested the bioactive compounds that act against gram-negative bacteria are majorly bromelain and saponin, while flavonoids and polyphenols are more potent in inhibit gram-positive bacteria. In the case of flavonoids and polyphenols, they work mainly in the peptidoglycan layer in Gram-positive bacteria by having polar properties (Suerni et al., 2013).

The bromelain is a proteolytic enzyme, and it is suggested that operate in weakening of outer membrane of gram-negative bacteria by proteins, thus leading to cellular damage (Zharfan & Mustika, 2017). On the other hand, saponin causes a change in membrane structure and function, increasing the permeability of the bacterial cell membrane, thus allowing antibacterial substances to easily enter cells, causing an interference in cellular metabolism while denaturing proteins in the membrane so that it is disintegrated (Karlina et al., 2013).

5.2.3.2 Antifungal activity

The antifungal activity of the hydroethanolic extracts obtained from pineapple peel and crown leaves was tested against a panel of six fungi (*Aspergillus fumigatus, Aspergillus niger, Aspergillus versicolor*, *Penicillium funiculosum*, *Penicillium aurantiogriseum* and

Trichoderma viride), and the results expressed in minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC), being presented in the **Table 11.**

Table 11: Antifungal activity of peel and crown leaves of *Ananas comosus* L. (MIC and MFC in mg/mL).

Samples		*A. fumigatus*	*A. niger*	*A. versicolor*	*P. funiculosum*	*P. aurantiogriseum*	*T. viride*
FA	**MIC**	0.50	0.25	0.50	0.25	1.00	0.25
	MFC	0.50	0.50	1.00	0.50	2.00	0.50
CA	**MIC**	0.50	0.25	1.00	0.25	2.00	0.25
	MFC	1.00	0.50	2.00	0.50	4.00	0.50
E211	**MIC**	1.00	1.00	2.00	1.00	2.00	1.00
	MFC	2.00	2.00	2.00	2.00	4.00	2.00
E224	**MIC**	1.00	1.00	1.00	0.50	1.00	0.50
	MFC	1.00	1.00	1.00	0.50	1.00	0.50

A. fumigatus: Aspergillus fumigatus; A. niger: Aspergillus niger; A. versicolor: Aspergillus versicolor; P. funiculosum: Penicillium funiculosum; P. aurantiogriseum: Penicillium aurantiogriseum; T. viride: Trichoderma viride. MIC: Minimum inhibitory concentration; MFC: Minimum fungicidal concentration. Sodium sulphite (E221) and potassium metabisulphite (E224) food additives were used as positive controls.

The results obtained demonstrate that both pineapple hydroethanolic extracts presented antifungal activity against all the six fungi analysed. The results presented showed that *A. niger*, *P. funicolosum* and *T. viride* were the most sensitive fungi for both extracts analysed with lower MIC values. It was even possible to verify that, for most of the fungi tested, the extracts showed better antifungal performance than the controls used (E211 and E224).

A study developed by Olakunle et al. (2019), compared the antifungal activity of four fruit peels (banana, pineapple, cashew and orange) and the results showed a greater antifungal activity of pineapple peel when tested against *Aspergillus niger* and *Alternaria alternata*. In turn, Chanda et al. (2010) intended to verify the influence of different solvents on the antimicrobial capacity of extracts, demonstrating that polar solvents (acetone and methanol) are more effective than nonpolar solvents (hexane and chloroform). In this study methanol extracts of *A. comosus* showed the best results against *Candida albicans*, *Candida glabrata*, *Cryptococcus luteoluscom* and *Candida tropicalis* with inhibition zones of 12 mm, 11.5 mm, 10.5 mm and 9.5 mm, respectively. As for nonpolar solvents, only chloroform showed activity against pathogenic fungi.

5.3 Characterization of the "súplicas" formulations

5.3.1 Nutritional composition of "súplicas"

For a better interpretation of the results, the following tables result of a two-way ANOVA, being divided in two sections, the upper represents the incorporation (control and pineapple peel) (I) but, included in the standard deviation of each incorporation are both the three storage times (0, 3 and 7 days), and, in the bottom section for each storage time (ST) both incorporations are included. Using this statistical tool, each factor can be analysed independently. When *p*-value I×ST > 0.05 a post-hoc classification was used, namely a student's T-test for I and a Tukey's test for ST. However, when *p*-value I×ST < 0.05, then, a significant interaction between both factors hinders an independent classification, and thus, for some cases, only general trends can be obtained through the estimated marginal means plots (EMM).

Table 12: Nutritional profile and sugar content in "súplicas" control and "súplicas" with pineapple peel extract evaluating storage time (ST).

		Moisture (g/100 g fw)	**Fat (g/100 g fw)**	**Proteins (g/100 g fw)**	**Ash (g/100 g fw)**	**Carbohydrates (g/100 g fw)**	**Energy (Kcal)**	**Energy (Kj)**	**Sucrose (g/100 g fw)**
Incorporation (I)	Control	6.5±0.2*	4.13±0.06	7.80±0.07*	0.34±0.01	80.9±0.2	393±1	1645±4	86±13
	Pineapple peel	7.4±0.2	3.56±0.04	7.90±0.01	0.36±0.01	80.7±0.2	386±1	1618±4	85±3
p-value (n=9)	T-test	<0.001	<0.001	0.005	<0.001	<0.001	<0.001	<0.001	0.708
Storage Time (ST)	0 Days	7.0±0.2	3.8±0.3	7.92±0.07	0.37±0.01	80.8±0.1	389±2	1630±9	91±2[b]
	3 Days	6.9±0.5	3.9±0.3	8.0±0.1	0.35±0.01	80.8±0.3	390±3	1633±14	82±3[a]
	7 Days	7.0±0.7	3.8±0.3	7.92±0.07	0.35±0.01	80.8±0.4	390±4	1631±19	82±3[a]
p-value (n=18)	Tukey's Test	0.089	0.004	0.050	<0.001	0.897	<0.001	<0.001	0.003
ST×I (n=27)	*p*-value	0.154	0.029	0.152	<0.001	<0.001	<0.001	<0.001	0.102

In each row, an asterisk (*) or a letter mean significant statistical differences, with an overall *p*-value of 0.05. The presented standard deviations were calculated from results obtained under different operational conditions. Therefore, these values should not be regarded as a measure of precision, rather as the range of the recorded values. T-test stands for Student's T-test.

Table 12 shows the influence of incorporation (I) and shelf life (ST) in each of the nutritional components analysed in the "súplicas". Carbohydrates were the macronutrients that stood out as the main macronutrient in "súplicas", followed by proteins. Regarding free sugars, only sucrose was detected in the samples, which would be expected considering that sugar was one of the ingredients used in making the cakes. Overall, for all components except moisture, proteins and sucrose, a significant interaction was found for ST and I, and thus no post-hoc classification could be performed. For moisture, the effect of ST did not induce statistically significant changes, although the incorporation did induce significant changes, revealing a higher amount of moisture in the "súplicas" with pineapple peel, meaning that the peel was responsible for increasing the moisture content in those samples. Regarding proteins, once again the pineapple peel was responsible for a statistically significant increase when compared to the control samples. Once again, ST did not show statistical influence on the protein fraction. Regarding sucrose, the incorporation of the pineapple peel did not show any significant effect, but the passage of time did show a significant statistical decrease in sucrose from T0 to T3 days, probably due to the breakdown of sucrose into fructose and glucose.

A study developed by Oliveira (2016), showed higher moisture content in the cake incorporated with 80% of pineapple peel flour and 20% of core (27.10%) than in cake with pulp flour (24.38%), this being justified due to the high fibre contents in the peel and core that together, preserve the water during the supply in its structure. Likewise, the protein content was also higher in the cake with the pineapple bio-residues (5,51%) when compared to the cake prepared with pulp flour (5.43%). Silva et al. (2018) reported a higher moisture content with 25.13% in muffins with pineapple peel, being above the stipulated standard, which should be a maximum of 14%. One of the reasons that may explain the maintenance of moisture in the "súplicas" in storage time, would be that glucose and fructose can absorb more water than sucrose (non-reducing sugars), by breaking the molecules over time (Oliveira, 2016). In relation to proteins, Adeoye et al. (2017) showed the highest protein value in cookies contained 50% and 40 % of pineapple peel flour (2.45%) comparing with traditional cookies (2.19%). In the work presented by de Toledo et al. (2017), the protein values were higher than this study, containing 8,14% in cookies with 5% of pineapple central axis.

Ash content is considered an important parameter to be analysed in foods, as it is related to the minerals presented in the food composition (de Toledo et al., 2017). Damasceno et al. (2016) reported a significant difference in ash content when comparing cereals bars containing 3% of pineapple peel flour (3.19%) when compared to traditional bars (2.81%). In turn, Oliveira (2016) highlighted a higher ash content in cakes prepared with pineapple peel flour (4.41%) when compared to cakes prepared with pulp flour (2.66%) or with central part of the fruit (2.42%).

