Evaluation of the suitability of quince peel extract for preserving quality attributes of plant-based smoothies during refrigerated storage

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ABSTRACT

The manufacture of nutritionally enriched and shelf-stable foods aligns with the modern consumer's demand for healthy products, formulated with sustainable natural ingredients. These challenges are also faced by the beverage industry, which mainly uses artificial additives given the scarcity of effective natural alternatives. Therefore, this work was carried out to evaluate the suitability of quince peel bioactive extract for preserving quality attributes of a plant-based smoothie during refrigerated storage. The extract was prepared following a previously optimized protocol and characterized for its composition in malic acid and phenolic compounds and *in vitro* antioxidant activity. Afterwards, a plant-based smoothie was prepared with and without added extract and analyzed during 60 days of storage at 4 ºC. Physicochemical (pH, color, total soluble solids (TSS), density) and compositional (proximate constituents, mineral elements, soluble sugars, organic acids, fatty acids) attributes of the smoothie formulations were evaluated using different analytical techniques, including official methods of analysis and chromatographic and spectroscopic techniques. The extract was rich in malic acid, flavonols (*O*-glycosylated quercetin derivatives), flavan-3-ols (β-type (epi)catechin trimers), and caffeoylquinic acids, and inhibited in some extent the formation of thiobarbituric acid reactive substances and the oxidative hemolysis. In general, the prepared smoothie was rich in carbohydrates (e.g., fructose, glucose), minerals (e.g., K, Mg, Mn, Cu), and dietary fiber. The pH of the smoothie samples was lower after 60 days of storage. The control smoothie underwent the greatest color changes compared to the initial sample during the first 14 days, while the color of the extract-added smoothie was better preserved during the same period of time and the greatest difference was observed after 60 days. Fructose and glucose contents increased during storage, while sucrose decreased during this time, which may be related to enzymatic or microbial activity. The citric acid content was higher in the extract-added smoothie at day 7 (as well as TSS), while increases occurred in the control after 14 and 21 days. After 60 days, the percentage of saturated and monounsaturated fatty acids was lower in the smoothie with added extract compared to the initial samples, while the percentage of polyunsaturated fatty acids was higher. Overall, while the addition of bioactive extract mainly impacted the hue angle (h°) and the fructose and oxalic acid contents, the storage time mainly affected the sucrose and also malic acid levels.

Keywords: quince peel extract, smoothies, refrigerated storage, nutritional composition, quality attributes, physicochemical stability, natural preservatives.

RESUMO

A produção de alimentos nutritivos e estáveis alinha-se com a procura do consumidor por produtos saudáveis e formulados com ingredientes naturais sustentáveis. Estes desafios também são enfrentados pela indústria de bebidas, a qual recorre sobretudo a aditivos artificiais dada a falta de alternativas naturais eficazes. Portanto, este estudo foi realizado para avaliar a adequação de um extrato bioativo de casca de marmelo para preservar atributos de qualidade de um smoothie durante o armazenamento refrigerado. O extrato foi preparado seguindo um protocolo previamente otimizado e caracterizado quanto à sua composição em ácido málico e compostos fenólicos e atividade antioxidante *in vitro*. Posteriormente, foi preparado um smoothie, com e sem adição de extrato, e analisado periodicamente durante 60 dias de armazenamento a 4 ºC. Os atributos físico-químicos (pH, cor, sólidos solúveis totais, densidade) e composicionais (composição centesimal, minerais, açúcares solúveis, ácidos orgânicos, ácidos gordos) das amostras foram avaliados usando diferentes técnicas analíticas, incluindo métodos oficiais de análise e técnicas cromatográficas e espectroscópicas. O extrato era rico em ácido málico, flavonóis (derivados *O*-glicosilados de quercetina), flavan-3-óis (trímeros de (epi)catequina do tipo β) e ácidos cafeoilquínicos, e inibiu em certa medida a formação de substâncias reativas ao ácido tiobarbitúrico e a hemólise oxidativa. Já o smoothie era rico em carboidratos (e.g., frutose, glucose), minerais (e.g., K, Mg, Mn, Cu) e fibra. O pH dos smoothies foi menor após 60 dias de armazenamento. O smoothie controle sofreu as maiores alterações de cor durante os primeiros 14 dias, comparativamente com a amostra inicial, enquanto a cor do smoothie com extrato foi melhor preservada durante o mesmo período e a maior diferença foi observada após 60 dias. Os teores de frutose e glucose aumentaram durante o armazenamento, enquanto a sacarose diminuiu, o que poderá dever-se à atividade enzimática ou microbiana. O ácido cítrico foi mais abundante no smoothie com extrato ao dia 7, enquanto o controlo sofreu aumentos após 14 e 21 dias. Ao dia 60, a percentagem de ácidos gordos saturados e monoinsaturados foi menor no smoothie com extrato, comparativamente às amostras iniciais, enquanto os ácidos gordos polinsaturados tenderam a aumentar. Em geral, embora a adição de extrato tenha afetado principalmente o ângulo de matiz (h°) e os teores de frutose e ácido oxálico, o tempo de armazenamento afetou sobretudo o teor de sacarose e também de ácido málico.

Palavras-chave: extrato de casca de marmelo, smoothies, armazenamento refrigerado, composição nutricional, estabilidade físico-química, conservante natural.

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ABBREVIATIONS

AAPH	2,2'-Azobis(2-amidinopropane) dihydrochloride
AAS	Atomic absorption spectroscopy
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
CIE	International Commission on Illumination
DAD	Diode array detector
DRI	Dietary reference intake
EC_{50}	Half-maximal effective concentration
EDTA	Ethylenediaminetetraacetic acid
FAME	Fatty acid methyl ester
FAO	Food and Agriculture Organization
FID	Flame ionization detector
HPLC	High-performance liquid chromatography
IC_{50}	Half-maximal inhibitory concentration
MDA	Malondialdehyde
MPa	Megapascal
MS	Mass spectrometry
MUFA	Monounsaturated fatty acids
NCD	Non-communicable diseases
PBS	Phosphate buffered saline
PUFA	Polyunsaturated fatty acids
RGB	Red, Green, Blue
SFA	Saturated fatty acids
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloroacetic acid
TDF	Total dietary fiber
TSS	Total soluble solids
UV-Vis	Ultraviolet-visible
WHO	World Health Organization

1. STATE OF THE ART

1.1. Beverages

Plant-based beverages refer to healthy drinks that are manufactured and processed from plant materials, such as grains, seeds, fruits, and vegetables, and extracted in water (Penha et al., 2021). They are low in calories and rich in dietary fiber, calcium, provitamin A and vitamin D and B-complex (Castillejo et al., 2016; Razola-Díaz et al., 2022). Plant-based beverages are commonly consumed as alternative to dairy products as they consist of a healthy combination of mono- and polyunsaturated fats and have zero concentration of lactose. As a result, plant-based beverages assist in reducing the risk of developing heart diseases, strokes, and diabetes (Aydar et al., 2020).

1.2. Classification of beverages

Beverages are consumed mainly for their nutritional value (e.g., milk and fruit juices), thirst-quenching qualities (e.g., non-alcoholic drinks), exciting nature (e.g., coffee and tea), and recreational value (e.g., alcoholic beverages). Beverages represent a heterogeneous class of commodities and there is no uniform regulation that defines how they should be classified. Primarily, beverages can be divided into natural and synthetic beverages. Natural drinks are all drinks that are obtained through natural ingredient use or through a natural transformation process (such as milk, fruit juice, and wine), whereas synthetic drinks are obtained through artificial compound mixing (such as aroma, coloring, and sugar syrup) (Bhattacharjee et al., 2017). Another type of beverage classification is based on the presence or absence of carbon dioxide. Carbon dioxide can be added to sugary beverages (such as cola drinks and carbonated water), or it can be produced naturally during fermentation processes, such as in wine and beer (Mudgil & Barak, 2018). Another beverage classification is based on alcohol content. Alcoholic beverages are characterized by the presence of ethanol produced by fermentation processes (Mudgil & Barak, 2018). This classification is the most common form used to divide beverages and is the one to be used hereafter.

1.2.1. Alcoholic beverages

An alcoholic beverage is a drink containing ethanol, commonly known as alcohol. Ethanol is a psychoactive drug, with a depressant effect. Significant blood alcohol content

may be considered legal drunkenness as it reduces attention and slows reaction speed (Garrisson et al., 2022). Alcohol has been widely consumed since prehistoric times by people around the world, as a component of the standard diet, for hygienic or medical reasons, for its relaxant and euphoric effects, for recreational purposes, for artistic inspiration, as aphrodisiacs, and for other reasons.

1.2.1.1 Fermented alcoholic beverages

During the fermentation process, certain yeasts decompose sugars in the feedstock in the absence of oxygen to form alcohol and carbon dioxide, a method for the production of ethanol, wine, and beer (Maicas, 2020). Low-alcohol drinks are produced by fermentation of sugar or starch-containing products, and high-alcohol ones are produced by distillation of these low-alcohol products.

- Beer: Beer is an alcoholic beverage made by brewing or fermenting cereals mash, especially malted barley, usually with the addition of hops as a flavoring agent (bitter taste) and as a stabilizer. A great many beers are brewed across the globe. Local traditions will give beers different names, giving the impression of a multitude of different styles. However, the basics of brewing beer are shared across national and cultural boundaries.
- Wine: Wine is an alcoholic beverage produced through the partial or total fermentation of grapes.
- Other fermented beverages: fruits and plants such as berries, elderberries, apples, cherries, dandelions, and rice can also be fermented. Examples include table wine, sparkling wine, fortified wine (vermouth), etc.

1.2.1.2. Distilled alcoholic beverages

A distilled beverage is a consumable ethanol-containing liquid obtained by distillation from a fermented feedstock such as fruits, vegetables, or cereal grains. The word spirits generally refer to distilled beverages low in sugars and containing at least 35% alcohol by volume. Popular spirits include absinthe, brandy, grappa, rum, tequila, vodka, and whisky, among others. A short description of these is presented below.

- Whiskey: Whiskey refers to a broad category of alcoholic beverages that are distilled from fermented grain mash and aged in wooden casks

(generally oak). Different grains are used for different varieties, including barley, malted barley, rye, malted rye, wheat, and maize (corn).

- Brandy: It is a general term for distilled wine that need to be aged, usually containing 40–60% alcohol. In addition to wine, this spirit can also be made from grape, pomace, or fermented fruit juice. It is normally consumed as an after-dinner drink. Brandy made from wine is generally colored with caramel to imitate the effect of long aging in wooden casks; pomace and fruit brandies are generally drunk unaged and are not usually colored.
- Rum: Rum is a distilled beverage made from sugarcane by-products such as molasses and sugarcane juice by a process of fermentation and distillation. The distillate, a clear liquid, is then usually aged in oak and other barrels. Rum is produced in a variety of styles. Light rums are commonly used in cocktails, while golden and dark rums are appropriate for use in cooking as well as cocktails. Premium brands of rum are also available that are made to be consumed neat or on the rocks.
- Vodka: Vodka is one of the world's most popular distilled beverages. It is a clear liquid containing water and ethanol purified by distillation from a fermented substance such as potatoes, grain, or sugar beet molasses, and an insignificant quantity of other substances: impurities and possibly flavorings. Except for various types of flavorings, vodka is a colorless liquid. Vodka usually has an alcohol content of 35% to 50% by volume. Vodka is a Russian delight.

1.2.2. Non-alcoholic beverages

According to the Codex Alimentarius, non-alcoholic beverages include waters and carbonated waters, fruit and vegetable juices and nectars, water-based flavored carbonated and non-carbonated drinks, and water-based brewed or steeped beverages such as coffee and tea (FAO/WHO, 2021). Furthermore, fruit and vegetable juices are described as unfermented but fermentable liquids obtained from the edible part of mature and fresh fruit usually by mechanical extraction or form fresh vegetables, respectively. In turn, fruit nectars are defined as unfermented but fermentable products obtained by adding water with or without the addition of sugar, honey, syrups, and/or sweeteners to fruit juice, concentrated fruit juice, fruit purees or concentrated fruit purees, or a mixture of those products, while vegetable nectars are products obtained by adding water with or

without the addition of sugar, honey, syrups, and/or sweeteners to vegetable juice or concentrated vegetable juice, or a mixture of those products. Furthermore, concentrates are prepared by removing water from fruit juice or nectar (FAO/WHO, 2021).

A non-alcoholic beverage is a beverage that contains no alcohol. Such drinks are generally drunk for refreshment, or to quench people's thirst. Non-alcoholic beverages can be mainly classified as hot and cold beverages.

1.2.2.1. Cold beverages

- Spring water/mineral water: Spring water is the water derived from underground formations from which water flows naturally (artesian) to the surface of the earth. Minerals become dissolved in the water as it moves through the underground rocks. This may give the water flavor and even carbon dioxide bubbles, depending upon the nature of the geology through which it passes. This is why spring water is often bottled and sold as mineral water. In turn, mineral water is water containing minerals or other dissolved substances that alter its taste or gives it therapeutic value. Salts, sulfur compounds, and gases are among the substances that can be dissolved in water. Mineral water can be prepared or can occur naturally.
- Aerated: These beverages are charged or aerated with carbonic gas. The charging with carbonic gas imparts the pleasant effervescent characteristic of these beverages. Carbonation occurs when carbon dioxide is dissolved in water or an aqueous solution. This process yields the "fizz" to carbonated water and sparkling mineral water. Examples: soda water, dry ginger, fizzy lemonade, ginger beer, cola soft drink, and others.
- Juice: Juice is prepared by mechanically squeezing or macerating fresh fruits or vegetables without the application of heat or solvents. Popular juices include but are not limited to, apple, orange, prune, lemon, grapefruit, cherry, pineapple, tomato, carrot, grape, strawberry, cranberry, pomegranate guava, sapota, and celery, as well as the mixture of some of them. It has become increasingly popular to combine a variety of fruits into single juice drinks. Juices are also used for cocktails and mixing with spirits.
- Squash: Squash is a highly sweetened (and often fruit-based) concentrate, which is diluted with a liquid, most commonly water, before drinking.

Typically, squash is created by mixing one part concentrate with four or five parts of water (depending on concentration and personal taste) directly into a glass or mug, or jug. Squashes are also mixed with spirits or cocktails. The most common flavors are orange, apple, blackcurrant, lemon, peppermint, mixed fruit, summer fruits, and lemon-lime.

- Syrup: Syrup is a thick, viscous liquid, containing a high concentration of dissolved sugar (60–65% Brix), but showing little tendency to crystallize. The main use of these concentrated sweet fruit flavorings is as a base for cocktails, fruit cups or mixed with soda water as a long drink.

1.2.2.2. Hot beverages

- Tea: Tea is one of the most widely consumed stimulant beverages in the world. It has a cooling, slightly bitter, astringent flavor. It has almost no carbohydrates, fat, or protein. Tea is a natural source of the amino acid theanine, methylxanthines such as caffeine and theobromine, and polyphenolic antioxidant catechins.
- Coffee: Coffee is a widely consumed stimulant beverage prepared from roasted seeds, commonly called coffee beans, of the coffee plant. Once brewed, coffee may be presented in a variety of ways. Drip brewed, percolated, or French-pressed/cafetière coffee may be served with no additives (colloquially known as black) or with either sugar, milk or cream, or both. When served cold, it is called iced coffee.
- Cocoa: It is a powder made from cacao seeds (beans) after they have been fermented, roasted, shelled, ground, and freed of most of their fat. A beverage is made by mixing this powder with sugar in hot water or milk. It is a rich source of theobromine which acts as a stimulant.

1.3. Economic and commercial issues

The increasing health consciousness among masses across the globe is creating a positive outlook for the market. Plant-based beverages provide hydration and serve as a source of various essential nutrients, vitamins, minerals, and dietary fiber that helps in lowering the risk of cardiovascular diseases, improving immunity, and strengthening bone health. In line with this, the widespread consumption of plant-based beverages

among the vegan population due to the rising prevalence of lactose intolerance and various kinds of food allergies is favoring the market growth. Apart from this, the launch of plant-based beverages formulated with additional healthy ingredients, such as oat-based options, are providing a considerable boost to the market growth. Additionally, the increasing demand for plant-based supplements in the sports and nutrition industry is positively impacting the market growth. Other factors, including rising expenditure capacities of consumers, the widespread product adoption in cafes and bakeries, significant advancements in processing technologies, and the increasing demand for ready-to-drink beverages due to the busy lifestyles and hectic schedules, are anticipated to drive the market further toward growth (IMARC Group, 2023).

1.4. Smoothies

In response of current consumption, there is a clear increased interest in more natural, nutritious, and healthier foods. Accordingly, natural fruit- and vegetable-based beverage companies like juices and smoothies have showed great growth. In that perspective, in order to increase the daily consumption of fruits and vegetables, today dairy-free smoothies represent an excellent and convenient alternative (Castillejo et al., 2016). The word smoothie comes from the English term “smooth” and defines a tender and creamy non-alcoholic drink with a thick texture similar to that of milkshakes (Cano-Lamadrid et al., 2020; Smith et al., 2013).

1.4.1. Composition

Smoothies are prepared with natural ingredients such as fruit and vegetable puree with fruit juice, and possibly dairy products (like yogurt, milk) or/and crushed ice cubes, which are blended without filtering and to be immediately consumed (Castillejo et al., 2016; Smith et al., 2013). Their preparation is based on the use of the entire fruit, which is processed from pulp to puree, with only the seeds and peel being removed. To develop different flavors and to obtain the appropriate texture of the final product, the juice from different fruits is used, as well as water in some cases. It is important to highlight that these beverages can be prepared without adding preservatives, stabilizers, or chemical correctors of pH and acidity. This type of product is the easiest form to eat fruits and vegetables and to increase its consumption between todays’ consumers with the current modern lifestyle (Cano-Lamadrid et al., 2020; Castillejo et al., 2016).

1.4.2. Nutritional interest

In general, fruit and vegetable smoothies contain high levels of dietary fiber and vitamin C, thus allowing the statement "source" of fiber and vitamin C to be made on the label of these food products, under the Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on food (European Union, 2006). Carbohydrates are usually the main constituents of these beverages, while fat and protein contents are generally low (Razola-Díaz et al., 2022).

Some smoothies are rich in soluble (pectin) and insoluble (cellulose and lignin) fiber, which delays the absorption of monosaccharides into the bloodstream and thus effectively regulate glucose-insulin homeostasis (Murphy et al., 2017). However, it has been recommended to moderate the consumption of juices and smoothies due to the possible excessive supply of energy from sugars. In addition, these products have a moderate or high glycemic index and, as a consequence, can cause a rapid increase in glucose and insulin levels. In general, the macronutrient composition of smoothies is similar to that found in two portions of fruit (Ruxton, 2008).

To prevent a rise in sugar consumption, health professionals sometimes discourage the consumption of smoothies because they are relatively high in energy and sugar, which can be around 30 g of total sugar per serving; most fruit smoothies contain a similar amount of soluble sugar to that found in one banana and a portion of mango or cherries (Ruxton, 2008). There is also concern that smoothie consumption may negatively affect dental health due to its sugar content or pH value.

The World Health Organization (WHO) is still alerting that non-communicable diseases (NCD) cause 71% of all deaths worldwide, and every year 15 million people die prematurely, *i.e.*, between the ages of 30 and 69 years, from NCD. Unfortunately, there is still a lack of public health actions about fruits and vegetables consumption (e.g., health education and health program promotion) (Castillejo et al., 2016; Hall et al., 2009). The recommended fruit and vegetables intake is 400 g per day. To encourage, to maintain and/or to increase their intake, the food industry comes with an alternative to raw fruits and vegetable intake, developing new products, easy-to-eat, and with longer shelf-life than regular fruits, such as smoothies (González-Tejedor et al., 2017).

1.4.3. Technological issues

The biggest difficulty in making smoothies is the short shelf life of the product due to its susceptibility to spoiling and quality decline. Because of this, it is necessary to

utilize modest thermal treatments during processing to extend the shelf-life while maintaining quality and low-temperature storage is advised (Di Cagno et al., 2011). To maintain the smoothie's nutritious value and sensory appeal, the thermal treatment should be as gentle as possible. Commercially, thermal treatments are used to inactivate spoilage enzymes in fruit purees and juices. These treatments typically take place at temperatures between 80 and 95 °C (Di Cagno et al., 2011). But these processes might lessen the phytochemical content of smoothies, decreasing the associated antioxidant capabilities. So far, not many publications are available on the effects of mild thermal processing and subsequent storage on quality changes of fresh plant-based smoothies.

Nowadays, a wide range of synthetic additives (e.g., sweeteners, preservatives, antioxidants) can be added to beverages such as juices and smoothies to preserve their quality during shelf life or enhancement sensory attributes. Examples of preservatives include sodium benzoate (E211), ascorbic acid (E300), citric acid (E330), and malic acid (E296) (M. M. Silva et al., 2019). Although sodium benzoate is an antimicrobial compound reported as non-toxic, some authors have observed some toxicity with mutagenic and cytotoxic effects on peripheral snag lymphocytes (Zhang & Ma, 2013). In the particular case of malic acid, this acidulant has been less used than citric acid due to its higher price (M. M. Silva et al., 2019).

1.5. Quince (*Cydonia oblonga* Mill.)

The quince, which corresponds to the species *Cydonia oblonga* Miller, is a shrub member of the Rosaceae family and thus related to apples and pears. The firm, highly acidic flesh of quince fruit (**Figure 1**) is astringent and unpleasant when consumed raw (Alesiani et al., 2010). Therefore, its pulp is used to make jam, marmalades, fresh fruit compote, jellies, dried slices, and wines, among other products. It is also used to a variety of goods, including beers and yogurts, because to its aromatic and useful qualities. This fruit is a potential choice in the food sector because the hydrocolloid and quince seed mucilage can be employed as thickeners and bulking agents in many food items (Hanan et al., 2020). This fruit is rich in pectin, tannins, vitamins, and minerals (Al-Zughbi & Krayem, 2022). Quince is known for its wide range of health-promoting properties, including its antioxidant, anti-inflammatory, antibacterial, anti-ulcerative, and anticancer properties (Rather et al., 2023). However, because to its sensory attributes and limited

understanding of its advantages by both farmers and consumers, this fruit is underutilized at the level of food processing, resulting in the generation of by-products such as its peel.

Figure 1 Quince fruits.

Overall, despite of all the nutritional composition and health benefits, the quince fruit is not appreciated fresh due to its pulp hardness, bitterness, and astringency (Silva et al., 2002, 2005). However, it has received increasing attention in the nutraceutical sector due to the presence of varied functional compounds and phytochemicals, as well as in the food packaging industry (Rather et al., 2023).

1.5.1. Production and consumption data of quince

The quince tree is originally from the Caucasus region and has gradually spread to Central Europe and Mediterranean countries. This species has potential to thrive in a variety of climates and can be successfully grown in latitudes as far north as Scotland (Abdollahi, 2019). Although its old reputation has been lost, the quince continues to be grown all over the world. As can be seen in the **Figure 2**, the world production of quinces has increased in the last years, reaching 697,563 tons in 2021 in a harvested area of 75894 ha (FAO, 2022). In Portugal, the quince production reached 5492 tons in 2017 (**Figure 2**), representing less than 1% of the world total. However, the FAOSTAT has no production data since 2018, nor does the Instituto Nacional de Estatística (INE, Portugal). At a global level, Turkey and China emerge as important world producers of quince, as well as Uzbekistan, Iran, Morocco, and Azerbaijan (FAOSTAT, 2022).

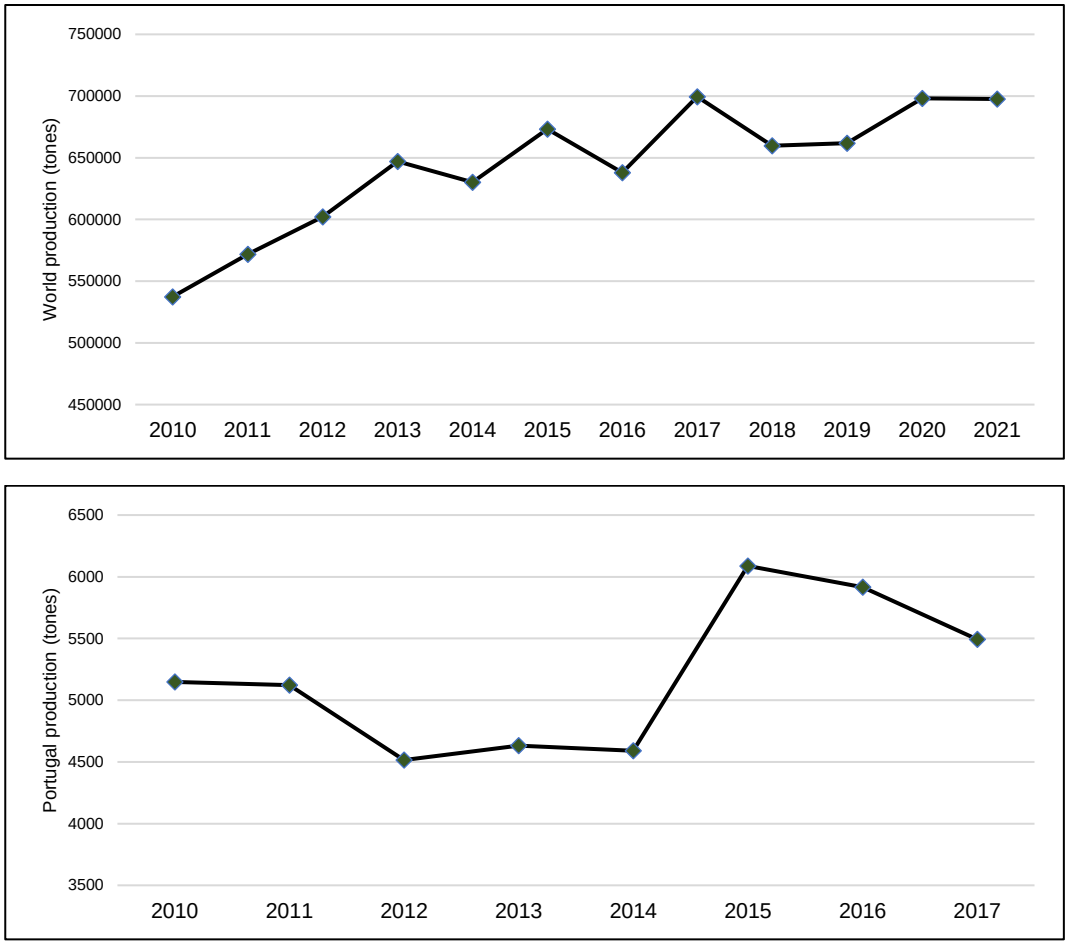


Figure 2 Data on quince production in (**A**) the world and (**B**) in Portugal since 2010. Source: FAOSTAT (2022).

1.5.2. Quince peel as a promising source of food preservatives

Quince is not appreciated fresh because of its hard pulp, bitterness, and astringency, but its ripe pulp is used to make marmalade, jams, jellies, and cakes, among other food products. Although the fruit peel is discarded as a by-product, it is rich in phenolic compounds, mainly flavan-3-ols, and malic acid (**Figure 3**) (Othman et al., 2022). It also has the potential to inhibit lipid peroxidation due to its antioxidant activity and antimicrobial effects against foodborne pathogens, which agreed with the highest levels of flavan-3-ol. According to Othman et al. (2022), quince peel extracts can be more effective than some synthetic food additives against fungal and bacterial strains, including *Salmonella* Typhimurium, *Staphylococcus aureus*, *Bacillus cereus*, *Aspergillus fumigatus*, *Aspergillus versicolor*, and *Trichoderma viride*. Due to this preservative potential of quince peel extracts, Pereira et al. (2023) optimized an extraction process to maximize the recovery of phenolic compounds and malic acid from quince peel. Both malic and phenolic rich extracts were produced, and the malic acid-rich extract was the

most effective in inhibiting the formation of thiobarbituric acid (TBA) reactive substances (TBARS) and the oxidative hemolysis, and also was more effective in inhibiting and killing the tested foodborne microorganisms. The preservative potential of the malic acid-rich extract was thus highlighted, but it was not tested in food matrices.

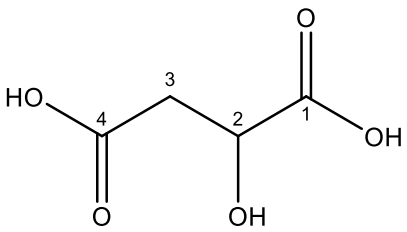


Figure 3 Chemical structure of malic acid.

2. OBJECTIVES

The MAIN OBJECTIVE of this study was to assess the suitability of quince peel bioactive extract for preserving quality attributes of a fruit and vegetable smoothie during refrigerated storage. The study comprised specific objectives related to the preparation and characterization of the bioactive extract and the evaluation of the stability and quality of the smoothie with and without the addition of bioactive extract during storage at 4 ºC.

Objectives related to the bioactive extract preparation and characterization:

- ✓ To produce the bioactive extract from quince peel.
- ✓ To evaluate the extract composition in phenolic compounds and malic acid.
- ✓ To evaluate the *in vitro* antioxidant activity of the extract.

Objectives related to the stability and quality evaluation of the fruit and vegetable smoothie during refrigerated storage:

- ✓ To evaluate physicochemical parameters of the smoothie formulations during shelf-life, namely pH, total soluble solids (TSS), color, and density.
- ✓ To assess the centesimal composition and energy value of the smoothie formulations at the beginning (day 0) and end of storage time (day 60), as well as the composition of mineral elements and fatty acid profile.
- ✓ To characterize the soluble sugar and organic acid profiles of the smoothie formulations throughout their shelf-life.

In addition to the aforementioned objectives, this master's work involved a comprehensive analysis and interpretation of the obtained results, culminating in the preparation of this dissertation.

3. MATERIAL AND METHODS

3.1. Materials

3.1.1. Plant material

Quince peels were supplied by a producer from Bragança, civil parish of Calvelhe, Portugal, in October 2021. After peeling the fruits for marmalade manufacture, the peels (~5 kg) were immediately frozen in plastic bags and taken to the laboratory in a thermal bag to be freeze-dried (FreeZone 4.5, Labconco, Kansas City, MO, USA). Afterwards, the dried material was ground and homogenized in a domestic food processor and passed through a 20-mesh sieve (≈ 0.9 mm aperture). The dried powder was vacuum-packed and stored at −20 °C until analysis.

The fresh fruits (orange, apple, and blueberry) and vegetables (carrot and beetroot) used to prepare the plant-based smoothie were purchased one day before its manufacture in a supermarket in Bragança, Portugal.

3.1.2. Biological material

Pig brain was supplied by a slaughterhouse in Bragança, Portugal, and immediately dissected into small pieces and stored at −20 °C until use. Sheep blood was freshly collected from healthy animals from the Bragança School of Agriculture herd, Portugal, in EDTA tubes for subsequent isolation of erythrocytes.

3.2. Standards and reagents

HPLC-grade acetonitrile (99.9%) was purchased from Fisher Scientific (Lisbon, Portugal). Cesium chloride and lanthanum chloride solution were acquired from Thermo Fisher Scientific Co. (Waltham, MS, USA). Standard mineral solutions were acquired from Panreac AppliChem (Barcelona, Spain). The phenolic standards (≥ 96% purity) of chlorogenic acid, *p*-coumaric acid, (+)-catechin, and quercetin-3-*O*-(6-acetylglucoside) were acquired from Extrasynthese (Genay Cedex, França). Ascorbic acid, iron sulphate, formic acid, metaphosphoric acid, phosphate buffered saline (PBS), thiobarbituric acid (TBA), trichloroacetic acid (TCA), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), the Supelco 37-component fatty acids methyl ester (FAME) mix (47885-U) standard, and standards of sugars ((D(-)-fructose, D(+)-glucose, and D(+)-saccharose) and organic acids (oxalic

acid, quinic acid, malic acid, shikimic acid, citric acid, succinic acid, and fumaric acid) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethanol and other reagents were supplied by common sources. Water was treated in a Milli-Q water purification system (TGI pure system, Houston, USA).

3.3. Quince peel extract preparation and characterization

3.3.1. Extraction

The quince peel extract was prepared following a solid-liquid extraction method previously described by Pereira et al. (2023), which aims to recover phenolic compounds and mainly malic acid from this plant material. The extraction was performed using a thermostated water bath, submersible magnetic stirrers (Cimarec, Thermo Scientific, San José, CA, USA), and sealed flasks to avoid solvent evaporation (**Figure 4**). The plant material (~1 g) was mixed with 35 mL of ethanol/water (54:46, *v*/*v*) at 30 g/L, submerged in the water bath at 93 °C, and stirred for 88 min. Then, the mixture was centrifuged at 4000×*g* for 10 min and the supernatant was collected. The ethanol was removed under reduced pressure in a rotary evaporator with the water bath not exceeding 40 ºC, while the aqueous fraction was freeze-dried until reaching constant weight. The extraction yield (%, *w/w*) was calculated and the dry extract was stored in a closed flask in a desiccator until characterization and incorporation into the plant-based smoothie formulation.

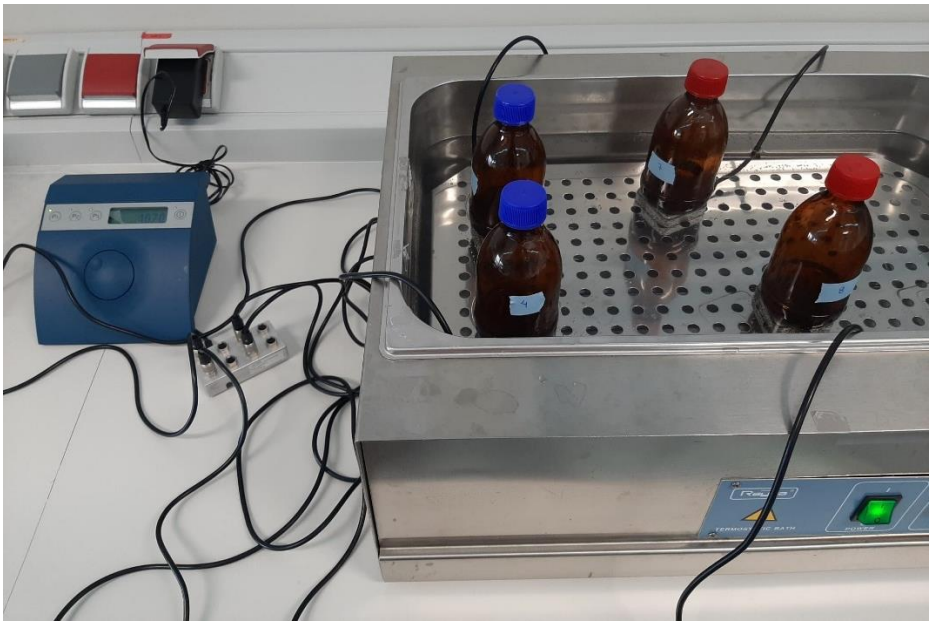


Figure 4 Illustration of the extraction system used to prepare the quince peel extract.