Regarding the crude fat content, a study by Reis Junior (2017) showed a significant difference in the reduction of lipids in hamburgers containing pineapple peel flour at concentrations of 5, 10, 15, 20 and 25% when compared to the control, concluding that the reduction in lipids was proportional to the addition of flour. Oliveira (2018) obtained similar results and conclude that pineapple peel flour could be considered a healthy ingredient that improves the nutritional aspect of hamburgers.

5.3.2. Composition in fatty acids in "súplicas"

Table 13 shows the effect of incorporation and storage time on the fatty acid content of the “súplicas”. Eleven individual fatty acids were identified and in **Table 13** only the majority are presented (with representation greater than 1%). Oleic acid (C18:1) was the most abundant individual fatty acid, followed by palmitic acid (C16:0). Monounsaturated fatty acids (MUFA) prevailed followed by saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) with very similar percentages. For all fatty acids a significant interaction was sought and thus no post-hoc classification was performed. Still, some general tendencies can be extracted from the EMM plots, shown in **Figures 17**. It can be understood for C18:1 (**Figure 17A**) that for the samples with pineapple peel, the amount of oleic acid in the control samples was overall higher than the samples with pineapple peel, probably due to the degradation of fats by enzymes present in the peel. Still, over the first three days there is an increase for the control sample that then decreases from T3 to T7, while all the amount in the incorporated samples remains mostly constant during the whole seven days. **Figure 17B)** shows the EMM for SFA, revealing that pineapple peel samples showed higher SFA amounts, which gradually decreased over time while the control sample increased from T0 to T3 and then decreased from T3 to T7.

This variation could be due to oxidation of fats in the control samples which did not have any compounds to protect them (contrarily to the samples with pineapple peel), and thus they decreased from T0 to T3. Then from T3 to T7, the increase could be explained by the degradation of MUFA to SFA by oxidation. Finally, this phenomenon is also reported in **Figure 17C** where there is a very high variation of MUFA over time in the control samples, while the samples with pineapple peel show a slight decreasing tendency. The control samples increased from T0 to T3 probably due to degradation of PUFA to MUFA and then decrease from T3 to T7, due to breakdown of MUFA to SFA.

Table 13: Fatty acids profile in the "súplicas" control and "súplicas" with with pineapple peel extract.

	Incorporation (I)			**Storage Time (ST)**				
	Control	**Pineapple peel**	***p*-value (n=9)**	**0 Days**	**3 Days**	**7 Days**	***p*-value (n=18)**	**ST×I (n=27)**
C16:0	20±2	23.6±0.4	<0.001	22±2	21±3	22.7±0.6	<0.001	<0.001
C16:1	2.5±0.2	2.96±0.08	<0.001	2.7±0.3	2.6±0.3	2.8±0.2	<0.001	<0.001
C18:1t	5.8±0.3	6.3±0.1	<0.001	6.0±0.2	5.9±0.6	6.22±0.08	<0.001	<0.001
C18:1c	49±3	44.0±0.7	<0.001	46±3	48±4	45.5±0.9	<0.001	<0.001
C18:2	19.3±0.5	20.0±0.2	<0.001	19.8±0.4	19.4±0.8	19.8±0.2	<0.001	<0.001
SFA	21±2	24.0±0.4	<0.001	22±2	21±3	23.1±0.7	<0.001	<0.001
MUFA	58±2	53.5±0.7	<0.001	55±2	57±4	54.8±0.7	<0.001	<0.001
PUFA	21.3±0.8	22.4±0.3	<0.001	22.0±0.6	21±1	22.1±0.2	<0.001	<0.001

In each row, an asterisk (*) means significant statistical differences, with an overall *p*-value of 0.05. The presented standard deviations were calculated from results obtained under different operational conditions. Therefore, these values should not be regarded as a measure of precision, rather as the range of the recorded values. T-test stands for Student's T-test.

Consisting of fatty acids, lipids are important components in the food industry because they confer desirable sensory characteristics to food, where the conversion of long-chain fatty acids into short-chain organic fatty acids, give unpleasant flavor and odor to products (Oliveira, 2018; Silva, 2012). Fats can affect the food texture by forming structures of crystalline networks and by disruption of structure by interfering with non-fat networks (Oliveira, 2018; Rios et al. 2014). In baked products, fat has numerous functions being responsible for the improve gas retention, lubrication, aeration, heat transfer in dough, and desirable texture in the final product (in breads), incorporation of air, softness, lubrication, mouthfeel, and structural and sensory properties (in cakes and biscuits) (Rios et al., 2014).

a)

b)

c)

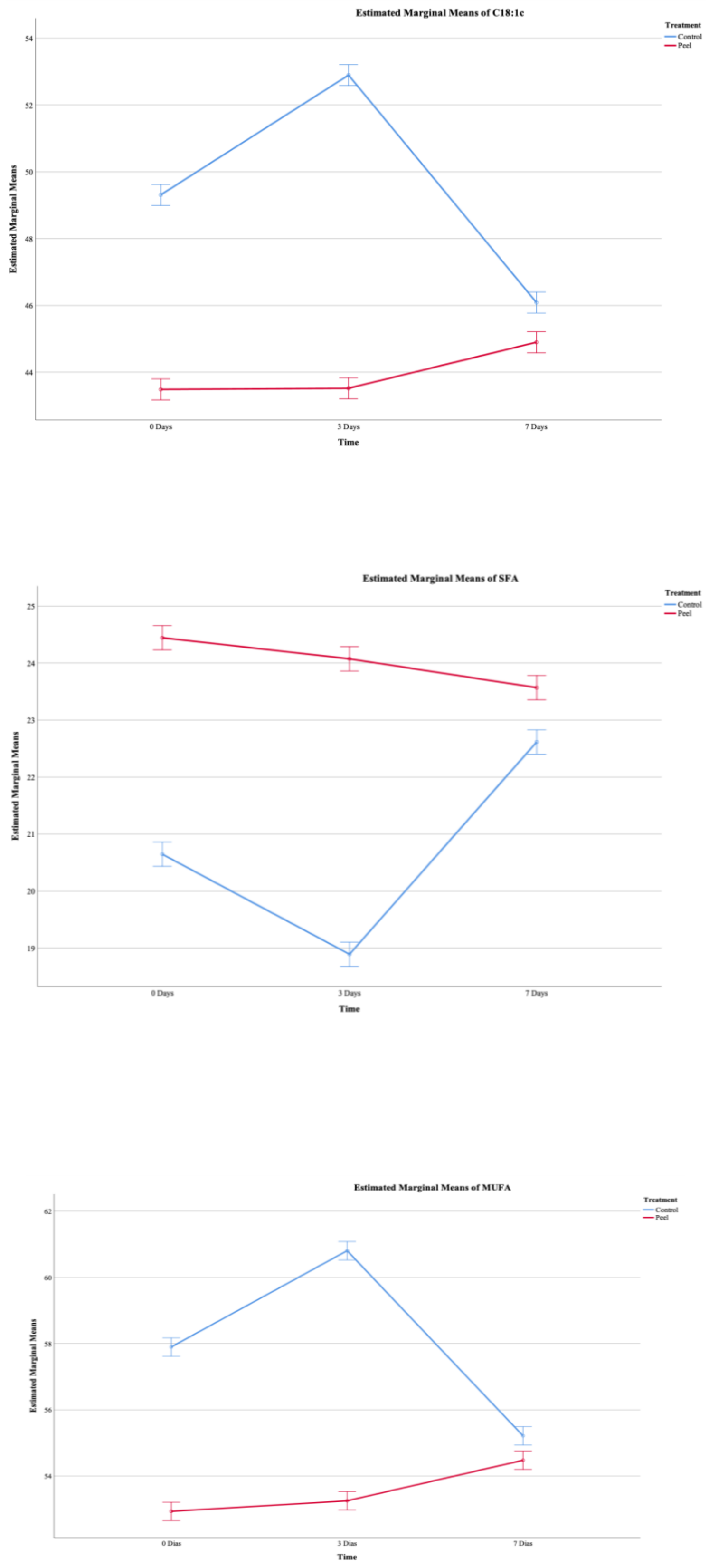


Figure 17: EMM plots of a) C18:1, b) SFA and c) MUFA.

5.3.3. Evaluation of the colour and antioxidant activity of "súplicas"

Table 14 show the results of the effect of extract incorporation and shelf life on antioxidant potential (through DPPH and RP assays) and on colour parameters (defined by CIE Lab coordinates of L^*, a^* and b^*) of the "súplicas" (**Figure 18**). Regarding the antioxidant activity analysis, there was a significant interaction for RP but for DPPH, the pineapple peel did seem to statistically improve the antioxidant activity of the "súplicas" reducing the EC_{50} from over 400 to 134 mg/mL. ST did not show any effects on the antioxidant activity of the snacks, probably due to a very low amount of moisture.