3.3.1. Analysis of phenolic compounds

Quince peel extract was dissolved in ethanol/water (20:80, *v/v*) at 10 mg/mL and filtered through a 0.2 μm pore size nylon membrane syringe filter into a glass vial.

The analysis was performed in a Dionex Ultimate 3000 HPLC system (Thermo Scientific, San Jose, CA, USA) equipped with a quaternary pump, an automatic injector thermostated at 5 ºC, a degasser, and a column compartment thermostated at 35 ºC. Chromatographic separation was made on a Waters Spherisorb S3 ODS-2 C18 reverse-phase column (4.6 × 150 mm, 3 μm; Milford, USA), using (A) formic acid/water (0.1%) and (B) acetonitrile as mobile phase at a flow rate of 0.5 mL/min. The elution in gradient mode was as follows: 10% to 15% B up to 5 min, 15-20% B up to 5 min, 20-25% B for 10 min, 25-35% B for 10 min, 35-50% B for 10 min, and rebalancing the column for 10 min (Bessada et al., 2016). Compound detection was achieved with a diode array detector (DAD) at the wavelengths of 280 and 370 nm.

The HPLC system was connected to an Ion Trap Linear LTQ XL mass spectrometer (ThermoFinnigan, San Jose, CA, USA) equipped with an electrospray ionization (ESI) source. The carrier gas was nitrogen (50 psi). The system worked with a spray voltage of 5 kV, at an initial temperature of 325 ºC and capillary voltage of -20 V. The voltage of the tube lens offset was maintained at -66 V. The spectra were recorded in negative ion mode between 100 and 1500 *m/z*. The collision energy used was 35 (arbitrary units).

The detected compounds were tentatively identified by comparing the obtained data (retention times and UV-Vis and mass spectra) with the available standards and literature. The calibration curves presented in **Table A1** were constructed with available phenolic standards and used in the quantitative analysis. Chromatographic data were collected and processed using Xcalibur™ software (Thermo Finnigan, San Jose, CA, USA). The results were expressed as mg per g of extract, thus same compounds were expressed in mg of equivalents of its basic constituent or similar compound.

3.3.2. Analysis of malic acid

Quince peel extract was dissolved in meta-phosphoric acid at 5 mg/mL and filtered through a 0.2 μm pore size nylon membrane syringe filter into a glass vial.

The analysis was performed in the Dionex Ultimate 3000 HPLC system referred above. Chromatographic separation was achieved in reverse phase on a C18 column (250 mm × 4.6 mm, 5 μm; Phenomenex, Torrance, CA, USA) thermostated at 35 ºC. The isocratic elution was made with sulfuric acid (3.6 mM) (Barros et al., 2013). Malic acid

was detected with the DAD detector at a wavelength of 215 nm and identified by comparing the retention time and UV-Vis spectrum of the sample peak with that of the commercial standard of this compound. Quantification was achieved using the eight-level calibration curve presented in **Table A1**. Chromatographic data were collected and processed using Xcalibur™ software. The results were expressed as g per g of extract.

3.3.3. Evaluation of antioxidant activity

The antioxidant activity of the quince peel extract was evaluated *in vitro* through the bioassay of thiobarbituric acid reactive substances (TBARS) formation inhibition and oxidative hemolysis inhibition. Phosphate-buffered saline (PBS, pH 7.4) was used to dissolve the malic acid-enriched extract and the range of concentrations prepared was established based on previous studies with quince peel extracts (Othman et al., 2022; Pereira et al., 2023). Trolox was used as a positive control and also dissolved in PBS.

3.3.3.1. TBARS formation inhibition capacity

A brain cell suspension was prepared by placing pig brain tissue and Tris-HCl buffer (20 mM, pH 7.4) at a ratio of 1:2 (*w/w*) in a Falcon tube. After vigorous mixing, the mixture was centrifuging at 3500 rpm for 10 min at 10 ºC and the supernatant was collected. In 2 mL eppendorfs, 200 µL of extract solution (0.027–3.419 mg/mL) or trolox (3.125–100 µg/mL) was mixed with 100 µL of ascorbic acid (0.1 mM), 100 µL of iron sulphate (10 mM), and 100 µL of the brain cell solution. After incubation at 37.5 ºC for 1 h, the oxidation reaction was stopped by adding 500 µL of trichloroacetic acid (28%, *w/v*). Then, 380 µL of TBA (2%, *w/v*) were added and the mixture was heated at 80 ºC for 20 min to promote the formation of the pink colored adducts between TBA and malondialdehyde (MDA) that resulted from the peroxidation of the polyunsaturated fatty acids of the brain cell membranes. The mixtures were centrifuged at 3,500 rpm for 5 min and the supernatants were transferred to a 96-well microplate to measure the color provided by the MDA-TBA adducts in an Elx800 microplate reader (BioTek Instruments, USA) at a wavelength of 532 nm (Pinela et al., 2012).

The percentage of TBARS formation inhibition was calculated using the equation:

$$Inhibition\ (\%) = \frac{C - E}{C} \times 100 \quad (1)$$

where *C* and *E* refer to the absorbance of the control and the extract solution, respectively.

The half-maximal effective concentration (EC_{50} value, μg/mL) was calculated from the graph of TBARS inhibition percentage against extract or trolox concentration. These graphs were constructed using GraphPad Prism® 8.

3.3.3.2. Oxidative hemolysis inhibition capacity

For isolation of erythrocytes from sheep blood, freshly collected samples were centrifuged at 1000 ×*g* for 5 min at 10 ºC. After discarding the plasma and buffy coats, the erythrocyte pellet was washed once with NaCl (150 mM) and three times with PBS (Evans et al., 2013). The erythrocytes were then resuspended in PBS at 2.8% (*v/v*).

In a flat bottom 48-well plate, 200 μL of erythrocyte solution were mixed with 400 μL of either: extract solution (12.9–414 μg/mL); trolox (3.91–125 μg/mL); PBS solution (negative control); or distilled water for hemolysis (baseline). After incubating the plate at 37 ºC for 10 min with shaking to adjust the temperature, 200 μL of AAPH (160 mM in PBS) were added and the optical density was measured at 690 nm in the microplate reader referred above, first at this time 0 and then at intervals of about 10 min until complete hemolysis occurs (Lockowandt et al., 2019).

The percentage of erythrocytes (*E*) that remained intact was calculated as follows:

$$E\ (\%) = (S_t - CH_0 / S_0 - CH_0) \times 100 \quad (2)$$

where S_t and S_0 correspond to the optical density of the sample at *t* and time 0, respectively, and CH_0 is the optical density of the baseline at time 0.

Then, the delayed time of hemolysis (Δt) was calculated as follows:

$$\Delta t\ (\text{min}) = Ht_{50}\ (\text{sample}) - Ht_{50}\ (\text{negative control}) \quad (3)$$

where Ht_{50} is the 50% hemolytic time (min) graphically obtained from the hemolysis curve of each extract or trolox concentration using GraphPad Prism® 8.

Lastly, the half-maximal inhibitory concentration (IC_{50} value, μg/mL) capable of promoting a Δt hemolysis delay of 60 and 120 min were calculated from the correlation between Δt values and extract or trolox concentrations.

3.4. Smoothie preparation, pasteurization, and storage

The smoothie was prepared with the fruits and vegetables presented in **Table 1**, which were selected due to their wide use in this kind of beverages and in order to include different micronutrients in the formulation (nutritional facts in attachment). This selection

was aided by the nutrition value calculator (https://www.nutritionvalue.org/). All fruits and vegetables were washed in tap water, drained, and then apples, carrots and beets were peeled and cut into small pieces. The orange juice was freshly prepared.

Table 1. Recipe of the prepared plant-based smoothie.

	Orange juice	**Apple**	**Carrot**	**Blueberries**	**Beetroot**	**Total**
Quantity	2 L	702 g	685 g	494 g	200 g	4.081 kg
Percentage (by weight)	49%	17%	17%	12%	5%	100%

After weighing, the ingredients were mixed and homogenized in a domestic blender until obtaining a smoothie with a homogeneous consistency. Afterwards, it was divided into two portions, one was added with quince peel extract at a concentration of 1 g/L (Moreira et al., 2021) and the other was an extract-free control smoothie. The smoothie formulations were packaged in 30 mL glass flasks and sealed as shown in **Figure 5**.

Figure 5 Appearance of the smoothie formulation before pasteurization.

The smoothies were pasteurized in a water bath at 70 °C for 2 min (Van de Velde et al., 2022), counting from the moment the set temperature was reached inside the flasks. The samples were then transferred to trays and covered with ice to cool down.

A group of smoothie samples with and without added quince peel bioactive extract was immediately analyzed (day 0) and the remaining samples were stored at 4 ± 1 ºC and taken for analysis after 7, 14, 21, 35, and 60 days.

3.5. Stability and quality assessing during shelf-life

The smoothie quality parameters analyzed during shelf life are specified in **Table 2**. While pH, TSS, color, soluble sugars, and organic acids were evaluated at six moments during shelf-life, density, proximate composition (moisture, protein, ash, fat, TDF, and carbohydrates), and mineral elements were analyzed on the first and last day of the study.

Table 2 Smoothie quality parameters evaluated during storage. The red color indicates that the parameter has been evaluated.

	Day 0	Day 7	Day 14	Day 21	Day 35	Day 60
pH	■	■	■	■	■	■
TSS	■	■	■	■	■	■
Color	■	■	■	■	■	■
Density	■					■
Centesimal composition	■					■
Mineral elements	■					■
Soluble sugars	■	■	■	■	■	■
Organic acids	■	■	■	■	■	■
Fatty acids	■					■

3.5.1. Physicochemical parameters

The pH was directly measured in each smoothie sample using a portable pH-meter (Hanna Instruments, Woonsocket, EI, USA).

The total soluble solids (TSS) content was determined by placing 1 drop of each smoothie sample on the prism of a digital hand refractometer (model HI 96801, Hanna Instruments, Woonsocket, RI, USA) and measured as °Brix.

The color parameters L^* (lightness; chromaticity from $^{(0)}$black to $^{(100)}$white), a^* (chromaticity from $^{(-)}$green to $^{(+)}$red), b^* (chromaticity from $^{(-)}$blue to $^{(+)}$yellow) were measured using a portable CR-400 colorimeter (Konica Minolta Sensing Inc., Tokyo, Japan) with illuminant D65 (standard defined by the International Commission on

Illumination (CIE) as roughly corresponding to the average midday light in Western Europe) and 8 mm diaphragm aperture. A standard white tile was used for calibration.

To represent the color of each smoothie, the *L**, *a**, and *b** values were converted into RGB (red, green, blue) color models using a website (http://colormine.org/). Furthermore, the saturation index (*C**) and hue angle (h°) were calculated from the parameters *a** and *b** as follows:

$$C^*_{ab} = (a^{*2} + b^{*2})^{1/2} \quad (4)$$

$$h° = arctan\,(b^*/a^*) \quad (5)$$

The total color difference (Δ*E**) between samples with and without extract and over shelf-life was calculated as follows:

$$\Delta E^* = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2} \quad (6)$$

where subscripts 2 and 1 of parameters *L**, *a** and *b** correspond to samples without extract and with extract or to samples at time 0 and time *t*.

The density was determined by gravimetry, by recording the weight of a volume of 5 cm^3 of each smoothie sample, and calculate as follows:

$$density = \frac{sample\ weight\ (g)}{sample\ volume\ (cm^3)} \quad (7)$$

3.5.2. Proximate composition and energy value

The smoothie samples at day 0 and 60 were analyzed for moisture content, using a PMB moisture analyzer (Adam Equipment, Kingston, Milton Keynes, UK), and then freeze-dried for subsequent determination of protein, fat, ash, and total dietary fiber (TDF) contents following the official methods of the Association of Official Analytical Chemists (AOAC International, 2019). These analyses were performed in triplicate and the results were converted from dry weight to fresh weight and then to volume based on the dry matter and density values, respectively.

3.5.2.1. Crude protein

The crude protein content was estimated by the macro-Kjeldahl method (AOAC 920.152), which involves a wet oxidation of the sample to reduce the organic nitrogen to ammonia, followed by distillation and titration steps. The freeze-dried smoothie samples (250 mg) were mixed with 15 mL of sulfuric acid and two tablets of catalyst, containing selenium and potassium sulfate, and then digested for about 2 h at 410 °C (Bloc Digest

12, JP Selecta, Barcelona) (**Figure 6A**). This mineralization process led to the formation of ammonium sulfate, which is a stable salt. After cooling the digestion tubes (**Figure 6B**), 25 mL of distilled water was added to increase the pH value. During distillation, the ammonium sulfate was decomposed by the addition of excess sodium hydroxide and the ammonia obtained was distilled into the container with boric acid, resulting in the formation of ammonium borate. The retained ammonia is then titrated with hydrochloric acid solution, using bromocresol green and methyl red as a mixed indicator solution. Both distillation and titration were carried out in the automatic equipment (Pro-Nitro-A, JP Selecta, Barcelona) shown in **Figure 6C**. A control was also prepared and analyzed with the same reagents to rule out possible interferences. The protein content was calculated by multiplying the nitrogen value given by the equipment by the conversion factor of 6.25, which is suitable for foods in general and considers that most proteins contain 16% nitrogen. The results were expressed as g per 100 mL.

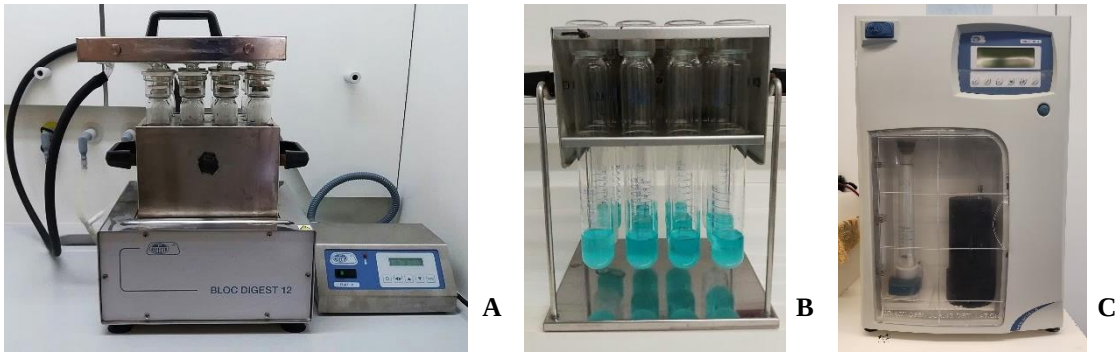


Figure 6 Illustration of the method used to determine the crude protein content; (**A**) digestion block, (**B**) digested samples; and (**C**) distillation and titration unit.

3.5.2.2. Crude fat

The crude fat was extracted from the freeze-dried smoothie samples (3 g) previously mixed with sodium sulphate anhydrous using a Soxhlet apparatus (**Figure 7**) with petroleum ether under reflux for about 7 h (AOAC 920.85). The crude fat was collected in a round flask, the solvent was removed by vacuum evaporation, and the flask with the extracted fat was oven-dried at 105 °C, cooled down in desiccator and weighted. The total fat content was determined as follows:

$$fat = \frac{(m_2 - m_1)}{m_0} \times 100 \quad (8)$$

where m_0 corresponds to the weighted sample, m_1 corresponds to the weight of empty extraction flask, and m_2 corresponds to the weight of extraction flask with fat after drying. The results were expressed as g per 100 mL.

Figure 7 Soxhlet apparatus used in the extraction of crude fat.

3.5.2.3. Ash

The freeze-dried smoothie samples (250 mg) were placed in porcelain crucibles previously calcined and weighed and then incinerated in a muffle furnace at 550 ± 15 °C until consistent weight was reached (AOAC 940.26). Afterward, the crucibles containing the incineration residue were cooled in a desiccator and weighed. The ash content was determined as follows:

$$ash = \frac{(m_2 - m_1)}{m_0} \times 100 \quad (9)$$

where m_0 corresponds to the weighted sample, m_1 corresponds to the weight of empty calcined crucible, and m_2 corresponds to the weight of crucible with incineration residue. The results were expressed as g per 100 mL.

3.5.2.4. Total dietary fiber

The total dietary fiber (TDF) content was determined by an enzymatic-gravimetric method (AOAC 985.29), using an assay kit from Sigma-Aldrich (Saint Louis, MO, EUA).