Table 14: Antioxidant analysis and colorimetric parameters in "súplicas" control and "súplicas" with pineapple peel extract evaluating storage time (ST).

		DPPH	**RP**	L^*	a^*	b^*
Incorporation (I)	Control	>400*	17±2	80.4±0.5	1.4±0.2	32±1
	Pineapple peel	134±53	20±4	80.9±0.6	1.5±0.2	31.7±0.5
p-value (n=9)	T-test	<0.001	<0.001	<0.001	0.196	<0.001
Storage Time (ST)	0 Days	237±173	17±2	80.9±0.6	1.4±0.1	32.5±0.9
	3 Days	278±125	19±4	80.2±0.6	1.4±0.1	32.4±0.7
	7 Days	292±111	20±4	80.7±0.3	1.6±0.2	31.0±0.5
p-value (n=18)	Tukey's Test	<0.001	<0.001	<0.001	<0.001	<0.001
ST×I (n=27)	*p*-value	0.101	<0.001	0.009	0.040	<0.001

In each row, an asterisk (*) means significant statistical differences, with an overall *p*-value of 0.05. The presented standard deviations were calculated from results obtained under different operational conditions. Therefore, these values should not be regarded as a measure of precision, rather as the range of the recorded values. T-test stands for Student's T-test. *L**luminosity; *a** chromatic axis from green (-) to red (+); *b**, chromatic axis from blue (-) to yellow (+).

For the colour parameter *b**, the coordinate that defines blue as negative and yellow values for positive values, where, over storage time, a decrease in yellow colour was evidenced in the samples and, for the seventh day, there was an increase in the red colour (*a**) in the "súplicas". Colour is an important feature of pastry products, not only by consumer preference, but also, depends on the physical-chemical characteristics of the mass and processes such as sugars, amino acid content, oven temperature, relative humidity and pH (Esteller & Lannes, 2008).

In the work carried out by Oliveira (2016), values of the colour parameters in cakes prepared with peel flour and core pineapple were higher than those presented by cakes prepared with pulp flour. This can be justified due to the high amount of reducing sugars present in the pulp flour when compared to the peel and core. The effects of colour parameters *L**, (+*a**) and (+*b**) caused differences in behavior in the cakes during storage possibly as a function of the water content as well as in this work, which could be justified by the association with the levels of sugars present in the flours, which enable greater water absorption and, therefore, the intensification or restriction of colour (Oliveira, 2016).

In the research conducted by Silva et al. (2018), the muffins prepared with pineapple peel residues showed a low luminosity (38.77) and the yellow intensity (30.87) was out of the intensity of red (11.00), consequently a low chroma index (32.77), meaning that the chroma closer the material is to the gray colour. Adeoye et al. (2017) reported that the pineapple peel flour gave smooth texture to cookies, but brown colour was imparted, and the colour gets darker as the levels of pineapple peel flour increases. However, in food formulations it is desirable that flour be lighter because flours with dark colours may limit possible food applications (Miri, 2020). For this reason and all parameters evaluated, the biscuits made from wheat-pineapple (90:10) peel flour were indicated having the highest mean scores and were close to 100% wheat cookies (Adeoye et al., 2017).

Figure 18: Colorimetric analysis in "súplicas" control and "súplicas" with pineapple peel extract in the storage time 0, 3 and 7 days.

In relation to the antioxidant analysis, one of the objectives of this work was to add value to a pastry product that normally has no functionality, being able to transfer to it, bioactive compounds of the bio-residues that present antioxidant activity. The same proposal was done by Toledo et al. (2019), where the use of fruit by-products (pineapple central axis, apple endocarp, and melon peels) in the formulation of cookies contributed to an increase of more than 100% in the phenolic compound's contents (from 7.80 ± 0.13 up to 16.91 ± 0.19 milligram of gallic acid equivalent per gram of sample (GAE/g), being the cookies made with apple by-product the most promising formulation. For the DPPH assays, the pineapple central axis values showed great capacity of antioxidant activity, being 7.14 ± 0.34 micromoles of Trolox equivalent per gram of sample (μmol TE/g) against 3.94 ± 0.70 μmol TE/g for the cookies control. The ABTS assays also reenforced the promising results, with

7.24 ± 0.16 µmol TE/g for the pineapple by-product and 5.39 ± 0.33µmol TE/g for the control.

Also, Sah et al. (2015) reported a stronger antimutagenic and antioxidant activities (evaluating the reducing power and scavenging capacity of 1,1-diphenyl-2-picrylhydrazyl; 2,2'-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid), and hydroxyl radicals), in crude water-soluble peptide extract of the probiotic yogurt with pineapple peel than control during storage time. All yogurt samples exhibited varying degrees of reducing power, scavenging capacities for DPPH, ABTS, and hydroxyl radicals. On the first day, the sample containing pineapple peel showed a reduction power of 0.39 versus 0.36 compared to the control and, for the last day (28th), 0.58 against 0.48 for the control only with starter culture (*Streptococcus thermophilus* + *Lactobacillus bulgaricus*). For the DPPH analysis expressed great results in the first day with the probiotic culture (*Lactobacillus acidophilus* + *Lactobacillus casei* + *Lactobacillus paracasei*), being 43.90 for the pineapple peel against 36.96 evaluated in the control. The results showed that pineapple peel could be used as a prebiotic ingredient in the manufacture of yogurts that would improve food nutrition and functionality (Sah et al., 2015).

The work conducted by Barros et al. (2020), presented significant values for antioxidant activity in cookies prepared with pineapple peel through ferric reducing antioxidant power assay, where the activity was proportional to the increase in the concentration of the peel flour.

6. CONCLUSION

The disposal of fruit and vegetable bio-waste is currently a matter of concern both from an economic and an environmental point of view. Thus, the potential use of this type of waste, characterized by its interesting nutritional composition and bioactive potential, has aroused growing interest from the industry. In this sense, the present study aimed to characterize two pineapple bioresidues (peel and crown leaves) in order to assess the potential for application as a natural ingredient.

In the characterization of pineapple peel and crown leaves, twenty-five phenolic compounds were identified in both extracts divided in phenolic acids and flavonoids. The main detected compounds were caffeic acid derivatives (namely caffeic acid-*O*-hexoside) and flavones (such as apigenin 6,8-*C*-diglucoside). Eleven phenolic acids and two flavonoids were identified in pineapple peel while on the crown leaves were identified twelve phenolic, nine flavonoids and two phenolic acids. None of the extracts showed toxicity and both showed antioxidant, antiproliferative and antimicrobial potential, with the pineapple peel extract standing out with the best results.

Due to its greater bioactive potential, the pineapple peel extract was selected to incorporate in a typical pastry product from the northeast of Portugal, the "súplicas" in order to study the potential of this residue as a natural ingredient. Thus, the effects of the incorporation of the extract on colour, nutritional profile, content in sugars and fatty acids, as well as the antioxidant activity were evaluated over the shelf life and by comparison with the traditional product. The results showed that no significant changes in the profile, only changes in proteins and moisture where, it can be explained by the high fiber content in the peel that preserved that water during the supply in its structure and also the breakdown of the molecules in glucose and fructose that can absorb more water than sucrose. In fatty acids the alterations were little noticeable, but the extract allowed reducing the variations between PUFA, MUFA and SFA. At the colour level, the variations were not significant and, as expected, the extract was able to statistically improve the antioxidant activity of the "súplicas" proving that, pineapple peel extract can be used in the food industry as a functionalizing agent with antioxidant activity without modifying the nutritional profiles, colour and composition of fatty acids in food.

With the results obtained by this research, we can conclude that many fruit and vegetable residues have valuable compounds for human health and that could be used to remove them from the environment to reduce the damage caused, adding value and return these wastes back into the economy. The incentive to research, through the dissemination of scientific work in the area, becomes extremely important and can give these wastes that would be discarded, a nutritional and market value, causing an increase in these foods and a greater generation of jobs for families that survive from agriculture.

7. REFERENCES

Abdullah, A., Hanafi M. (2008) Characterisation of solid and liquid pineapple waste. Reaktor, 12, 48-52.

Abreu, R.M.V., Ferreira, I.C.F.R., Calhelha, R.C., Lima, R.T., Vasconcelos, M.H., Adega, F., Chaves, R. & Queiroz, MJ.R.P. (2011). Anti-hepatocellular carcinoma activity using human HepG2 cells and hepatotoxicity of 6-substituted methyl 3-aminothieno [3,2-b] pyridine-2-carboxylate derivatives: In vitro evaluation, cell cycle analysis and QSAR studies. European Journal of Medicinal Chemistry, 46, 5800-5806.