The freeze-dried smoothie samples (260 mg) were incubated (or gelatinized) with heat-stable α-amylase (pH 6.0) in a water bath at 95 °C for 15 min. Then, the samples were enzymatically digested with protease (pH 7.5), in a water bath at 60 °C for 30 min, and amyloglucosidase (pH 4.5), in a water bath at 60 °C for 30 min, to remove protein and starch. After overnight ethanol precipitation followed by filtration, the residues were successively washed with 78% ethanol, 95% ethanol, and acetone, and then oven-dried at 105 °C and weighed. The protein (AOAC 920.152) and ash (AOAC 940.26) contents were determined by macro-Kjeldahl nitrogen analysis (N × 6.25) or incineration at 525 °C, respectively, for subsequent calculation of the total dietary fiber content as follows:

$$TDF = \frac{(R_{sample} - P_{sample} - A_{sample})}{SW} \times 100 \quad (10)$$

where *R* corresponds to the average residue weight, *P* corresponds to the average protein weight, *A* corresponds to the average ash weight, and *SW* corresponds to the average sample weight. The results were expressed as g per 100 mL.

3.5.2.5. Carbohydrates

The carbohydrate content was estimated by weight difference using the following equation and the results were expressed as g per 100 mL.

$$carbs = 100 - (\text{g}\, moisture + \text{g}\, protein + \text{g}\, fat + \text{g}\, ash + \text{g}\, TDF) \quad (11)$$

3.5.2.6. Energy

The energy value was calculated according to the Regulation (EC) No. 1169/2011 of the European Parliament and of the Council (European Union, 2011), based on the following conversion factors: 9 kcal/g for fat, 4 kcal/g for proteins and carbohydrates, and 2 kcal/g for fiber. The results were expressed as kcal per 100 mL.

3.5.3. Mineral elements

Mineral elements were analyzed by atomic absorption spectroscopy (AAS) using a Perkin Elmer PinAAcle 900T Spectrometer (Waltham, MA, USA). Potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), zinc (Zn), and iron (Fe) were analyzed by flame ionization AAS, while a graphite furnace AAS was used for manganese (Mn) and copper (Cu). The freeze-dried smoothie samples (~250 mg) were placed in digestion tubes with 10 mL of concentrated nitric acid. The digestion was performed by setting the ramp

temperature program: 15 min until 200 °C with a microwave power of 1200 W, followed by more 15 min at the same temperature and power conditions. After cooling down, the obtained solutions were diluted up to 50 mL with deionized water and analyzed by AAS, with prior treatment for specific elements. For the determination of K and Na, the sample was diluted in a cesium chloride solution (1 g/L); for Ca and Mg, the sample was diluted in a lanthanum chloride solution (1 g/L); for Mn and Cu, a magnesium nitrate solution (1 g/L) was used as a matrix modifier; and Fe and Zn were directly analyzed. The quantification of elements was achieved by comparing the absorbance responses with calibration curves prepared from standard solutions. The results were expressed as mg per 100 mL for K, Na, Ca, and Mg and as µg per 100 mL for Fe, Mn, Cu, and Zn.

3.5.4. Soluble sugars

Each smoothie sample was filtered first through Whatman No. 4 paper and then through a 0.2 µm pore size nylon membrane syringe filter into a glass vial. The analysis was performed in a HPLC system (Knauer, Smartline 1000 system, Berlin, Germany) coupled to a refractive index (RI) detector (Knauer Smartline 2300). Chromatographic separation was made in isocratic mode on a 100-5 NH_2 Eurospher column (4.6 × 250 mm, 5 µm, Knauer) using acetonitrile/deionized water (70:30, *v/v*) as mobile phase, at a flow rate of 1 mL/min (Barros et al., 2013). Free sugars were identified by chromatographic comparisons with commercial sugar standards and quantified using calibration curves constructed with for the same standards (**Table A1**). The results were expressed as g per 100 mL of filtered smoothie.

3.5.5. Organic acids

Each smoothie sample was prepared as described for soluble sugars (section 3.5.4.) and the analysis was performed as described for malic acid (section 3.3.2.). The detection was performed with the DAD detector programmed for wavelengths of 215 and 245 nm. Organic acids were identified by comparing the retention time and UV-Vis spectrum of the sample peaks with those of commercial standards and subsequently quantified using eight-level calibration curves constructed for each compound (**Table A1)**. The results were expressed as g per 100 mL of filtered smoothie.

3.5.6. Fatty acids

The crude fat obtained after Soxhlet extraction was vortex mixed with 5 mL of methanol/sulphury acid 95%/toluene (2:1:1, *v/v/v*) and transesterified for 12 h in a water bath at 50 ºC with shaking (160 rpm). Then, approximately 3 mL of deionized water were added to obtain phase separation by vortex mixing; the fatty acids methyl ester (FAME) mixture was mixed with 3 mL of diethyl ether by vortex agitation and the upper phase was carefully transferred to a glass vial for dehydration with sodium sulphate anhydrous. The sample was then filtered through a 0.2 µm nylon syringe filter into a 2 mL glass vial.

The analysis was performed in a YOUNG IN Chromass 6500 gas chromatography system (YL Instruments, Anyang, Korea) equipped with a *split/splitless* injector set at 250 ºC and a flame ionization detector (FID) set at 260 ºC. Chromatographic separation was made on a Zebron™ ZB-FAME column (30 m × 0.25 mm, 0.20 µm; Phenomenex, Lisbon, Portugal) operating under the following oven temperature program: initial temperature of 100 ºC, held for 2 min, increase at 10 ºC/min to 140 ºC, followed by a 3 ºC/min ramp to 190 ºC, a 30 °C/min ramp to 260 °C and held for 2 min. Hydrogen was used as carrier gas at the flow rate of 1.2 mL/min.

Fatty acids were identified by comparing the relative retention times of the sample FAME peaks those of the standard, using the Clarity DataApex 4.0 Software (Prague, Czech Republic). The results were expressed as relative percentage (%) of each fatty acid.

3.6. Statistical analysis

The results were expressed as mean ± standard deviation. Differences among samples were assessed using one-way analysis of variance (ANOVA). The fulfilment of the ANOVA requirements was tested by means of the Shapiro Wilk's and the Levene's tests. The dependent variables were compared using Tukey's HSD or Tamhane's T2 multiple comparison tests, when homoscedasticity was verified (p-value > 0.05) or not (p-value < 0.05), respectively. The statistical tests were performed at a 5% significance level using SPSS Statistics (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Furthermore, a linear discriminant analysis (LDA) was performed to compare the different smoothie samples, also considering the variables extract addition and storage time. The stepwise technique and the Wilk's λ test with an F-value of 3.84 for entering and 2.71 for removal of variables were applied.

4. RESULTS AND DISCUSSION

4.1. Quince peel extract characteristics

The quince peel extract prepared to be added to the plant-based smoothie as a natural preservative was characterized regarding its composition of phenolic compounds and malic acid and *in vitro* antioxidant activity *via* lipid oxidation inhibition.

4.1.1. Composition in malic acid and phenolic compounds

The quince peel extract was obtained following an extraction method previously optimized to obtain a bioactive extract rich in malic acid and phenolic compounds. The process yielded about 32.2% (*w/w*) of extract, a value lower than that obtained by Pereira et al. (2023), which can be justified by the lower solid/liquid ratio used in this study.

Regarding malic acid, 71.66 ± 1.26 mg/g extract were quantified, a value in line with the 75.68 ± 2.00 mg/g extract obtained by Pereira et al. (2023). This dicarboxylic acid is used as a food preservative with the number E296. While it is almost as sour as citric acid, it provides a slightly stimulating and smoother sour taste and is beneficial in low-energy drinks, where it masks the unpleasant flavors of some artificial sweeteners (Gurtler & Mai, 2014).

Figure 8 shows the HPLC chromatogram of the phenolic profile of the extract and the chromatographic data used in the tentative identification of the detected compounds are presented in **Table 3**. The phenolic profile of the quince peel extract is similar to that previously described by Othman et al. (2022) and Pereira et al. (2023). Therefore, the detected compounds were tentatively identified by comparing the chromatographic data in **Table 3** with those described in these studies. However, the identity of some peaks was not assigned due to fragmentation issues, which is why the number of identified phenolic compounds is lower than that described by the aforementioned authors. On the other hand, it was possible to detect the previously undetected compound **6**, namely quercetin-3-*O*-hexosyl-dideoxyhexoside. It was identified based on the deprotonated ion $[M-H]^-$ at *m/z* 755 and a MS^2 fragment ion at *m/z* 301 (the quercetin aglycone), corresponding to the loss of one a hexosyl moiety (−162 u) and two deoxyhexosyl units (146 u + 146 u).

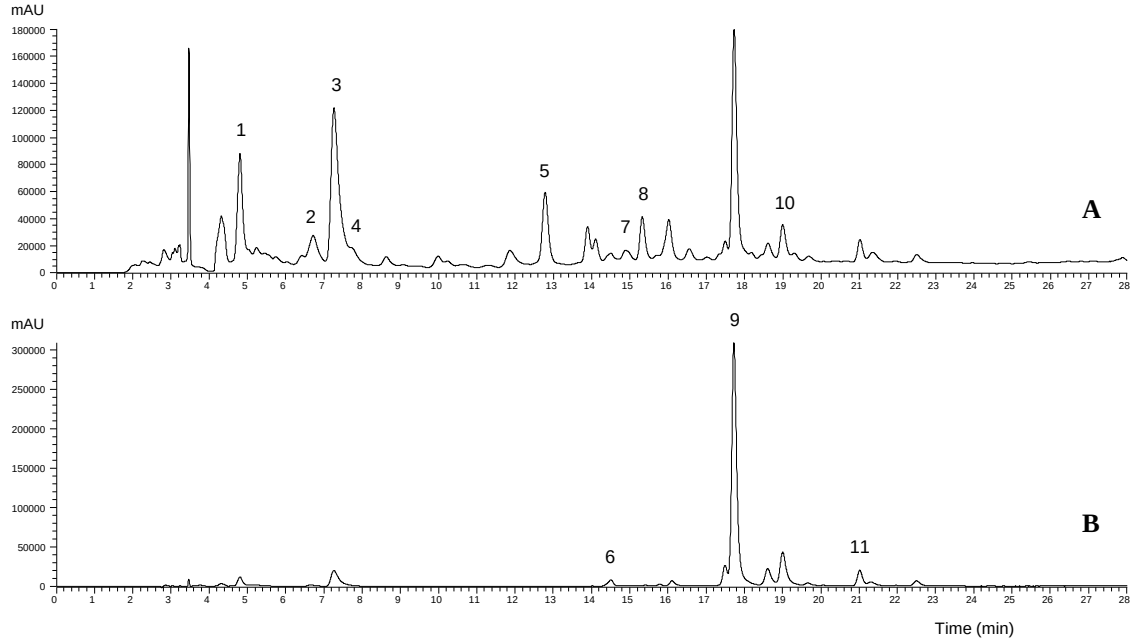


Figure 8 HPLC chromatogram of the phenolic profile of quince peel extract recorded at (**A**) 280 nm and (**B**) 370 nm. Compound identification is presented in **Table 3**.

Table 3 Content of phenolic compounds identified in quince peel extract. The retention time (R*t*), maximum absorption wavelength in the visible region, and deprotonated ion are presented.

Peak	**R*t*** **(min)**	**λ_{max}** **(nm)**	**$[M-H]^-$** **(*m/z*)**	**Tentative identification**	**Content** **(mg/g extract)**
1[A]	4.80	325	353	3-*O*-Caffeoylquinic acid	0.548 ±0.012
2[B]	6.72	292	337	3-*O*-*p*-Coumaroylquinic acid	0.095 ± 0.006
3[A]	7.26	326	353	*cis*-5-*O*-Caffeoylquinic acid	1.093 ± 0.013
4[A]	7.64	323	353	*trans*-5-*O*-Caffeoylquinic acid	0.177 ± 0.004
5[C]	12.78	276	865	β-Type (epi)catechin trimer	0.751 ± 0.017
6[D]	14.50	353	755	Quercetin-3-*O*-hexosyl-dideoxyhexoside	0.482 ± 0.001
7[C]	14.89	280	1153	β-Type (epi)catechin tetramer	0.180 ± 0.008
8[C]	15.33	271	865	β-type (epi)catechin trimer	0.427 ± 0.005
9[D]	17.73	355	609	Quercetin-*O*-deoxyhexoside-hexoside	1.275 ± 0.016
10[C]	19.00	268	863	Procyanidin with A-type linkage	0.580 ± 0.020
11[D]	21.02	357	593	Kaempferol-*O*-deoxyhexosyl-hexoside	0.515 ± 0.007
				Σ Phenolic acids	1.91 ± 0.03
				Σ Flavan-3-ols	1.94 ± 0.01
				Σ Flavonols	2.27 ± 0.02
				Σ Phenolic compounds	6.12 ± 0.02

Superscript letters indicate the standards used in quantification: A: chlorogenic acid; B: *p*-coumaric acid; C: catechin; and D: quercetin-3-*O*-(6-acetylglucoside). The quantification results are presented as mean ± standard deviation.

As shown in **Table 3**, the phenolic fraction consisted of flavonols (*O*-glycosylated quercetin and kaempferol derivatives), flavan-3-ols (two β-type (epi)catechin trimers and one tetramer and a procyanidin with A-type linkage), and phenolic acids (caffeoylquinic acids). Quercetin-*O*-deoxyhexoside-hexoside (compound **9**, 1.28 mg/g extract; whose aglycone is represented in **Figure 9A**), β-type (epi)catechin trimer (compounds **5** and **8**, 1.18 mg/g extract), and *cis*-5-*O*-caffeoylquinic acid (**Figure 9B**; compound **3**, 1.09 mg/g extract) were the most abundant phenolic compounds, which agreed with previous reports (Othman et al., 2022; Pereira et al., 2023). Although Pereira et al. (2023) reached a total of 8.1 mg of phenolic compound per gram of quince peel extract with the same extraction method, it is worth noting that a higher number of phenolic compounds was quantified by the authors, which could justify this difference. Despite this, these other phenolic compounds may be present in our extract because the peaks identified in previous works also appear in the chromatographic profile (**Figure 8**), but their identity could not be confirmed due to the fragmentation problem. In the case of flavonol glycosides, its concentration was higher in our extract than in those of Pereira et al. (2023). In turn, the content (4.27–4.70 mg/g extract) of phenolic compounds described by Othman et al. (2022) is lower than that quantified in the present study, due to the lower levels of phenolic acids and flavonol glycosides obtained by the authors.

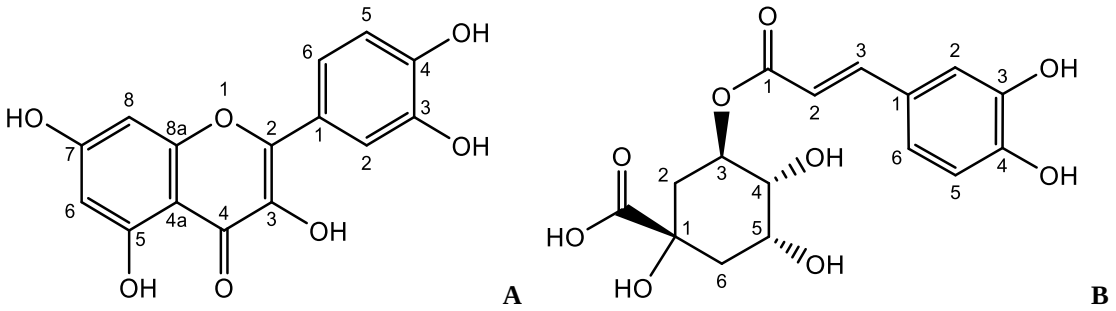


Figure 9 Chemical structures of (**A**) quercetin aglycone and (**B**) 5-*O*-caffeoylquinic acid.

Caffeoylquinic acids have been used in the food industry as natural preservatives to help extend the shelf-life of foods and to protect them from oxidation (Santana-Gálvez et al., 2017). These antioxidants are substrates of the catecholase activity of polyphenol oxidase and, therefore, can influence oxidation and color change processes during food manufacture (Maghsoudlou et al., 2019). The antioxidant activity of flavonoids is also well documented in the literature and catechins stand out among the most powerful compounds of this class (Hassanpour & Doroudi, 2023).

4.1.2. *In vitro* antioxidant activity

The antioxidant activity of quince peel extract was evaluated by two cell-based *in vitro* assays and the results are shown in **Table 4**. The lower the EC_{50} and IC_{50} values used to express the results, the greater the antioxidant activity of the extract.

Table 4 Antioxidant activity of quince peel extract and trolox *via* TBARS formation inhibition and oxidative hemolysis inhibition.

		Quince peel extract	Trolox
TBARS formation inhibition (EC_{50}, µg/mL)		89.31 ± 0.55	5.39 ± 0.28
Hemolysis inhibition (IC_{50}, µg/mL)	Δ*t* 60 min	93.31 ± 6.51	21.47 ± 0.17
	Δ*t* 120 min	247.58 ± 13.74	43.49 ± 0.25

The results are presented as mean ± standard deviation.

The TBARS assay is based on a Fenton-like system to induce lipid peroxidation *in vitro* (Bedlovičová et al., 2020) and provided information on the ability of quince peel extract to inhibit the formation of reactive compounds such as malondialdehyde (MDA). This aldehyde resulted from the oxidation of unsaturated fatty acids from pig brain tissue used as substrate and it was quantified spectrophotometrically at 532 nm after the formation of a pink chromogen with TBA, as illustrated in **Figure 10**.

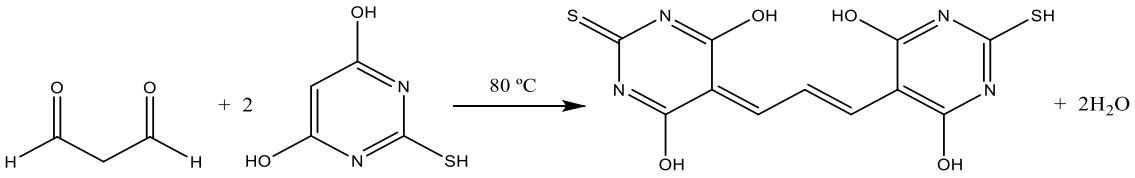
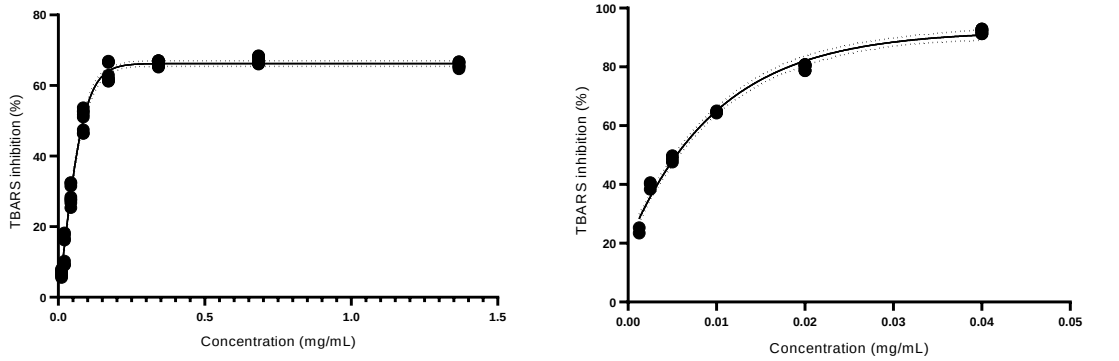


Figure 10 Reaction of malondialdehyde (MDA) with two molecules of thiobarbituric acid (TBA) forming a pink chromogen (MDA-TBA) and two water molecules.

As presented in **Table 4**, an EC_{50} value of 89.31 µg/mL was obtained for quince peel extract, while a lower EC_{50} value of 5.39 µg/mL was obtained for trolox, the commercial antioxidant used as a positive control. These values were calculated from the graphs of inhibition percentage against extract or trolox concentration shown in **Figure 11A** and **B**, respectively. Compared to the literature, the EC_{50} value obtained in this study for the extract is higher than that (EC_{50} of 26 µg/mL) described for a malic acid-rich extract obtained under the same extraction conditions, but it is comparable with that (EC_{50} of 83 µg/mL) obtained for a phenolic-rich extract, both obtained from quince peel through optimized extraction processes targeting distinct constituents (Pereira et al., 2023). A

lower IC_{50} value (60.3 µg/mL) was also reported for a hydroethanolic extract obtained by a routine solid-liquid extraction method from this quince by-product (Othman, 2022).



A **B**

Figure 11 Graphs showing percentage inhibition of TBARS with changing concentration of (**A**) malic acid-enriched extract and (**B**) Trolox, which were used to calculate the EC_{50} values (µg/mL). The well dilution factor was considered when calculating the concentrations shown in the graphs.

The IC_{50} values presented in **Table 4** for the oxidative hemolysis inhibition assay translate the extract and trolox concentrations required to protect 50% of the erythrocyte population from the damage caused by AAPH-derived free radicals for 60 and 120 min. These IC_{50} were calculated after determining the Ht_{50} values from the hemolysis curves of each tested extract and trolox concentration shown in **Figure 12A** and **B**, respectively, and subsequently subtracting the Ht_{50} value of the negative control (PBS) to obtain the delayed time of hemolysis (Δt). Then, the Δt values were correlated with the extract and trolox concentrations to calculate the IC_{50} value for the two periods of time (**Figure 13**).

The analysis of the results showed that 93.31 and 247.58 µg/mL are the quince peel extract concentrations necessary to protect the erythrocyte population for Δt of 60 and 120 min, respectively. These IC_{50} values are somewhat comparable with those previously reported for the antihemolytic activity of a malic acid-rich extract (105 µg/mL for a 60 min Δt and 234 µg/mL for a 120 min Δt) (Pereira et al., 2023) and a hydroethanolic extract (115 µg/mL for a 60 min Δt and 230 µg/mL for a 120 min Δt) obtained by a routine method (Othman, 2022) from quince peel. It is also worth noting that this assay monitored the antioxidant effect over time, as it depends on factors such as the short-term and long-term reaction kinetics of antioxidant compounds and their reaction rate with specific radicals (Antolovich et al., 2002). Hence, some antioxidants can react faster and become depleted in the reaction system, while others may offer prolonged protection.

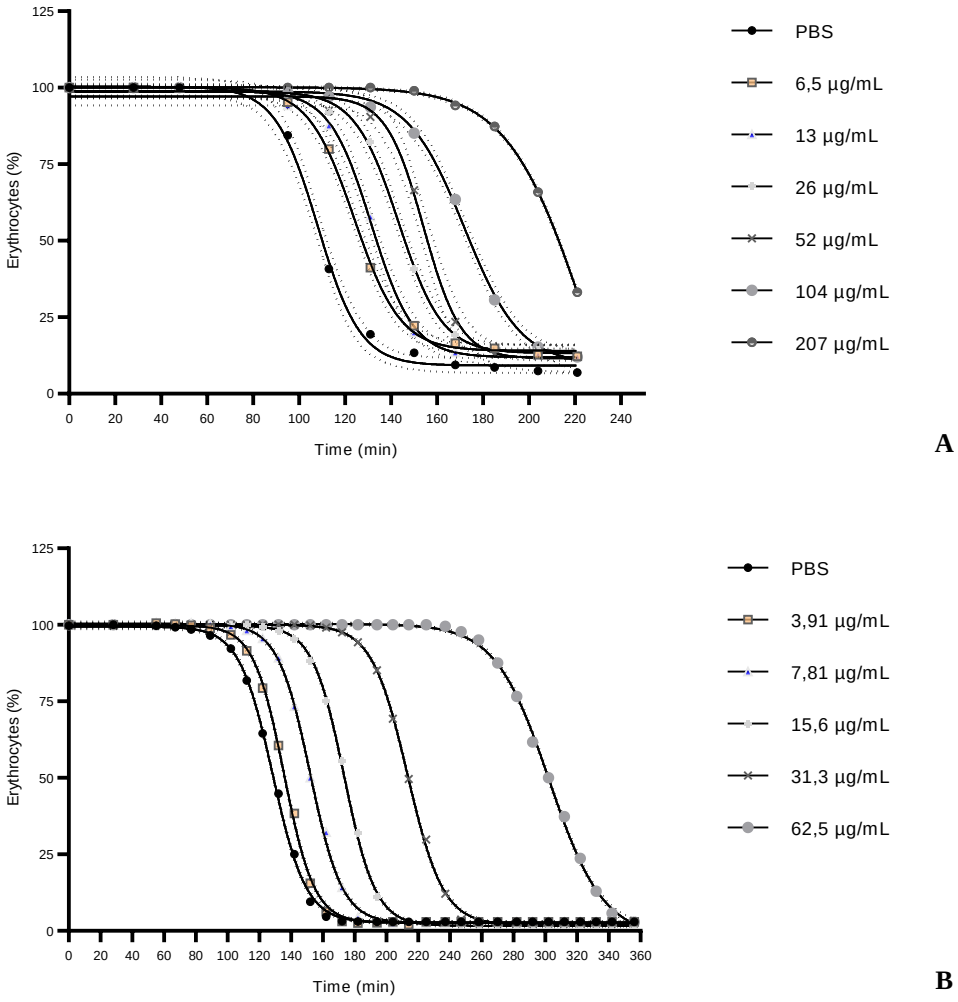


Figure 12 Hemolysis curves of each concentration tested of (**A**) quince peel extract and (**B**) trolox, for which Ht_{50} (min) values were determined using GraphPad Prism® 8. The well dilution factor was considered when calculating the concentrations shown in the graphs.

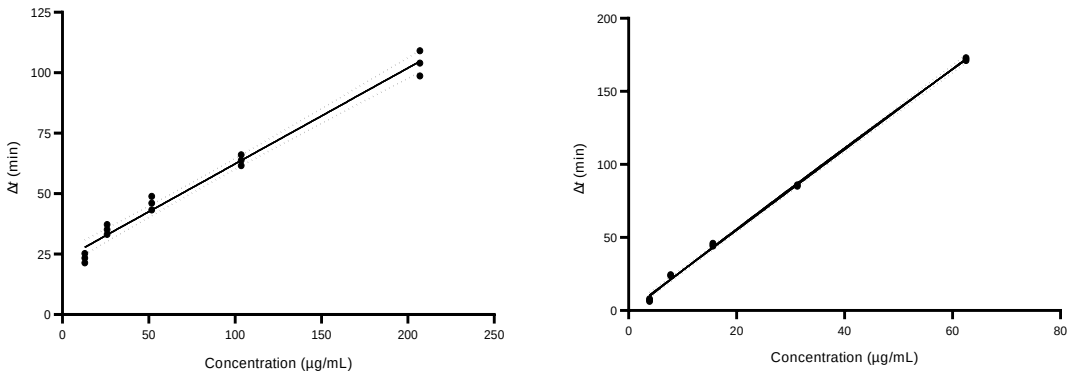


Figure 13 Linear correlations between Δ*t* values and tested concentrations of (**A**) extract and (**B**) trolox and respective calibration curves ($y = 0.3765x + 25.722$; $R^2 = 0.9994$ and $y = 2.7625x - 0.2833$; $R^2 = 0.9987$) used to calculate the IC_{50} values.

Pereira et al. (2023) compared the antioxidant activity of quince peel extracts with that of calcium ascorbate (E223) and sodium metabisulfite (E302) and found that the extracts had a greater antioxidant capacity than these food additives used as antioxidants. These quince peel extracts, and especially the malic acid-enriched extract, have also been reported to have antimicrobial activity against foodborne bacteria and fungi, standing out in some way when compared with the synthetic food additives sodium benzoate (E211) and potassium metabisulfite (E224) against some microorganisms, such as *Bacillus cereus*, *Staphylococcus aureus*, *Enterobacter cloacae*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella* Typhimurium and *Aspergillus* spp. The antibacterial activity of organic acids may be partially related to their ability to diffuse freely across bacterial membranes in their uncharged state and then to dissociate inside the bacterial cell (Bushell et al., 2019). Overall, these findings suggested that quince peel extract could be a natural alternative to slow down oxidative and microbial degradation phenomena in foods and extend their shelf-life. Therefore, the following sections of this master dissertation describe the suitability of quince peel extract to preserve quality attributes of a plant-based smoothie.

4.2. Smoothies' quality and stability during refrigerated storage

The implementation of adequate preservation methods is the main challenge of the smoothie production industry, so that the shelf-life of the product increases without using artificial additives or negatively affecting the organoleptic and nutritional characteristics. Therefore, after producing the fruit and vegetable smoothie formulation, with and without added bioactive quince peel extract, one group was immediately analyzed (day 0) and the remaining samples were stored at 4 ºC and analyzed after 7, 14, 21, 35, and 60 days.

4.2.1. pH, TSS, color, and density

As shown in the line chart of **Figure 14**, the initial pH of the plant-based smoothie formulations was ≈ 3.61 and decreased to 3.50 during the 60 days of refrigerated storage. Smoothie samples with 35 or more days of storage presented a pH value statistically different (p-value < 0.05) from that of the fresh smoothies. Furthermore, control samples showed greater acidification on day 14 than those with bioactive extract. Despite this, the pH value of samples with and without added extract did not show significant differences (p-value > 0.05) at subsequent or previous storage times.

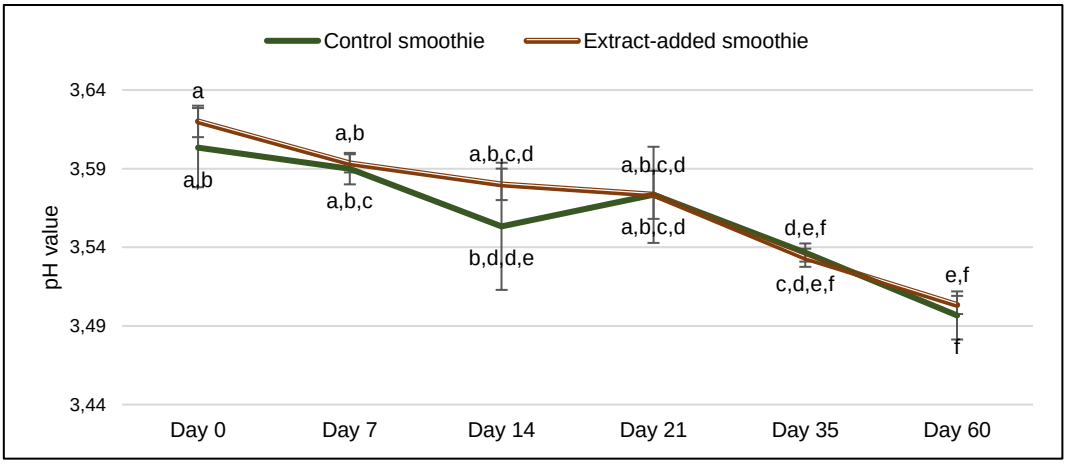


Figure 14 pH values of the plant-based smoothie, with and without added quince peel bioactive extract, during refrigerated storage. Different letters indicate statistically significant differences (*p*-value < 0.05) between samples.

The pH values of our smoothie samples are slightly lower than that the pH 3.93 of a smoothie prepared with orange juice (59%), apples (15%), carrots (15%), beet greens (6%), and beet stems (5%), while the pH of the same smoothie added with citric acid presented a pH value of 3.54 (which evidenced the acidifying effect of this natural antimicrobial compound) (Nieva et al., 2022). In general, the pH of plant-based smoothies tends to be acidic. Razola-Díaz et al. (2022) reported pH values ranging from 3.31 to 4.06 for different smoothies available on the Spanish market. Castillejo et al. (2016) studied smoothie formulations containing tomato, red pepper, broccoli, carrot, and spices and the pH of these fresh smoothies was around 4.3 and did not change significantly after thermal treatment or during storage either at either 5 or 20 °C. However, Aderinola (2018) reported a pH towards to neutral (6.3–6.51) for fruit smoothies supplemented with *Moringa oleifera* leaves. The acidic pH is useful to ensure microbiological stability and, together with pasteurization treatments, can prevent the proliferation of microorganisms and increase the food shelf-life (Han et al., 2022). Ingredients such as apples, oranges, and red fruits are suitable to help reduce the pH of smoothies (Razola-Díaz et al., 2022).

The smoothie processing involves the breakdown of plant parenchyma, which leads to a dispersed solution consisting in a liquid phase and a solid phase composed of insoluble solids. The TSS indicates the concentration of sugars, organic acids, and other soluble compounds in a solution and is often used to determine the sweetness of fruits, vegetables, and other plant-based products. At 20°C, the Brix is usually considered equivalent to the percentage of sucrose in the solution (10 °Brix equivalent to 10% sugar). The TSS of the smoothie samples during refrigerated storage is shown in **Figure 15**. The

TSS of both formulations, with and without added extract, evolved from 12.33 ºBrix at the beginning of the experiment to 12.27–12.30 ºBrix at day 60. In general, there were no major changes during shelf-life. Even so, the sample with added extract showed a significantly (p-value < 0.05) higher content on day 7 than at time 0 and from day 21 onwards. These results could be somehow related to the added extract, which has a high concentration of soluble sugars in addition to malic acid (Pereira et al., 2023). Furthermore, the applied thermal pasteurization treatment may have triggered the gradual release of intracellular soluble solids (Castillejo et al., 2016).