Adeoye, B. K., Alao, A. I., & Famurewa, J. A. V. (2017). Quality evaluation biscuits produced from wheat and pineapple peel flour. Applied Tropical Agriculture, 22(2), 210-217.

Alexandre, H. V. Silva, F. L. H. Da, Gomes, J. P.; Silva, O. S. Da & Carvalho, J. P.(2014). Isotermas de dessorção de resíduos de abacaxi. In: XX Congresso Brasileiro de Engenharia Química – COBEQ, São Paulo: Blucher, 3472-3479.

Ali, M. M., Hashim, N., Abd Aziz, S., & Lasekan, O. (2020). Pineapple (Ananas comosus): A comprehensive review of nutritional values, volatile compounds, health benefits, and potential food products. Food Research International, 109675.

AOAC. (2016). Official methods of analysis of AOAC International, 20th edition. Association of Official Analytical Chemists International; Arlington; USA.

Arjeh, E., Akhavan, H.R., Barzegar, M.& Carbonell-Barrachina, A.A. (2020) Bio-active compounds and functional properties of pistachio hull: A review. Trends in Food Science & Technology, 97, 55–64.

Atmani, D., Chaher N., Berboucha, M., Debbache, N., Boudaoud, H.& Curr. (2009) Nutrition & Food Science, 5, 225–237.

Azizan, A., Xin, L. A., Abdul Hamid, N. A., Maulidiani, M., Mediani, A., Abdul Ghafar, S. Z., Zolkeflee, N. K. Z. & Abas, F. (2020). Potentially bioactive metabolites from pineapple waste extracts and their antioxidant and α-glucosidase inhibitory activities by 1H NMR. Foods, 9(2), 173.

Azmir, J. & Sarker, Md Z. & Rahman, M. & Khan, M. S. & Awang, M. & Ferdosh, S. & Jahurul, MHA & Ghafoor, K. & Norulaini, NAN & Omar, AKM. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. Journal of Food Engineering, 117.

Banerjee, S., Ranganathan, V., Patti, A. & Arora, A. (2018). Valorisation of pineapple wastes for food and therapeutic applications. Trends in Food Science & Technology, 82, 60–70.

Barros, L. D. S., Magalhães, A.L. M., Filho, P. S. L, Steel, C.J., Júnior, M. R. M., & Pinho, L. X. (2020). Potencial nutricional e tecnológico de cookies elaborados com farinha de casca de abacaxi. Dans CBCP - Congresso On-line Brasileiro de Tecnologia de Cereais e Panificação. Even3.

Barros, L., Falcão, Barros, L., Falcão, S., Baptista, P., Freire, C., Vilas-Boas, M., Ferreira, I.C.F.R., (2008). Antioxidant activity of Agaricus sp. mushrooms by chemical, biochemical and electrochemical assays. Food Chemistry, 111, 61-66.

Barros, L., Oliveira, S., Carvalho, A.M., & Ferreira, I.C.F.R. (2010). In vitro antioxidant properties and characterization in nutrients and phytochemicals of six medicinal plants from the Portuguese folk medicine. Industrial Crops and Products, 32, 572–579.

Barros, L., Pereira, C., & Ferreira, I.C.F.R. (2013b). Optimized analysis of organic acids in edible mushrooms from Portugal by ultra-fast liquid chromatography and photodiode array detection. Food Analytical Methods, 6, 309–316.

Barros, L., Pereira, E., Calhelha, R.C., Dueñas, M., Carvalho, A.M., Santos-Buelga, C.& Ferreira, I.C.F.R. (2013a) Bioactivity and chemical characterization in hydrophilic and lipophilic compounds of Chenopodium ambrosioides L. Journal of Functional Foods, 5, 1732–1740.

Barros, L.,Cabrita, L., Vilas Boas, M., Carvalho, A. M., Ferreira, I. C. F. R. (2011). Chemical, biochemical and electrochemical assays to evaluate phytochemicals and antioxidant activity of wild plants. Food Chemistry, 127, 1600-1608.

Bart, H.J. (2011) Extraction of natural products from plants-an Introduction Wiley-VCH Verlag GmbH & Co, KGaA, Kaiserslautern, Germany.

Battino, M., Forbes-Hernandez, T. Y.& Giampieri, F. (2021) Plant-based bioactive compounds: healthy promoters and protective agents, Trends in Food Science & Technology, ISSN 0924-2244.

Bergamaschi, K. B. (2010) Capacidade antioxidante e composição química de resíduos vegetais visando seu aproveitamento. Dissertação (Mestrado e Ciência e Tecnologia de Alimentos). Escola Superior de Agricultura “Luiz Queiroz”, Universidade de São Paulo, Piracicaba.

Bessada, S. M., Barreira, J. C. M., Barros, L., Ferreira, I. C. F. R., & Oliveira, M. B. P. P. (2016). Phenolic profile and antioxidant activity of Coleostephus myconis (L.) Rchb. f.: An underexploited and highly disseminated species. Industrial Crops and Products, 89, 45–51.

Birt, D.F., Jeffery, E. (2013) Flavonoids. Advances in Nutrition. 4, 576–577.

Brito, T. B. N., Lima, L. R. S., Santos, M. C. B., Moreira, R. F. A., Cameron, L. C., Fai, A. E. C., & Ferreira, M. S. L. (2021). Antimicrobial, antioxidant, volatile and phenolic profiles of cabbage-stalk and pineapple-crown flour revealed by GC-MS and UPLC-MSE. Food Chemistry, 339, 127882.

Cádiz-Gurrea, M. de la L., Villegas-Aguilar, M. del C., Leyva-Jiménez, F. J., Pimentel-Moral, S., Fernández-Ochoa, Á., Alañón, M. E., & Segura-Carretero, A. (2020). Revalorization of bioactive compounds from tropical fruit by-products and industrial applications by means of sustainable approaches. Food Research International, 138, 109786.

Cai, M. (2019). Fruit-based functional food. The role of alternative and innovative food ingredients and products in consumer wellness, Academic Press, 35.

Caleja, C., Ribeiro, A., Oliveira, M.B.P.P., Barreiro, M.F. & Ferreira, I.C.F.R. (2017). The use of phenolic compounds as nutraceuticals or functional food ingredients. Review Article. Current Pharmaceutical Design, 23, 2787-2806.

Caló, E., & Khutoryanskiy, V. V. (2015). Biomedical applications of hydrogels: A review of patents and commercial products. European Polymer Journal, 65, 252-267.

Campos, D. A., Coscueta, E. R., Vilas-Boas, A. A., Silva, S., Teixeira, J. A., Pastrana, L. M., & Pintado, M. M. (2020b). Impact of functional flours from pineapple by-products on human intestinal microbiota. Journal of Functional Foods, 67, 103830.

Campos, D.A.; Gómez-García, R., Vilas-Boas, A.A., Madureira, A.R. & Pintado, M.M. (2020a) Management of fruit industrial by-products—a case study on circular economy approach. molecules, 25, 320.

Carocho, M., Barros, L., Calhelha, R.C., Ćirić, A., Soković, M., Santos-Buelga, C., Morales, P., & Ferreira, I.C.F.R. (2015). Melissa officinalis L. decoctions as functional beverages: a bioactive approach and chemical characterization. Food & Function, 6, 2240-2248.

Catalán, V., Frühbeck, G., & Gómez-Ambrosi, J. (2018). Inflammatory and oxidative stress markers in skeletal muscle of obese subjects. Obesity, 163-189.

Chanda, S., Baravalia, Y., Kaneria, M. & Rakholiya, K. (2010): Current research technology and education topics in applied microbiology and microbial biotechnology. A. Mendez-Vilas (Ed), 444 - 450.

Chen, Z., Sun, Y., Wang, G., Zhang, Y., Zhang, Q., Zhang, Y., Li J. & Wang, Y. (2021). De novo biosynthesis of c-arabinosylated flavones by utilization of indica rice c-glycosyltransferases. Bioresources and Bioprocessing, 8(1), 1-13.

Cheok, C.Y., Adzahan, N.M., Rahman, R.A., Abedin, N.H.Z., Hussain, N., Sulaiman, R. & Chong, G.H. (2018) Current trends of tropical fruit waste utilization. Critical Reviews in Food Science and Nutrition, 58(3), 335–61.

Colombo, R., Yariwake, J. H., Queiroz, E. F., Ndjoko, K. & Hostettmann, K. (2013) LC-MS/MS Analysis of sugarcane extracts and differentiation of monosaccharides moieties of flavone c-glycosides, Journal of Liquid Chromatography & Related Technologies, 36(2), 239-248.