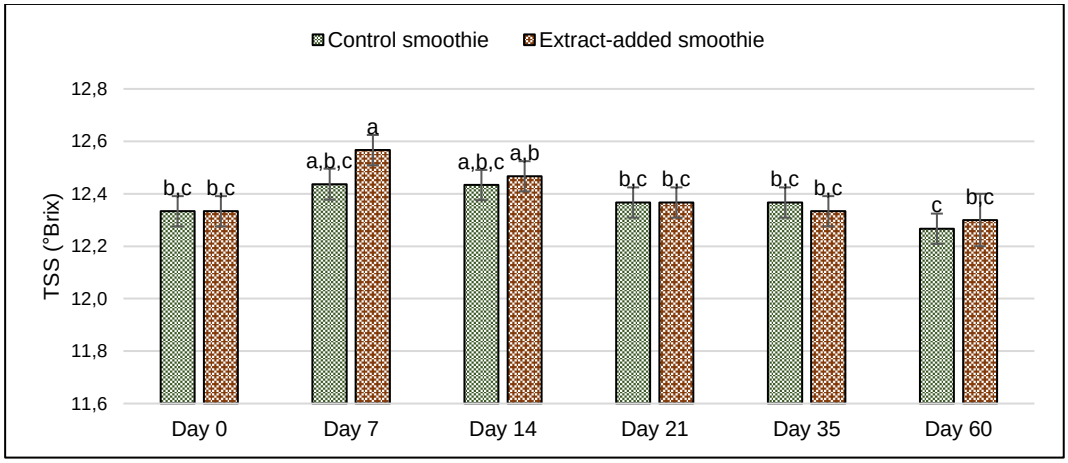
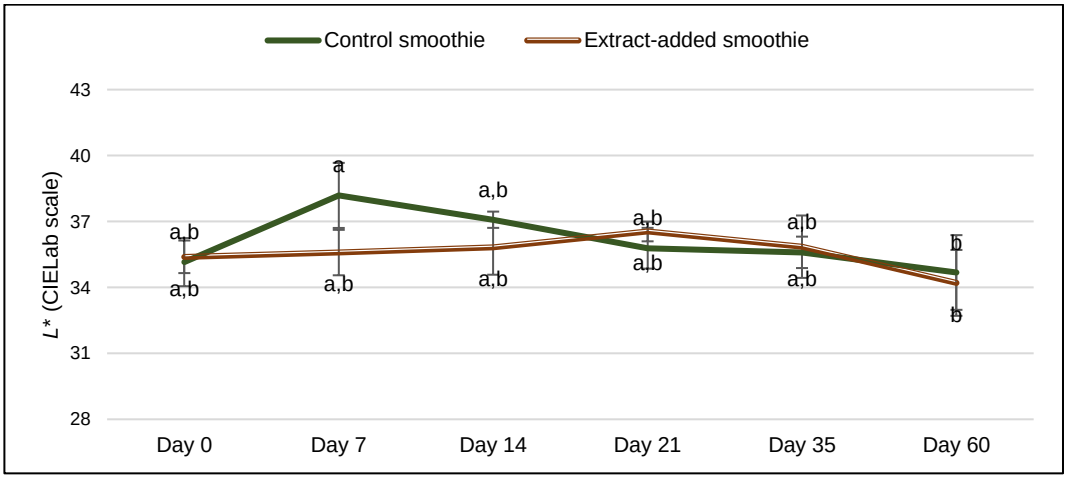


Figure 15 Total soluble solids (TSS) content of the plant-based smoothie, with and without added quince peel bioactive extract, during refrigerated storage. Different letters indicate statistically significant differences (p-value < 0.05) between samples.

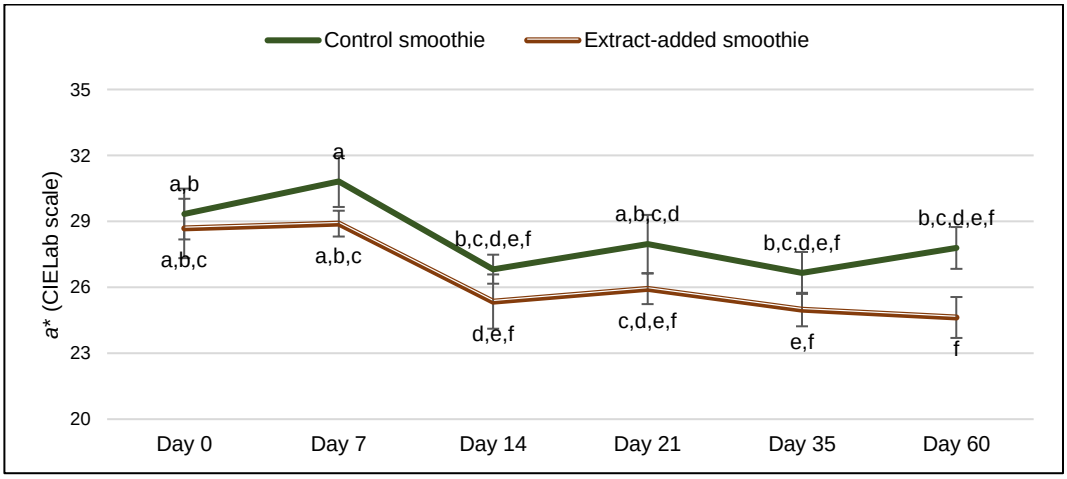
The TSS content of the plant-based smoothie prepared in this study is higher than that described for other formulations congaing tomato, carrots, pepper, and broccoli (7.07–8.37 °Brix) (Castillejo et al., 2016) and orange juice, apples, carrots, beet greens, and beet stems (10.60 ºBrix, which increased to 11.33 with the addition of citric acid) (Nieva et al., 2022). Therefore, the TSS content is related to the type and proportion of fruits and vegetables used in the preparation of the smoothie. In our formulation, apples and blueberries may have contributed to the high TSS value. According to Aprea et al. (2017) and Hera et al. (2023), the TSS content can vary from about 9.5 to 15.8 ºBrix in apples and from 11.30 to 19.22 ºBrix in blueberries, respectively, with sweeter varieties having higher TSS levels.

The color resulting from different fruit and vegetable pigments, such as carotenoids, anthocyanins, chlorophylls, and betalains, is an important parameter used by consumer to judge the quality of foods, especially plant-based beverages like smoothies. The lightness

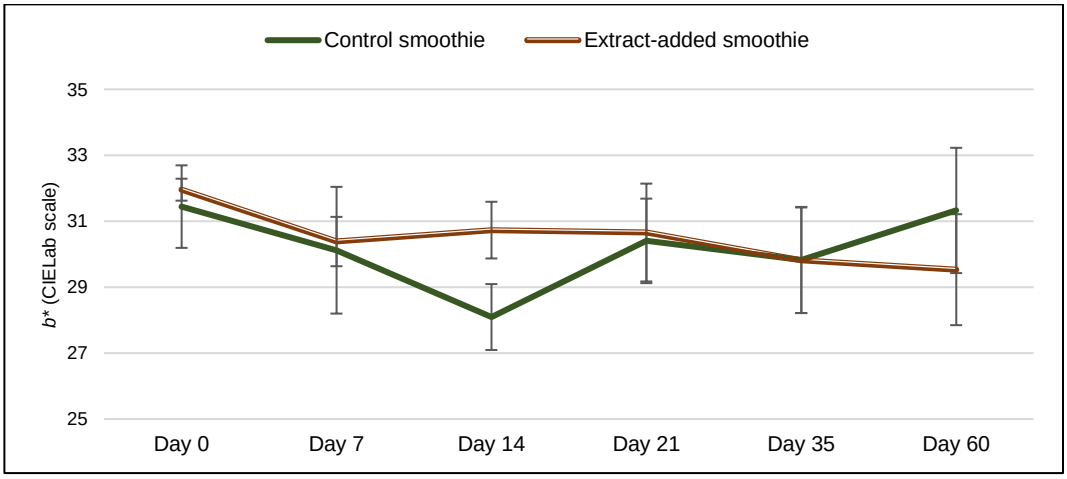
(*L**), redness (*a**) and yellowness (*b**) values of the plant-based smoothie, with and without added quince peel extract, during refrigerated storage are shown in **Figure 16**.



A



B



C

Figure 16 Lightness (*L**), redness (*a**) and yellowness (*b**) of the plant-based smoothie, with and without added quince peel bioactive extract, during refrigerated storage. Different letters indicate statistically significant differences (p-value < 0.05) between samples.

In general, the *L** value of the smoothie samples remained relatively constant during storage. However, there was a statistically significant difference between the control smoothie at day 7 and both smoothie samples at the end of storage (**Figure 16A**). For the *a** parameter, a significant decrease was observed between days 7 and 21 in both control and extract-added smoothies (**Figure 16B**). Although the control smoothie appeared to be redder than the extract-added smoothie over shelf-time, the extract variable did not cause statistically significant differences. In turn, the *b** parameter did not change among samples (**Figure 16C**), thus presenting the same shade of yellow.

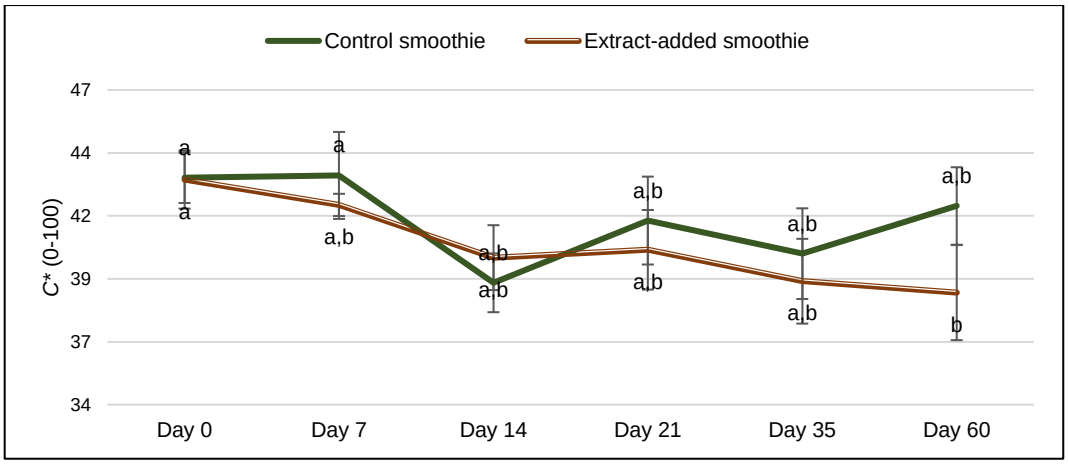
To represent the color of smoothies, the *L**, *a**, and *b** values were converted into RGB values in order to obtain the colors shown in **Table 5**. This visual representation demonstrated that the slightly lighter color of the control samples at day 7 mentioned above is noticeable by the human eye, as well as other small variations in color tone.

Table 5 RGB (red, green, blue) color model of the plant-based smoothie, with and without added quince peel bioactive extract, during refrigerated storage.

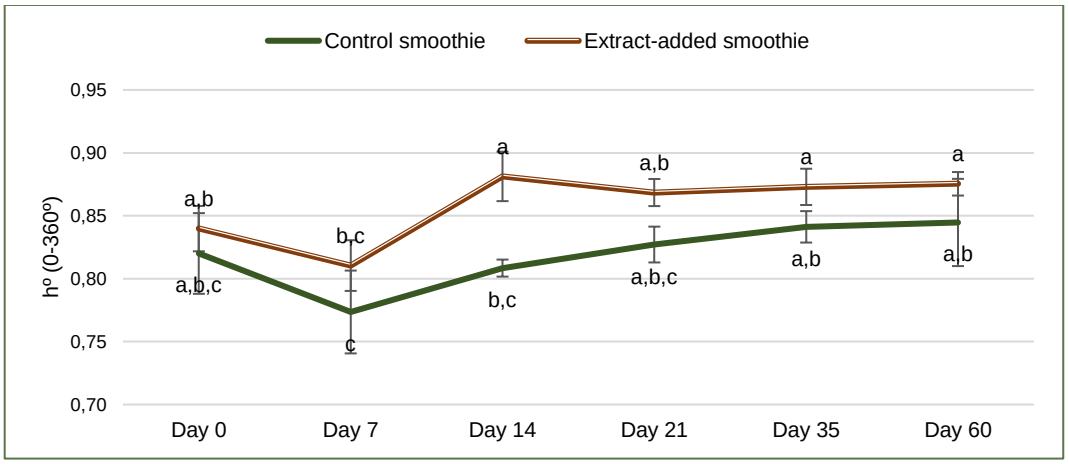
	Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 35**	**Day 60**
Control smoothie						
Extract-added smoothie						

The *a** and *b** parameters were also used to calculate the saturation index (*C**) and the hue angle (hº) and results are illustrated in **Figure 17**. The term "saturation" refers to the color intensity or vividness, with it being highly saturated if it is vibrant and pure and less saturated if it appears more muted or washed out. In turn, the hue angle helps describe the overall color tone of a product (e.g., red, green, and blue) and is measured in degrees, ranging from 0 to 360º. Together, these parameters help characterize and quantify the color of foodstuff, which is important in food manufacturing and quality control. As shown in **Figure 17A**, the *C** value of the extract-added smoothie gradually decreased during storage, and at day 60, the value was significantly lower (p-value < 0.05) compared to the initial sample. On the other hand, the control samples showed some fluctuations, particularly at days 14 and 60, although the general trend also appears to be decreasing. In the case of hº, values close to 0º indicate a red color. As shown in **Figure 17B**, there was a slight decreasing trend until day 7, followed by an increase, more pronounced in the extract-added smoothie, and then the hue values remained constant until day 60. In general, the hue angle of the extract-added smoothie samples was slightly higher than that

of the control, but this difference was only significant (p-value < 0.05) on day 14. Overall, these results show that the hue/red of both smoothie formulations at day 60 did not differ from the initial samples, while the color saturation also did not change significantly in any formulation during the first 35 days of refrigerated storage.



A



B

Figure 17 Evolution of the (**A**) saturation index (C*) and (**B**) hue angle (h_{ab}) of the plant-based smoothie, with and without added quince peel bioactive extract, during refrigerated storage. Different letters indicate statistically significant differences (p-value < 0.05) between samples.

Figure 18A and **B** illustrates the total color difference (ΔE*) caused by the variables "storage time" and "extract addition", respectively. The control smoothie underwent the greatest color changes in relation to the initial sample during the first 14 days of storage, while the color of the extract-added smoothie was better preserved during the same period of time and the greatest difference was observed at day 60 (**Figure 18A**). This effect somehow highlights the preservative potential of the antioxidant extract. Furthermore, the extract did not induce major color changes in the smoothie at time 0, but these were more noticeable at subsequent times, especially on the last day of storage (**Figure 18B**).

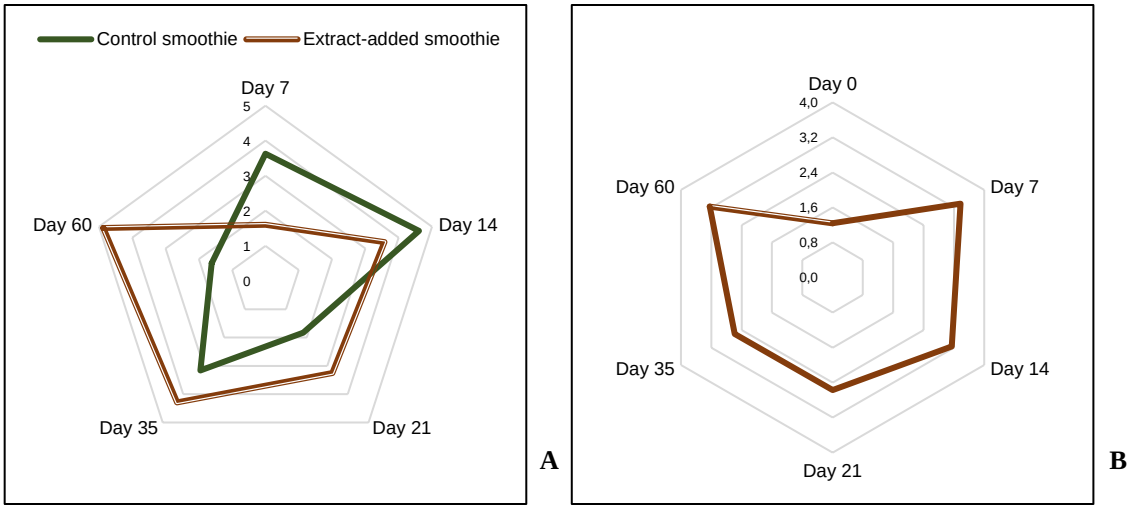


Figure 18 Total color difference (ΔE^*) between plant-based smoothie samples when considering the variables (**A**) "storage time" and (**B**) "extract addition".

Density is another important quality parameters because it contributes to the texture and mouthfeel of smoothies. A denser smoothie may be more substantial and satisfying and have a higher nutrient content, potentially leading to increased satiety and a greater feeling of fullness. As shown in **Table 6**, all smoothie samples had similar density, which was not significantly affected (p-value > 0.05) by either the addition of bioactive extract or the storage time. A comparable density of 1.06 g/cm^3 was previously reported for smoothies prepared with orange, papaya and melon juice, carrot puree, and soymilk or skimmed milk submitted to pasteurization by heat (80 ºC for 3 min) or high pressure (550–650 MPa for 3 min at 20 ºC) (Andrés et al., 2016).

Table 6 Density of the plant-based smoothie, with and without added quince peel bioactive extract, at time 0 and after 60 days of refrigerated storage.

	Control smoothie		**Extract-added smoothie**	
	Day 0	**Day 60**	**Day 0**	**Day 60**
Density (g/cm^3)	1.05 ± 0.01	1.06 ± 0.02	1.04 ± 0.91	1.05 ± 0.01

The results are presented as mean ± standard deviation.

4.2.2. Centesimal composition and energy value

The centesimal composition of the plant-based smoothie, with and without added quince peel bioactive extract, at time 0 and after 60 days of refrigerated storage is shown in **Table 7**. All smoothies had a moisture content of around 82 g/100 mL. Carbohydrates were the most abundant nutrients, with about 10.47 g/100 mL, followed by total dietary

fiber (TDF) and ash with approximately 1.1 g/100 mL. The protein content was relatively low, not exceeding 0.63 g/100 mL. All of these centesimal constituents of the smoothie remained stable with the addition of bioactive extract and during the shelf-life, with no statistical differences (p-value > 0.05) between samples being observed. In turn, crude fat was the constituent detected in the lowest quantity in the smoothie samples (≤ 0.064 g/100 mL), and those with added extract tended to have a slightly lower content. Furthermore, a 100 mL portion of smoothie did not exceed 46.34 kcal, making it a beverage suitable for low-calorie diets. This value is between 38 and 82 kcal generally reported for plant-based smoothies (Razola-Díaz et al., 2022). In addition to dietary fiber and minerals, smoothies may also contain water-soluble vitamins and bioactive compounds such as phenolic compounds, carotenoids, and betalains (Septembre-Malaterre et al., 2018).

Table 7 Centesimal composition and energy value of the plant-based smoothie, with and without added quince peel bioactive extract, at time 0 and after 60 days of refrigerated storage.

	Control smoothie		**Extract-added smoothie**	
	Day 0	**Day 60**	**Day 0**	**Day 60**
Moisture (g/100 mL)	82.17 ± 0.38	81.51 ± 0.38	82.47 ± 0.35	82.21 ± 0.10
Protein (g/100 mL)	0.61 ± 0.01	0.62 ± 0.02	0.64 ± 0.04	0.66 ± 0.05
Fat (g/100 mL)	0.079 ± 0.003 [a]	0.079 ± 0.002 [a]	0.049 ± 0.002 [b]	0.049 ± 0.001 [b]
Ash (g/100 mL)	1.06 ± 0.12	1.15 ± 0.03	1.07 ± 0.01	1.13 ± 0.02
TDF (g/100 mL)	1.06 ± 0.04	1.10 ± 0.01	1.01 ± 0.08	1.02 ± 0.03
Carbohydrates (g/100 mL)	10.58 ± 0.46	10.24 ± 0.40	10.65 ± 0.33	10.43 ± 0.08
Energy (kcal/100 mL)	47.59 ± 1.82	46.34 ± 1.65	47.62 ± 1.31	46.84 ± 0.20

The results are presented as mean ± standard deviation. In each line, different letters indicate statistically significant differences (p-value < 0.05) between samples.

Comparing our plant-based smoothie with others described in the literature in terms of nutrient composition becomes difficult, as the fruit and vegetable composition of these beverages can be very variable. For example, Aderinola (2018) reported variable moisture (49.24–78.62%), protein (5.47–19.37%), fiber (5.14–9.39%), ash (1.01–9.71), crude fat (0.72–1.86%), and carbohydrate (3.65–16.99%) contents for pineapple (43.5–45%), banana (38.5–40%), and apple (13.5–15%) smoothies supplemented with up to 4.5% of *Moringa oleifera* leaves as a source of protein. The higher levels of these macronutrients compared to those of our smoothie formulation may be partially related to the lower

moisture content. Furthermore, the author used unpeeled apples, which somewhat contributed to increasing the dietary fiber content.

In a previous study, Razola-Díaz et al. (2022) evaluated the nutritional quality of smoothies available on the Spanish market and concluded that these beverages stand out mainly for their carbohydrate content (7.9–14.6 g/100 mL), among which simple free sugars can correspond to 100% of carbohydrates. Additionally, smoothies containing orange, mango, and banana were those associated with a higher carbohydrate content. As verified in our study, the smoothies were not characterized by a high fiber content. This constituent ranged from 0.3–1.8 g/100 mL, apples, and raspberries were the ingredients that contributed the most fiber. In turn, the protein (0.0–0.9 g/100 mL) and fat (0.0–3.2 g/100 mL) content was generally insignificant. In fact, fruits and vegetables are generally not characterized as raw materials rich in these macronutrients. In the case of crude fat, the highest levels were related to the presence of coconut, such as coconut milk, coconut drink or coconut-based preparations. Therefore, the smoothie formulation prepared in this study has a similar composition to those on the market.

4.2.3. Mineral elements composition

Mineral elements play a crucial role in maintaining various biological functions of the human body (Gharibzahedi & Jafari, 2017). Therefore, the combination of a variety of mineral-rich ingredients into smoothies can be a tasty and nutritious way to obtain essential mineral elements. **Table 8** presents the mineral composition of the prepared smoothie formulations at time 0 and after 60 days of refrigerated storage. Potassium was by far the most abundant element, with 100 mL of smoothie providing about 7.8% of the dietary reference intake (DRI) of this mineral element (European Parliament & Council of the European Union, 2011), which contributes to the normal functioning of the nervous system, muscle function, and the maintenance of blood pressure (Haddy et al., 2006).

According to the WHO, high sodium intake (> 2 g/day, equivalent to 5 g salt/day) and inadequate potassium intake (< 3.5 g/day) contribute to high blood pressure and increase the risk of heart disease and stroke (World Health Organization, 2022). As shown in **Table 8**, a sodium content of about 14.69 mg/100 mL was quantified in the smoothie samples and those with added extract had a lower level at day 60 compared to the control at time 0. Thus, the stipulated limit of 2 g/day was far from being exceeded with 100 mL of smoothie. Furthermore, the sodium/potassium ratio was very low (≈ 0.09).

Table 8 Mineral element composition of the plant-based smoothie, with and without added quince peel bioactive extract, at time 0 and after 60 days of refrigerated storage.

	Control smoothie		**Extract-added smoothie**	
	Day 0	**Day 60**	**Day 0**	**Day 60**
Potassium, K (mg/100 mL)	154.0 ± 3.2	156.9 ± 3.8	157.9 ± 0.9	157.7 ± 1.4
Sodium, Na (mg/100 mL)	15.44 ± 0.91 [a]	14.59 ± 0.37 [a,b]	14.84 ± 0.25 [a,b]	13.90 ± 0.33 [b]
Calcium, Ca (mg/100 mL)	4.67 ± 0.11	4.65 ± 0.11	4.76 ± 0.13	4.53 ± 0.10
Magnesium, Mg (mg/100 mL)	9.20 ± 0.62	8.54 ± 0.12	9.11 ± 0.33	8.67 ± 0.12
Iron, Fe (µg/100 mL)	88.66 ± 4.01 [c]	122.6 ± 4.9 [a]	104.6 ± 5.4 [b]	96.34 ± 7.70 [b,c]
Manganese, Mn (µg/100 mL)	109.0 ± 12.8	120.3 ± 17.5	120.0 ± 26.3	96.54 ± 3.52
Copper, Cu (µg/100 mL)	48.33 ± 3.00	52.66 ± 2.03	50.58 ± 0.12	49.59 ± 1.00
Zinc, Zn (µg/100 mL)	50.63 ± 12.33	44.25 ± 7.99	54.88 ± 0.57	44.33 ± 4.86

The results are presented as mean ± standard deviation. In each line, different letters indicate statistically significant differences (p-value < 0.05) between samples.

A 100 mL serving of the prepared fruit and vegetable smoothie contained about 2.4% of the DRI for magnesium (which is 375 mg/day) (European Parliament & Council of the European Union, 2011), a mineral involved in hundreds of enzymatic reactions and essential for protein synthesis, neuromuscular conduction, cardiac contractility, energy metabolism, and immune system function (Al Alawi et al., 2021). In turn, the calcium content did not exceed 4.76 mg/100 mL (**Table 8**). The levels of this element could have been higher if green vegetables had been included in the formulation.

Regarding trace elements, the smoothie contained interesting levels of manganese and copper, since a 100 mL serving contained 5.7 and 5.0% of the DRI for these elements (European Parliament & Council of the European Union, 2011). Manganese is involved in the formation and maintenance of connective tissue and vital for proper and normal growth of human bone structure, while copper is a structural part in many enzymes and essential for the proper functioning of organs and metabolic processes (Mehri, 2020). In turn, the iron and zinc levels were around 103 and 48.5 µg/100 mL, respectively (**Table 8**). However, the contributions to the DRI of these elements did not exceed 1%.

This study showed that most of the mineral elements quantified in the smoothie was not affected by the addition of extract or storage time. Their concentrations were higher than those described for other plant-based smoothie formulations (Aderinola, 2018). Furthermore, the sodium/potassium ratio was higher (0.63–0.94) than that obtained for our formulation (Castillejo et al., 2016).

4.2.4. Soluble sugars composition

Soluble sugars are one of the main components of fruit juices and are responsible for the sweetness of smoothies, therefore being an important quality attribute. **Figure 19** shows a representative HPLC chromatogram of the soluble sugars in a filtered smoothie sample, where it is possible to observe the peaks corresponding to fructose, glucose, and sucrose. Fructose was the most abundant soluble sugar, followed by glucose and lower amounts of sucrose. The levels of these molecules quantified in each sample during storage are shown in **Figure 20**. There was a tendency for fructose and glucose levels to increase and sucrose levels to decrease during storage, as also illustrated in **Figure 19**. With the exception of formulations at time zero, smoothie samples with added extract tended to have higher levels of both reducing sugars than control samples. For fructose, this difference was significant (p-values < 0.05) on days 7 and 14 (**Figure 20A**), while for glucose it was significant on days 14 and 21 (**Figure 20B**). According to Pereira et al. (2023), fructose is by far the most abundant soluble sugar in quince peel extract, followed by glucose and minor amounts of sucrose. However, the addition of the bioactive extract to the smoothie formulations did not change the content of any free soluble sugar, since the day zero samples did not differ significantly (p-values > 0.05) from each other.

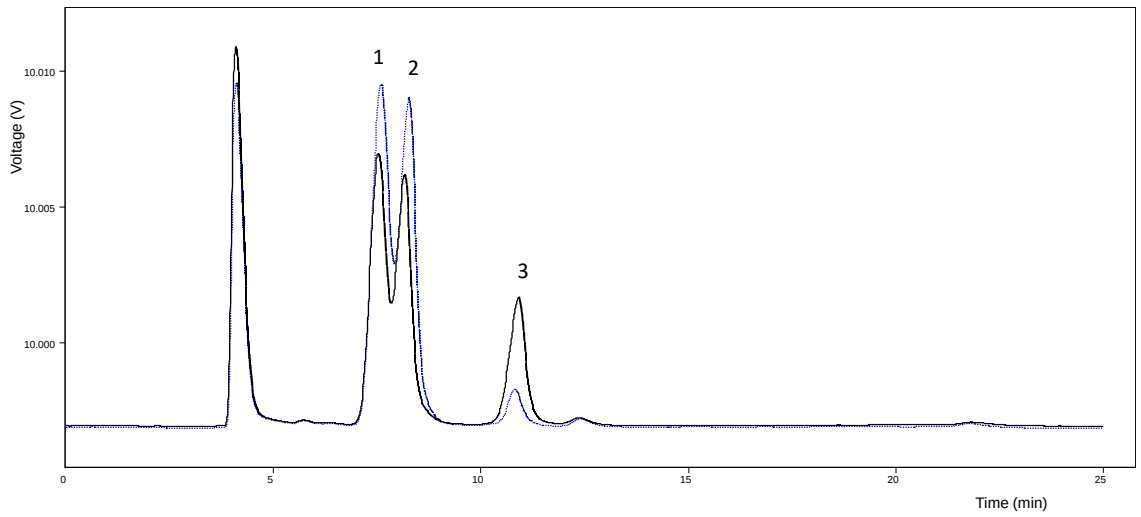


Figure 19 HPLC chromatogram of soluble sugars in extract-added smoothie on day 0 (—) and day 60 (·····) of refrigerated storage. 1 – fructose; 2 – glucose; and 3 – sucrose.

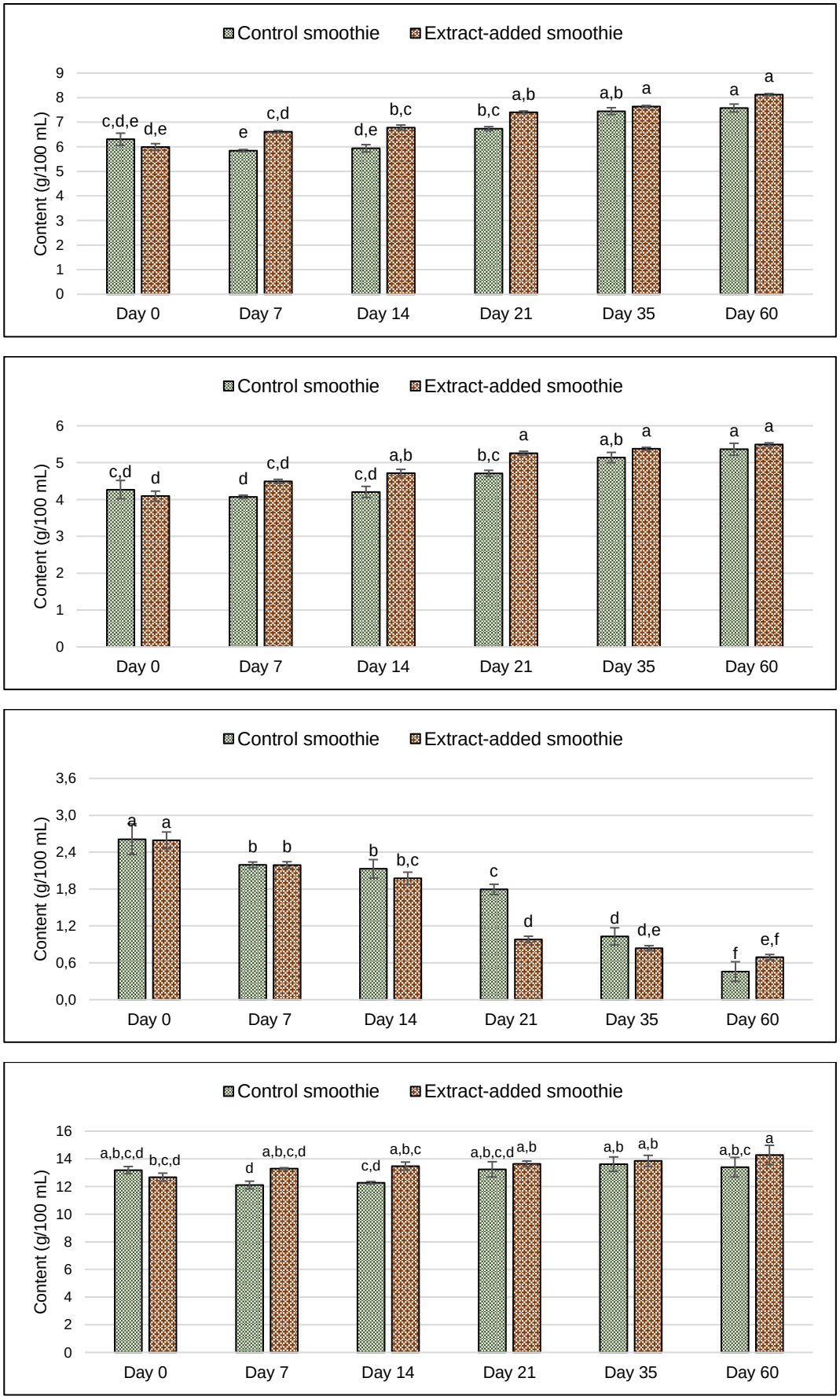


Figure 20 Content of (**A**) fructose, (**B**) glucose, (**C**) sucrose, and (**D**) total soluble sugars in the filtered smoothie samples, with and without added quince peel extract, during refrigerated storage.

The reduction in sucrose concentrations observed in **Figure 20C** may be related to its conversion into organic acids thought microbial metabolic activity, or to its hydrolysis into fructose and glucose by sucrose-metabolizing enzymes such as invertase (also known as β-fructofuranosidase) (Kaddumukasa et al., 2017; Nieva et al., 2022). In fact, the variations in sucrose content were opposite to those in fructose and glucose content in the samples, while the total soluble free sugar content was relatively stable (**Figure 20D**). These results agree with those previously obtained by Huang et al. (2015) for sugarcane juice and which were related to sucrose invertase activity. The authors also reported that the activity of this enzyme is reduced by 82.86% by thermal pasteurization, thus not being completely inactivated. On the other hand, it is known that cold stored potato tubers accumulate reducing sugars derived from the breakdown of starch into sucrose, which is then converted into glucose and fructose by vacuolar acid invertase. This metabolic process is known as cold-induced sweetening (Wiberley-Bradford et al., 2014).

4.2.5. Organic acids composition

Figure 21A and **B** shows a representative HPLC chromatogram of the organic acids from the mixture standard solution and a smoothie sample, respectively. Oxalic, malic, and citric acids were quantified in all smoothie samples and the result are presented in **Figure 22**. Trace amounts of succinic and fumaric acids were also detected.