Contreras-Calderón, J., Calderón-Jaimes, L., Guerra-Hernández, E. & García-Villanova, B. (2011) Antioxidant capacity, phenolic content and vitamin C in pulp, peel and seed from 24 exotic fruits from Colombia. Food Research International, Barking, 44, 2047–2053.

Crestani, M., Hawerroth, F.J., Carvalho, F.I.F., Oliveira, A.C., Barbieri, R.L. (2010) From the Americas to the world – origin, domestication and dispersion of pineapple. Ciência Rural, 40(6), 1473-1483

Da Silva, R.P.F.F., Rocha-Santos, T.A.P., Duarte, A.C. (2016) Supercritical fluid extraction of bioactive compounds. TrAC- Trends in Analytical Chemistry, 76, 40–51.

Daglia, M. (2012) Polyphenols as antimicrobial agents. Current Opinion in Biotechnology, 23, 174-181.

Dai, J., Mumper, R.J. (2010) Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules, 15, 7313-7352.

Dai, J., Mumper, RJ (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules, 15, 7313-7352

Damasceno, K. A., Gonçalves, C. A. A., Pereira, G. D. S., Costa, L.L., Campagnol, P. C. B., De Almeida, P. L., & Arantes-Pereira, L. (2016). Development of cereal bars containing pineapple peel flour (Ananas comosus L. Merril). Journal of Food Quality, 39(5), 417-424.

De la Rosa, L. A., Moreno-Escamilla, J. O., Rodrigo-García, J., & Alvarez-Parrilla, E. (2019). Phenolic compounds. postharvest physiology and biochemistry of fruits and vegetables, 253–271.

De Toledo, N. M. V., Nunes, L. P., da Silva, P. P. M., Spoto, M. H. F., & Canniatti-Brazaca, S. G. (2017). Influence of pineapple, apple and melon by-products on cookies: physicochemical and sensory aspects. International Journal of Food Science & Technology, 52(5), 1185-1192.

Dhukani, A. (2013). Ananas comosus (L.) Merr, Bromeliaceae.

Di Mattia, C.D., Sacchetti, G., Mastrocola, D., Sarker, D.K. & Pittia, P. (2010). Surface properties of phenolic compounds and their influence on the dispersion degree and oxidative stability of olive oil O/W emulsions. Food Hydrocolloids, 24, 652–658.

Dias, M. I., Barros, L., Morales, P., Cámara, M., Alves, M. J., Oliveira, M. B. P., et al. (2016). Wild Fragaria vesca L. fruits: A rich source of bioactive phytochemicals. Food & Function, 7, 4523–4532.

Difonzo, G., Vollmer, K., Caponio, F., Pasqualone, A., Carle, R., & Steingass, C.B. (2019). Caracterização e classificação do suco de abacaxi (Ananas comosus [L.] Merr.) Da polpa e da casca. Food Control, 96, 260-270.

Dutta, S., & Bhattacharyya, D. (2013). Enzymatic, antimicrobial and toxicity studies of the aqueous extract of Ananas comosus (pineapple) crown leaf. Journal of Ethnopharmacology, 150(2), 451-457.

Ellis, B.E., Towers, G.H.N. (1970). Biogenesis of rosmarinic acid in Mentha. Biochemical Journal, 118, 291–297.

Esfanjani, F. A., Assadpour, E., & Jafari, S. M. (2018). Improving the bioavailability of phenolic compounds by loading them within lipid-based nanocarriers. Trends in Food Science & Technology, 76, 56–66.

Espinel-Ingroff, A. (2001). Comparison of the E-test with the NCCLS M38-P Method for antifungal susceptibility testing of common and emerging pathogenic filamentous fungi. Journal of Clinical Microbiology, 39(4), 1360-1367.

Esteller, M. S., & Lannes, S. C. S. (2008). Production and characterization of sponge-dough bread using scalded rye. Journal of Texture Studies, 39(1), 56-67.

European Parliamentary Research Service (EPRS) (2016). Tackling food waste, The EU's contribution to a global issue. Katsarova I. Retrieved from: https://www.europarl.europa.eu/RegData/etudes/BRIE/2016/593563/EPRS_BRI(2016)593563_EN.pdf. Accessed: 19th November, 2020.

Evans, B.C., Nelson, C.E., Yu, S.S., Beavers, K.R., Kim, A.J., Li, H., Nelson, H.M., Giorgio, T.D. & Duvall, C.L. (2013). Ex vivo red blood cell hemolysis assay for the evaluation of pH-responsive endosomolytic agents for cytosolic delivery of biomacromolecular drugs. Journal of Visualized Experiments, e50166.

Farag, M. A., Handoussa, H., Fekry, M. I., & Wessjohann, L. A. (2016). Metabolite profiling in 18 Saudi date palm fruit cultivars and their antioxidant potential via UPLC-qTOF-MS and multivariate data analyses. Food & Function, 7(2), 1077-1086.

Farias, G. (2021). Abordagem bibliográfica sobre o suco de abacaxi (Ananas comosus L.) e resíduos do seu processamento: composição físico-química e bioacessibilidade de compostos fenólicos.

Feitosa, B. F., Oliveira, E. N. A. de, Oliveira Neto, J. O. de, Oliveira, D. B. de, & Feitosa, R. M. (2019). Cinética de secagem dos resíduos da agroindústria processadora de polpa de frutas. Energia na Agricultura, 1808-8759.

Fernandes, Â., Antonio, A. L., Barreira, J. C. M., Oliveira, M. B. P. P., Martins, A., & Ferreira, I. C. F. R. (2012). Effects of gamma irradiation on physical parameters of Lactarius deliciosus wild edible mushrooms. Postharvest Biology and Technology, 74, 79–84.

Fernandes, S. S., Coelho, M. S., & Salas-Mellado, M. de las M. (2019). Bioactive compounds as ingredients of functional foods. Bioactive Compounds, 129–142.

Ferreira, I. C. F. R., Martins, N., & Barros, L. (2017). Phenolic compounds and its bioavailability. Advances in Food and Nutrition Research, 1-44.

Ferreres, F., Magalhães, S. C. Q., Gil-Izquierdo, A., Valentão, P., Cabrita, A. R., Fonseca, A. J., & Andrade, P. B. (2017). HPLC-DAD-ESI/MSn profiling of phenolic compounds from Lathyrus cicera L. seeds. Food Chemistry, 214, 678-685.

Food and Agriculture Organization of the United Nations (FAO) (2017). Global initiative on food loss and waste, Italy, Retrieved from: http://www.fao.org/3/a-i7657e.pdf. Accessed: 18th November, 2020.

Food and Agriculture Organization of the United Nations (FAO) (2018). Major tropical fruits market review 2018, Rome, Italy. Retrieved from: http://www.fao.org/3/ca5692en/ca5692en.pdf. Accessed: 7th September, 2020.

Food and Agriculture Organization of the United Nations (FAO) (2020a). Major tropical fruits preliminary results 2019, Rome, Italy. Retrieved from: http://www.fao.org/3/ca7566en/CA7566EN.pdf. Accessed: 5th April, 2021.

Food and Agriculture Organization of the United Nations (FAO) (2020b). Medium-term Outlook: Prospects for global production and trade in bananas and tropical fruits 2019 to 2028, Rome, Italy. Retrieved from: http://www.fao.org/3/ca7568en/ca7568en.pdf Accessed: 9th November, 2020.

Food and Agriculture Organization of the United Nations (FAO) (2020c). Food loss and waste must be reduced for greater food security and environmental sustainability Retrieved from: http://www.fao.org/news/story/en/item/1310271/icode/ Accessed: 18th November, 2020.

Franke, A. A., Custer, L. J., Arakaki, C., & Murphy, S. P. (2004). Vitamin C and flavonoid levels of fruits and vegetables consumed in Hawaii. Journal of Food Composition and Analysis, 17(1), 1-35.

Garcia-Fuentes, A. R., Wirtz, S., Vos, E., & Verhagen, H. (2015). Short review of sulphites as food additives. European Journal of Nutrition & Food Safety, 113-120.

Ge, X., Jing, L., Zhao, K., Su, C., Zhang, B., Zhang, Q., Li, W. (2020). The phenolic compounds profile, quantitative analysis and antioxidant activity of four naked barley grains with different colour. Food Chemistry, 127655.

Giacomelli, E.J., P.Y., C. (1981) O abacaxi no Brasil. Campinas: Fundação Cargil, 101p.

Gómez, F. S. & Pablos, M.P.A. (2016). Pineapple waste extract for preventing oxidation in model food systems. Journal of Food Science, 81(7), 1622–1628.

Gonçalves, G.A., Soares, A.A., Correa, R.C.G., Barros, L., Haminiuk C.W.I., Peralta R.M., Ferreira I.C.F.R., Bracht A. (2017) Merlot grape pomace hydroalcoholic extract improves the oxidative and inflammatory states of rats with adjuvant-induced arthritis. Journal of Functional Foods, 33, 408-418.

Goudarzi, M., Mehdipour, M., Hajikhani, B., Sadeghinejad, S., & Sadeghi-Nejad, B. (2019). Antibacterial properties of citrus limon and pineapple extracts on oral pathogenic bacteria (Streptococcus mutans and Streptococcus sanguis). International Journal of Enteric Pathogens, 7(3), 99-103.

Goufo, P., Trindade H. (2015). Factors influencing antioxidant compounds in rice. Critical Reviews in Food Science and Nutrition, 57 (5), 893-922.

Harnly, J. M., Doherty, R. F., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Bhagwat, S., & Gebhardt, S. (2006). Flavonoid content of US fruits, vegetables, and nuts. Journal of Agricultural and Food Chemistry, 54(26), 9966-9977.

Hernandez, Y., Lobo, M.G., González, M. (2009). Factors affecting sample extraction in the liquid chromatographic determination of organic acids in papaya and pineapple. Food Chemistry, 114, 734 - 41.

Hossain, F. (2016). World pineapple production: an overview. African Journal of Food, Agriculture, Nutrition and Development. 16.

Hossain, M. A., & Rahman, S. M. M. (2011). Total phenolics, flavonoids and antioxidant activity of tropical fruit pineapple. Food Research International, 44(3), 672–676.

Hsu, C. T., Chang, Y. H., & Shiau, S. Y. (2019). Colour, antioxidation, and texture of dough and Chinese steamed bread enriched with pitaya peel powder. Cereal Chemistry, 96(1), 76-85.

Hu X., Hu K., Zeng L. & Huang H. (2010) Hydrogels prepared from pineapple peel cellulose using ionic liquid and their characterization and primary sodium salicylate release study Carbohydrate Polymers, 82 (1), 62-68.

Ignat, I., Volf, I. & Popa, V. I. A. (2011) Critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. Food Chemistry, 126 (4), 1821-1835.

Ito, F., Sono, Y., & Ito, T. (2019). Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: oxidative stress in diabetes, atherosclerosis, and chronic inflammation. Antioxidants, 8(3), 72.

Ji, C., Kong, C.X., Mei, Z.L., Li, J. (2017). A review of the anaerobic digestion of fruit and vegetable waste. Appl. Biochem. Biotechnol., 183 (3), 906-922.

Jimenez-Lopez, C., Pereira, A.G., Lourenço-Lopes, C., Garcia-Oliveira, P., Cassani, L., Fraga, M., Prieto, M.A., Simal-Gandara, J., (2020). Main bioactive phenolic compounds in marine algae and their mechanisms of action supporting potential health benefits, Food Chemistry, 341, 128262.

Jovanović, M., Milutinović, M., Kostić, M., Miladinović, B., Kitić, N., Branković, S., & Kitić, D. (2018). Antioxidant capacity of pineapple (Ananas comosus (L.) Merr.) extracts and juice. Journal of Lekovite Sirovine, 38, 27-30.

Karimi, A., Kazemi, M., Samani, S. A., Simal-Gandara J. (2021) Bioactive compounds from by-products of eggplant: Functional properties, potential applications and advanes in valorization methods, Trends in Food Science & Technology, 112, 518-531.

Karlina, C. Y., Muslimin, I., Trimulyono G. (2013) Aktivitas antibakteri ekstrak herba krokot (Portulaca oleraceae L.) terhadap Staphylococcus aureus dan Escherichia coli. Jornual LenteraBio, 2(1), 87-93.

Karthikeyan, O.P., Trably, E., Mehariya, S., Bernet, N., Wong, J.W, H. (2018), Carrere Pré tratamento de resíduos alimentares para recuperação de metano e hidrogênio: uma revisão. Bioresource Technology, 249, 1025-1039.

Kim, G. D., Park, Y. S., Jin, Y. H., & Park, C. S. (2015). Production and applications of rosmarinic acid and structurally related compounds. Applied Microbiology and Biotechnology, 99(5), 2083-2092.

Lasekan O., Hussein F.K. (2018). Classification of different pineapple varieties grown in Malaysia based on volatile fingerprinting and sensory analysis. Chemistry Central Journal, 12 (1), 1-12.

Lattanzio, V. (2013). Phenolic compounds: introduction. Natural Products, 1543–1580.

Le, N.A. (2014) Lipoprotein-associated oxidative stress: A new twist to the postprandial hypothesis. International Journal of Molecular Sciences, 16, 401-419.

Li, T., Shen, P., Liu, W., Liu, C., Liang, R., Yan, N., & Chen, J. (2014). Major polyphenolics in pineapple peels and their antioxidant interactions. International Journal of Food Properties, 17(8), 1805-1817.

Libro, R., Giacoppo, S. & Thangavelu, S. R., Bramanti, P. & Mazzon, E. (2016). Natural phytochemicals in the treatment and prevention of dementia: an overview. Molecules, 21, 518.

Lipinski, B., Kitinoja, L., Searchinger T., Hanson C. (2013). Reducing Food Loss and Waste. Working Paper, Instalment 2 of Creating a Sustainable Food Future. Washington, DC: World Resources Institute. Lisa

Lobo, M.G., Paull, R.E. (Eds.) (2017) Handbook of pineapple technology: production, postharvest science, processing and nutrition, John Wiley & Sons, Chichester, UK, Hoboken, NJ.

Lockowandt, L., Pinela, J., Roriz, C.L.; Pereira, C., Abreu, R.M.V., Calhelha, R.C., Alves, M.J., Barros, L., Bredol, M., Ferreira, I.C.F.R (2019) Chemical features and bioactivities of cornflower (Centaurea cyanus L.) capitula: The blue flowers and the unexplored non-edible part. Industrial Crops and Products, 128, 496–503.

Lourenço, S.C, Campos, D.A, Gómez-García, R., Pintado, M., Oliveira, M.C., Santos, D.I., Corrêa-Filho, L.C. & Alves, VD (2021). Otimização da extração de antioxidantes naturais da casca de abacaxi e sua estabilização por spray drying. Foods, 10 (6), 1255.

Ma, C., S.-y. Xiao, Z.-g. Li, W. Wang and L.-j. Du (2007). Characterization of active phenolic components in the ethanolic extract of Ananas comosus L. leaves using high-performance liquid chromatography with diode array detection and tandem mass spectrometry. Journal of Chromatography, 1165(1), 39-44.

Manchón, N., Rostagno, M., Vivaracho, L., Pedrola, M. y D'Arrigo, M. (2015). Métodos avanzados para el análisis, conservación y extracción de compuestos fenólicos en alimentos (Doctorado). Universidad de Valladolid.

Mandal, D. (2019). Food preservative chemistry: Effects and side effects. Journal of the Indian Chemical Society, 96(12), 1519-1528.

Mandim, F., Petropoulos, S. A., Dias, M. I., Pinela, J., Kostic, M., Soković, M., Santos-Buelga, C. & Barros, L. (2021). Seasonal variation in bioactive properties and phenolic composition of cardoon (Cynara cardunculus var. altilis) bracts. Food Chemistry, 336, 127744.

Manousi, N., Sarakatsianos, I., & Samanidou, V. (2019). Extraction techniques of phenolic compounds and other bioactive compounds from medicinal and aromatic plants. Engineering Tools in The Beverage Industry, 283–314.

Martillanes S., Rocha-Pimienta J., Cabrera-Bañegil M., Martín-Vertedor D., Delgado-Adámez J. (2017), Application of phenolic compounds for food preservation: Food additive and active packaging. M. Soto-Hernánez, M. Palma-Tenango, R. García-Mateos (Eds.), Phenolic compounds. Biological activity, InTechOpen, London, UK, 40-58.

Martín Ortega, A. M., & Segura Campos, M. R. (2019). Bioactive compounds as therapeutic alternatives. Bioactive Compounds, 247–264.

Martins, S., Mussatto, S.I., Martínez-Avila, G., Montañez-Saenz, J. Aguilar, C.N., J.A. (2011). Teixeira Bioactive phenolic compounds: Production and extraction by solid-state fermentation. A review. Biotechnology Advances, 29 (3), 365-373.

Miri, J. D. C. (2020). Desenvolvimento de mistura para bolo com adição de farinha da casca do abacaxi (Ananas comosus L. Merril) e farinha de banana verde (Musa spp).

Naczk, M., Shahidi, F. (2004). Extractions and analysis of phenolics in food. Journal of Chromatography A, 1054 (1), 95- 111.

Niedzwiecki, A., Roomi, M. W., Kalinovsky, T., & Rath, M. (2016). Anticancer efficacy of polyphenols and their combinations. Nutrients, 8(9), 552.

Ogwu, M. C., Omorotionmwan, F. O. O., & Ogwu, H. I. (2019). Antibacterial characteristics and bacteria composition of pineapple (Ananas comosus [Linn.] Merr.) peel and pulp. Food and Health, 5(1), 1-11.

Okoh, M. & Obadiah, H. & Aiyamenkue, J. (2019). Antimicrobial activities of pineapple peel. Biological Reports, 4, 11.

Oksana, S., Marian, B., Mahendra, R. & Hong, B. S. (2012) Plant phenolic compounds for food, pharmaceutical and cosmetics production, Journal of Medicinal Plants Research, 6, 2526-2539.

Olakunle, O. O., Joy, B. D., & Irene, O. J. (2019). Antifungal activity and phytochemical analysis of selected fruit peels. Journal of Biology and Medicine, 3(1), 040-043.

Oliveira, A. A. N. (2018). Avaliação da oxidação lipídica em hambúrguer de carne bovina adicionado de farinha da casca do abacaxi (Ananas comosus (L.) Merril) como antioxidante natural.

Oliveira, A. D. S. (2016). Elaboração de farinha de polpa, casca e cilindro central de abacaxi cv. pérola para produção de bolo.

Panzella, L. (2020). Natural phenolic compounds for health, food and cosmetic applications. antioxidants (basel). Multidisciplinary Digital Publishing Institute, 9, 427.

Pardo, M., Cassellis, M., Escobedo, R., Garcia, E. (2014) Chemical characterisation of the industrial residues of the pineapple (Ananas comosus) Journal of Agricultural Chemistry and Environment, 3 (2), 53-56.

Paritosh, K., Kushwaha, S.K., Yadav, M., Pareek, N., Chawade, A., Vivekanand V. (2017) Food waste to energy: an overview of sustainable approaches for food waste management and nutrient recycling. BioMed Research International.

Paull, R. E., & Chen, N. J. (2020). Tropical fruits: Pineapples. Controlled and modified atmospheres for fresh and fresh-cut produce, Academic Press, 381–388.

Paull, R.E. & Lobo, M.G. (2012) Pineapple. in: tropical and subtropical fruits postharvest physiology, processing and packaging. (Eds M. Siddiq, J. Ahmed, M.G. Lobo & F. Ozadali), 333–357. Ames, IO: Wiley-Blackwell.

Pereira, E., Barros, L., Martins, A., & Ferreira, I. C. F. R. (2012). Towards chemical and nutritional inventory of Portuguese wild edible mushrooms in different habitats. Food Chemistry, 130, 394 - 403.

Pérez-Sánchez, A., Barrajón-Catalán, E., Herranz-López, M., Castillo, J., & Micol, V. (2016). Lemon balm extract (Melissa officinalis, L.) promotes melanogenesis and prevents UVB-induced oxidative stress and DNA damage in a skin cell model. Journal of Dermatological Science, 84(2), 169-177.

Piedade, J.; Canniatti-Brazaca, S. G. (2003) Comparação entre o efeito do resíduo do abacaxizeiro (caules e folhas) e da pectina cítrica de alta metoxilação no nível de colesterol sangüíneo em ratos. Ciência e Tecnologia de Alimentos, 23, 2.

Pool, H., Campos-Vega, R., Herrera-Hernández, M.G., García-Solis, P., García-Gasca, T., Sánchez, I.C., et al. (2018) Development of genistein-PEGylated silica hybrid nanomaterials with enhanced antioxidant and antiproliferative properties on HT29 human colon cancer cells. American Journal of Translational Research, 10, 2306.

Punbusayakul, N. (2018). Antimicrobial activity of pineapple peel extract. Functional foods: trends in research and markets. Department of food science. Faculty of Science. Burapha University.

Rahaiee, S., Assadpour, E., Esfanjani, A. F., Silva, A. S., & Jafari, S. M. (2020). Application of nano/microencapsulated phenolic compounds against cancer. Advances in Colloid and Interface Science, 102153.

Ramallo, L.A.; Mascheroni, R.H. (2012). Quality evaluation of pineapple fruit during drying process. Food and Bioproducts Processing, 90, 275-283.

Rashmi, H. B., & Negi, P. S. (2020). Phenolic acids from vegetables: A review on processing stability and health benefits. Food Research International, 109298.

Reis Junior, W. J. D. (2017). Utilização de farinha da casca do abacaxi (Ananas comosus (L.) Merr.) para desenvolvimento de hambúrguer bovino com teor reduzido de gordura.

Ribeiro A., Caleja C., Barros L., Santos-Buelga C., Barreiro M.F., Ferreira I.C. (2016) Rosemary extracts in functional foods: extraction, chemical characterization and incorporation of free and microencapsulated forms in cottage cheese. Food & Function, 7(5), 2185-96.

Rios, R. V., Pessanha, M. D. F., Almeida, P. F. de, Viana, C. L., & Lannes, S. C. da S. (2014). Application of fats in some food products. Food Science and Technology, 34(1), 3–15.

Roda, A., & Lambri, M. (2019). Food uses of pineapple waste and by-products: a review. International Journal of Food Science and Technology, 54, 1009-1017.

Rodrigues, P. (2017). Os desperdícios por trás do alimento que vai para o lixo. Empresa Brasileira de Pesquisa Agropecuária – Embrapa. Retrieved from:

https://www.embrapa.br/busca-de-noticias/-/noticia/28827919/os-desperdicios-por-tras-do-alimento-que-vai-para-o-lixo. Accessed: 18th November, 2020.

Roha, S., Zainal S., Noriham, A., Nadzirah, K. (2013) Determination of sugar content in pineapple waste variety N36. International Food Research Journal, 20 (4), 1941-19.

Sagar, N. A., Pareek, S., Sharma, S., Yahia, E. M., & Lobo, M. G. (2018). Fruit and vegetable waste: Bioactive compounds, their extraction, and possible utilization. Comprehensive Reviews in Food Science and Food Safety, 17(3), 512–531.

Sah, B. N. P., Vasiljevic, T., McKechnie, S., & Donkor, O. N. (2015). Effect of refrigerated storage on probiotic viability and the production and stability of antimutagenic and antioxidant peptides in yogurt supplemented with pineapple peel. Journal of Dairy Science, 98, 5905–5916.

Sah, B. N. P., Vasiljevic, T., McKechnie, S., & Donkor, O. N. (2016). Antibacterial and antiproliferative peptides in synbiotic yogurt—release and stability during refrigerated storage. Journal of dairy science, 99(6), 4233-4242.

Salvi, Rajput (1995) Pineapple in handbook of fruit science and technology. Production, composition, storage and processing. Marcel Dekker, Inc, New York,681-691.

Sanewski, Garth M. (2011) Genetic diversity in pineapple. Chronica Horticulturae 51 (3), 9-12.

Saravanan, P., Muthuvelayudham, R., Viruthagiri, T., (2013), Enhanced production of cellulase from pineapple waste by Response Surface Methodology. Journal of Engineering, Article 979547, 8.

Sari, L., Angraeni, L., Hamauzu, Y., Setha, S. & Naradisorn, M. (2017). Effect of UV-C on phenolic compound contents in 'phulae' pineapple (Ananas comosus L cv. Phulae). Scientia Horticulturae, 213, 314-320.

Schieber, A.; Stintzing, F.C.; Carle, R. (2001) By-products of plant food processing as a source of functional compounds: recent developments. Trends Food Science Technology, Cambridge, 12 (11), 401-413.

Selani, M. M., Shirado, G. A., Margiotta, G. B., Rasera, M. L., Marabesi, A. C., Piedade, S. M., ... & Canniatti-Brazaca, S. G. (2016). Pineapple by-product and canola oil as partial fat replacers in low-fat beef burger: Effects on oxidative stability, cholesterol content and fatty acid profile. Meat Science, 115, 9-15.

Sharma, P., Gaur, V.K, Kim, S.-H., Pandey, A. (2020) Microbial strategies for bio-transforming food waste int resources. Bioresource Technology, 299, 122580.

Silva, D.I., Nogueira, G.D., Duzzioni, A.G., Barrozo, M.A., (2013) Mudanças nos constituintes antioxidantes no resíduo de abacaxi (Ananas comosus) durante o processo de secagem. Industrial Crops and Products, 50, 557 – 562.

Silva, J.S. (2012) Barra de cereais elaboradas com farinha de semente de abóbora (dissertação). Lavras, MG: Programa de Pós Graduação em Agroquímica, Universidade Federal de Lavras.

Silva, M. M., & Lidon, F. (2016b). Food preservatives–An overview on applications and side effects. Emirates Journal of Food and Agriculture, 366-373.

Silva, N. F. I., de Lima, A. R. C., Santos, F. S., Pereira, P. K. G., & Silva, M. J. S. (2018). Aproveitamento de resíduos de abacaxi (Ananas comosus) para elaboração de muffins. Gestão Integrada de Resíduos, 53.

Silva, Neliton & Adaime, Ricardo & Zucchi, Roberto. (2016a). Pragas agrícolas e florestais na amazônia. Brasília, Embrapa.

Stefani, T. (2020) Metabolomic and biodirected phytochemical study of Hechtia glomerata, evaluation of its antibacterial and cytotoxic activity, and determination of the mechanism of action of one active compound. Doctorado thesis, Universidad Autónoma de Nuevo León.

Steingass, C. B., Glock, M. P., Schweiggert, R. M., & Carle, R. (2015). Studies into the phenolic patterns of different tissues of pineapple (Ananas comosus [L.] Merr.) infructescence by HPLC-DAD-ESI-MS n and GC-MS analysis. Analytical and Bioanalytical Chemistry, 407 (21), 6463-6479.

Steingass, C.B., Glock, M.P., Lieb, V.M., Carle, R. (2017). Light-induced alterations of pineapple (Ananas comosus [L.] Merr.) juice volatiles during accelerated ageing and mass spectrometric studies into their precursors. Food Research International, 100, 366-374.

Steingass, C.B., Vollmer, K., Lux, P.E., Dell, C., Carle, R., Schweiggert, R.M. (2020) HPLC-DAD-APCI-MSn analysis of the genuine carotenoid pattern of pineapple (Ananas comosus [L.] Merr.) infructescence. Food Research International, 127, 108709.

Suerni, E., Alwi, M., Guli, M.M. (2013) Uji daya hambat ekstrak buah nanas (Ananas comosus L.Merr.), salak (Salacca edulis Reinw.) dan mangga kweni (Mangifera odorata Griff.) terhadap daya hambat Staphylococcus aureus. Jurnal Biocelebes, 7, 1.

Sun, G.-M., Zhang, X.-M., Soler, A., & Marie-Alphonsine, P. (2016). Nutritional composition of pineapple (Ananas comosus (L.) Merr.). Nutritional Composition of Fruit Cultivars, 609–637.

Sun, X., Li, X., Shen, X., Wang, J., & Yuan, Q. (2020). Recent advances in microbial production of phenolic compounds. Chinese Journal of Chemical Engineering, 30, 54-61.

Takata, R. H. and P. J. Scheuer (1976). solation of glyceryl esters of caffeic and p coumaric acids from pineapple stems. Lloydia 39(6), 409-411.

Takebayashi, J., Iwahashi, N., Ishimi, Y., Tai, A., 2012. Development of a simple 96-well plate method for evaluation of antioxidant activity based on the oxidative haemolysis inhibition assay (OxHLIA). Food Chemistry, 134, 606-610.

Tanaka, T., Nishimura, A., Kouno, I., Nonaka, G. I., & Yang, C. R. (1997). Four new caffeic acid metabolites, yunnaneic acids EH, from Salvia yunnanensis. Chemical and Pharmaceutical Bulletin, 45(10), 1596-1600.

Taofiq, O., Calhelha, R.C., Heleno, S., Barros, L., Martins, A., Santos-Buelga, C., Queiroz, M.J.R.P. & Ferreira, I.C.F.R. (2015) The contribution of phenolic acids to the anti-inflammatory activity of mushrooms: Screening in phenolic extracts, individual parent molecules and synthesized glucuronated and methylated derivatives. Food Research International., 76, 821–827.

Testai, L., De Leo, M., Flori, L., Polini, B., Braca, A., Nieri, P., Pistelli1, L. & Calderone, V. (2021). Contribution of irisin pathway in protective effects of mandarin juice (Citrus reticulata Blanco) on metabolic syndrome in rats fed with high fat diet. Phytotherapy Research.

Toledo, N. M. V., Mondoni, J., Harada-Padermo, S. S., Vela-Paredes, R. S., Berni, P. R. A., Selani, M. M., & Canniatti-Brazaca, S. G. (2019). Characterization of apple, pineapple, and melon by-products and their application in cookie formulations as an alternative to enhance the antioxidant capacity. Journal of Food Processing and Preservation, 14100.

Ueno, T., Ozawa, Y., Ishikawa, M., Nakanishi, K., Kimura, T. (2003) Lactic acid production using two food processing wastes, canned pineapple syrup and grape invertase, as substrate and enzyme. Biotechnology Letters, 25 (7), 573-577.

Upadhyay, A. & Lama, J. & Tawata, S. (2010). Utilization of pineapple waste: a review. Journal of Food Science and Technology Nepal, 6, 10.

Van der Linden, A., Reichel, A., Bio-waste in Europe - turning challenges into opportunities, European Environment Agency. (2020). doi:10.2800/630938. Retrieved from: https://www.eea.europa.eu/publications/bio-waste-in-europe.: 17th of october 2020.

Vankar, P.S. (2004). Essential oils and fragrances from natural sources. Resonance, 9 (4), 30-41.

Varzakas, T., Zakynthinos, G., Verpoort, F. (2016). Functional foods. Foods 5, 1–32.

Vasiljevic, T. (2020). Pineapple. Valorisation of fruit processing by-products, Academic Press, 203–225.

Vauzour, D. (2014) Effect of flavonoids on learning, memory and neurocognitive performance: Relevance and potential implications for Alzheimer's disease pathophysiology. Journal of the Science of Food and Agriculture, 94, 1042–1056.

Ventura, S.P.M., Silva, F.A., Quental, M.V., Mondal, D., Freire, M.G., Coutinho, J.A.P. (2017), Ionic-liquid-mediated extraction and separation processes for bioactive compounds: past, present, and future trends. Chemical Reviews, 117(10), 6984-7052.

Vichai, V., & Kirtikara, K. (2006). Sulforhodamine B colorimetric assay for cytotoxicity screening. Nature protocols, 1(3), 1112-1116.

Vuolo, M.M., Lima, V.S., Maróstica, M.R.J. (2019). Phenolic compounds: structure, classification, and antioxidant power. In: In: Campos MRS, Bioactive compounds: health benefits and potential applications. Elsevier, 1, 33-50.

Wakasa, K. (1989). Pineapple (Ananas comosus L. Merr.). Biotechnology in Agriculture and Forestry, 13–29.

Wang, L., Liu, S., Zhang, X., Xing, J., Liu, Z., Song, F. (2016). A strategy for identification and structural characterization of compounds from Gardenia jasminoides by integrating macroporous resin column chromatography and liquid chromatography-tandem mass spectrometry combined with ion-mobility spectrometry. Journal of Chromatography A, 1452, 47-57.

Wang, W., Y. Ding, D. M. Xing, J. P. Wang and L. J. Du (2006). Studies on phenolic constituents from leaves of pineapple (Ananas comosus). Zhongguo Zhongyao Zazhi 31(15),1242-1244.

Wen, L., R. E. Wrolstad and V. L. Hsu (1999). Characterization of sinapyl derivatives in pineapple (Ananas comosus [L.] Merill) juice. Journal of Agricultural and Food Chemistry, 47(3), 850-853.

Wijayati, N., Rini, A. R. S., & Supartono, S. (2016). Hand sanitizer with pineapple peel extract as antibacterial against Staphylococcus aureus and Escherichia coli. Proceeding of ICMSE, 3(1), C-40.

Zainal-Abidin, M. H., Hayyan, M., Hayyan, A., & Jayakumar, N. S. (2017). New horizons in the extraction of bioactive compounds using deep eutectic solvents: A review. Analytica Chimica Acta, 979, 1–23.

Zamperlini, G. P. (2010) Crescimento e desenvolvimento fotoquímico do processo fotossintético em abacaxizeiro 'Vitória'. 2010. 60 p. Dissertação (Mestrado em Biologia Vegetal) - Universidade Federal do Espírito Santo, Vitória.

Zhang, X.M., Du, L.Q., Sun, G.M., Gong, D.Q., Chen, J.Y., Li, W.C., Xie, J.H., (2007). Changes in organic acid concentrations and the relative enzyme activities during the development of Cayenne pineapple fruit. Journal of Fruit Science, 24 (3), 381–384.

Zharfan, R., Purwono, P. & Mustika, A. (2017). Antimicrobial activity of pineapple (Ananas comosus L. Merr) extract against multidrug-resistant of Pseudomonas aeruginosa: an in vitro study. Indonesian Journal of Tropical and Infectious Disease, 6(5), 118-123.

Zheng, L.Y., Sun, G. M., Liu, Y.G., Lv, L.L., Yang, W.X., Zhao, W.F. & Chang, B. W. (2012). Aroma volatile compounds from two fresh pineapple varieties in China. International Journal of Molecular Sciences, 13, 7383-92.

Zillich, O. V., Schweiggert-Weisz, U., Eisner, P., & Kerscher, M. (2015). Polyphenols as active ingredients for cosmetic products. International Journal of Cosmetic Science, 37(5), 455-464.