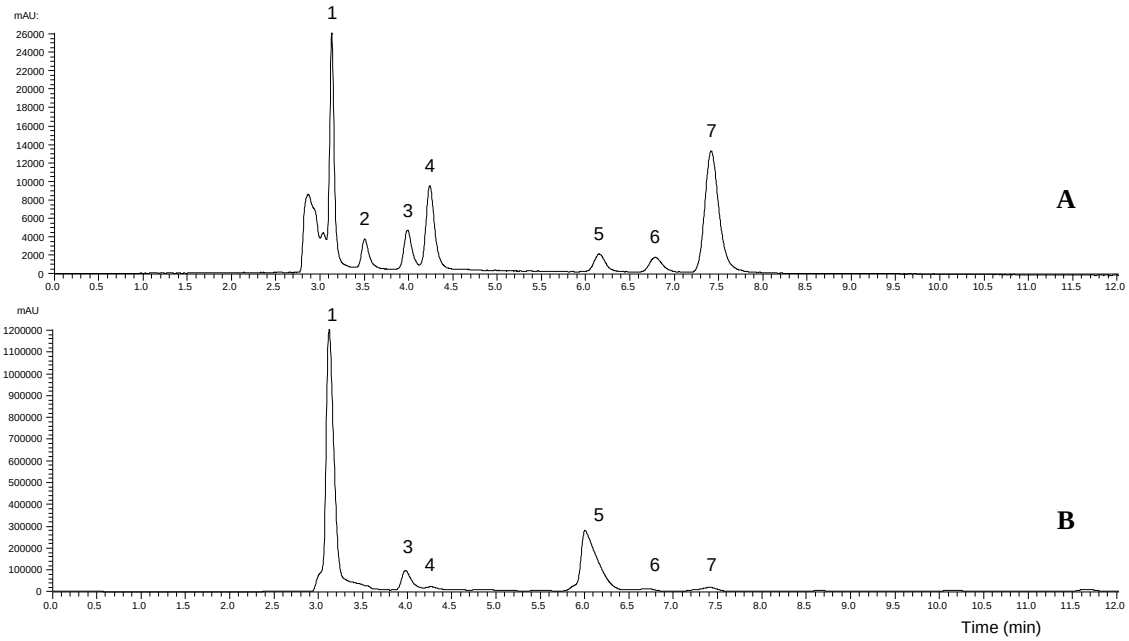
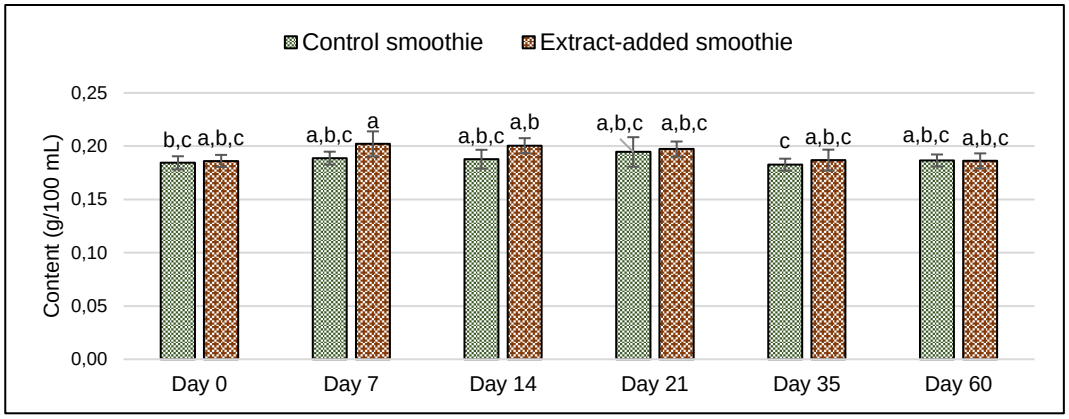
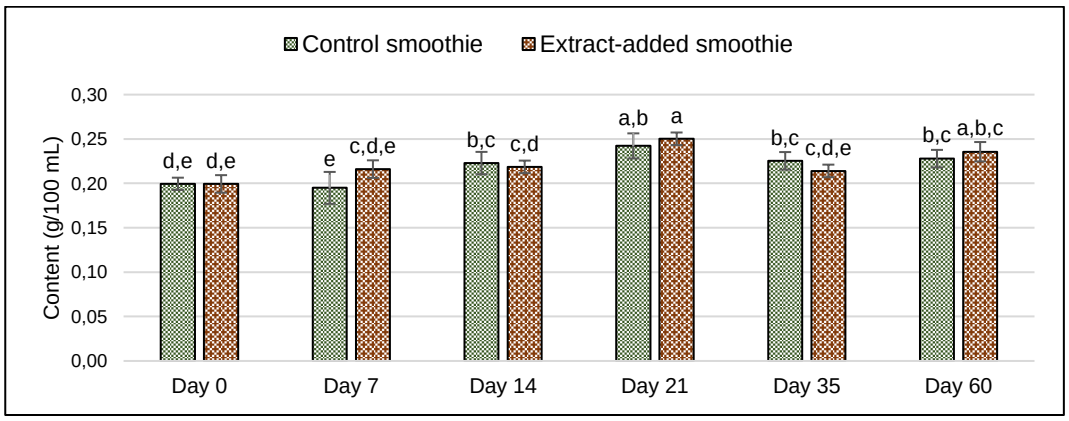


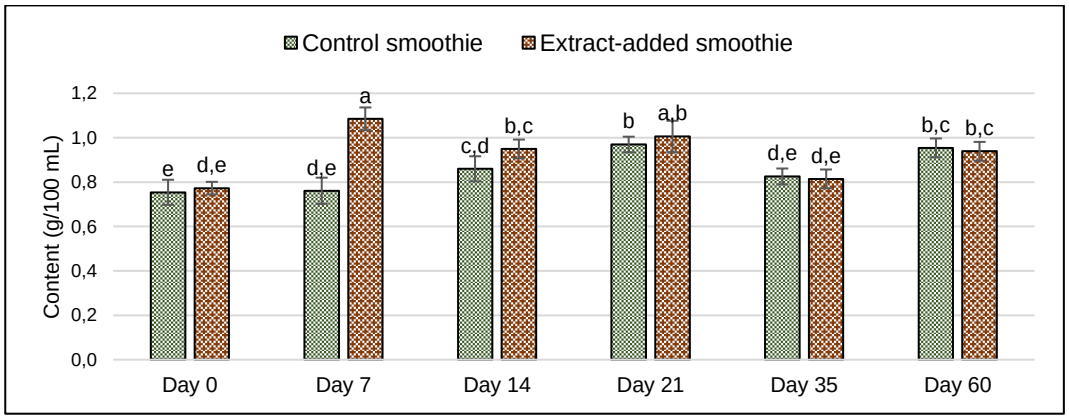
Figure 21 HPLC chromatogram of organic acids of (**A**) mixture standard solution and (**B**) extract-added smoothie at day 0, recorded at 215 nm. 1 – oxalic acid; 2 – quinic acid; 3 – malic acid; 4 – shikimic acid; 5 – citric acid; succinic acid; and 7 – fumaric acid.



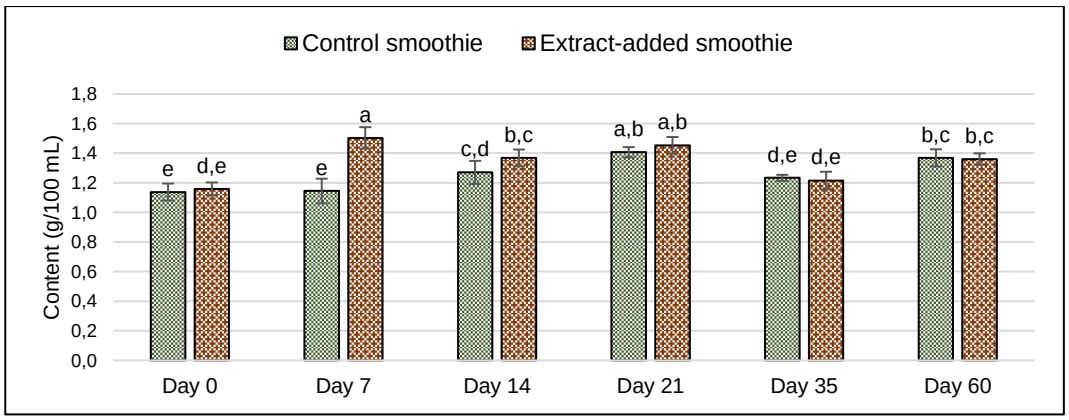
A



B



C



D

Figure 22 Content of (**A**) oxalic acid, (**B**) malic acid, (**C**) citric acid, and (**D**) total organic acids in the filtered smoothie samples, with and without added quince peel extract, during refrigerated storage.

Citric acid was the most abundant organic acid detected in the smoothie samples, accounting for about 68% of the total organic acid content, followed by malic acid and then oxalic acid. Although naturally present in fruits and vegetables, citric acid is used in the food and beverage sector as a preservative (E330) due to its antioxidant activity, and as flavor enhancer to balance the sweetness of juices and soft beverages (Di Lorenzo et al., 2022). As verified for soluble sugars, the addition of bioactive quince peel extract did not induce a significant effect on the organic acid content in the samples analyzed on the day they were prepared. During refrigerated storage, oxalic acid was relatively stable in both smoothie formulations (**Figure 22A**), while the malic acid content gradually increased, reaching the highest concentration after 21 days (**Figure 22B**). For citric acid, a large increase in the formulation with added bioactive extract occurred after 7 days of storage (in agreement with the increase in TSS), while successive significant increases (p-value < 0.05) occurred in the control formulation after 14 and 21 days (**Figure 22C**). Furthermore, there was a decrease in the levels of this organic acid after 35 days, followed by a further increase at the end of the storage time. The increase in citric acid content may be related to possible microbial activity, characterized by utilizing sugars and generating acids through energy-generating metabolic processes. The increasing trend in citric/total acid content is consistent with previous shelf-life studied conducted with fruit/vegetable smoothies (Castillejo et al., 2016; Jafari et al., 2021).

According to the Nutrition Calculator and Recipe Builder output (see attached file), ascorbic acid was expected to be present in the smoothie formulation, but this vitamin was not detected in any sample (with DAD at 245 nm). This antioxidant may have been degraded by the heat treatment applied to pasteurize the beverage. The use of mild or nonthermal pasteurization treatments could result in higher vitamin C in this beverage.

4.2.6. Fatty acids profile

Although the crude fat content of the prepared smoothie formulations was quite low (**Table 7**), the fatty acid profile may give some clues about the preservation status of these beverages. As shown in **Table 9** and in the representative chromatogram in **Figure 23**, eleven fatty acids were identified in the studied samples. The polyunsaturated fatty acid (PUFA) class was the most representative of the lipid fraction, given the high percentage of linoleic (C18:2*n*6c) and α-linolenic (C18:3*n*3) acids, followed by saturated fatty acids (SFA), among which palmitic (C16:0), stearic (C18:0), and behenic (C22:0) acids were the most abundant. High percentages of oleic acid (C18:1*n*9c), the only monounsaturated

fatty acid (MUFA) detected, were also found. In terms of lipid quality indices, PUFA/SFA ratios were greater than 0.45 and PUFA *n*6/*n*3 ratios were lower than 4.0, as recommended for a healthy diet (Chen & Liu, 2020).

Table 9 Fatty acid profile of the plant-based smoothie, with and without added quince peel bioactive extract, at time 0 and after 60 days of refrigerated storage.

	Control smoothie		**Extract-added smoothie**	
Fatty acids	**Day 0**	**Day 60**	**Day 0**	**Day 60**
C12:0	0.53 ± 0.02 ^{b}	0.54 ± 0.02 ^{b}	0.64 ± 0.02 ^{a}	0.39 ± 0.02 ^{c}
C14:0	1.49 ± 0.07 ^{a}	1.31 ± 0.09 ^{b}	1.27 ± 0.10 ^{b}	0.93 ± 0.05 ^{c}
C15:0	1.36 ± 0.06 ^{a}	0.84 ± 0.06 ^{b}	0.70 ± 0.08 ^{c}	0.85 ± 0.06 ^{b}
C16:0	21.80 ± 0.85 ^{a}	22.23 ± 0.85 ^{a}	22.62 ± 0.85 ^{a}	19.96 ± 0.54 ^{b}
C17:0	1.70 ± 0.05 ^{a}	1.49 ± 0.07 ^{b}	1.21 ± 0.07 ^{c}	1.09 ± 0.07 ^{c}
C18:0	7.61 ± 0.22 ^{a}	6.35 ± 0.32 ^{b}	7.20 ± 0.32 ^{a}	5.44 ± 0.32 ^{c}
C18:1*n*9c	19.16 ± 0.38 ^{a}	19.12 ± 0.54 ^{a}	18.94 ± 0.54 ^{a}	16.19 ± 0.54 ^{b}
C18:2*n*6c	28.13 ± 0.75 ^{c}	32.21 ± 1.06 ^{b}	28.39 ± 1.06 ^{c}	35.07 ± 1.06 ^{a}
C18:3*n*3	13.26 ± 0.07 ^{c}	11.75 ± 0.27 ^{d}	14.71 ± 0.10 ^{b}	15.81 ± 0.27 ^{a}
C20:0	1.57 ± 0.10 ^{a}	1.20 ± 0.07 ^{c}	1.21 ± 0.07 ^{c}	1.38 ± 0.07 ^{b}
C22:0	3.38 ± 0.11 ^{a}	2.96 ± 0.16 ^{b}	3.11 ± 0.16 ^{a,b}	2.89 ± 0.11 ^{b}
Classes				
SFA	39.45 ± 1.13 ^{a}	36.92 ± 1.68 ^{a}	37.96 ± 1.64 ^{a}	32.93 ± 1.62 ^{b}
MUFA	19.16 ± 0.38 ^{a}	19.12 ± 0.54 ^{a}	18.94 ± 0.54 ^{a}	16.19 ± 0.54 ^{b}
PUFA	41.39 ± 0.68 ^{c}	43.96 ± 1.33 ^{b}	43.10 ± 1.16 ^{b,c}	50.89 ± 1.33 ^{a}
Indices				
PUFA/SFA	1.05 ± 0.01	1.19 ± 0.02	1.14 ± 0.02	1.55 ± 0.04
PUFA *n*3/*n*6	2.12 ± 0.07	1.93 ± 0.06	2.74 ± 0.03	2.22 ± 0.03

C12:0 – lauric acid; C14:0 – myristic acid; C15:0 – pentadecanoic acid; C16:0 – palmitic acid; C17:0 – heptadecanoic acid; C18:0 – stearic acid; C18:1*n*9c – oleic acid; C18:2*n*6c – linoleic acid; C18:3*n*3 – α-linolenic acid; C20:0 - arachidic acid; C22:0 – behenic acid; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids. The results are presented as mean ± standard deviation.

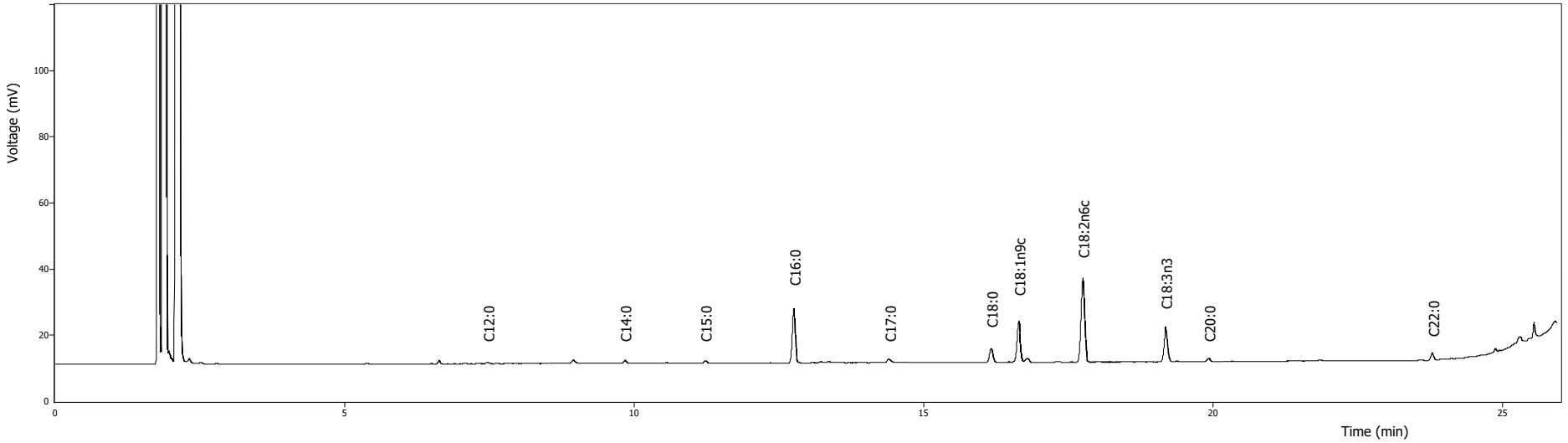


Figure 23 CG chromatogram of fatty acids in extract-added smoothie after 60 days of refrigerated storage. Detected fatty acids: C12:0 – lauric acid; C14:0 – myristic acid; C15:0 – pentadecanoic acid; C16:0 – palmitic acid; C17:0 – heptadecanoic acid; C18:0 – stearic acid; C18:1*n*9c – oleic acid; C18:2*n*6c – linoleic acid; C18:3*n*3 – α-linolenic acid; C20:0 - arachidic acid; C22:0 – behenic acid; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids. The results are presented as mean ± standard deviation.

The addition of bioactive extract to the smoothie did not cause significant effects on the percentage of the three main fatty acids (C18:2*n*6c, C16:0, and C18:1*n*9c; which structure is shown in **Figure 24**) nor on the classes of fatty acids at time zero (**Table 9**). However, some relative changes emerged during storage. At day 60, the percentage of SFA (including C16:0, C18:0, and C22:0) and MUFA (C18:1*n*9c) was significantly lower in the formulations with added extract compared to the initial samples, while the percentage of PUFA (C18:2*n*6c and C18:3*n*3) was significantly higher (p-values < 0.05). Therefore, these results highlighted a potential positive effect of the bioactive extract used as a natural preservative.

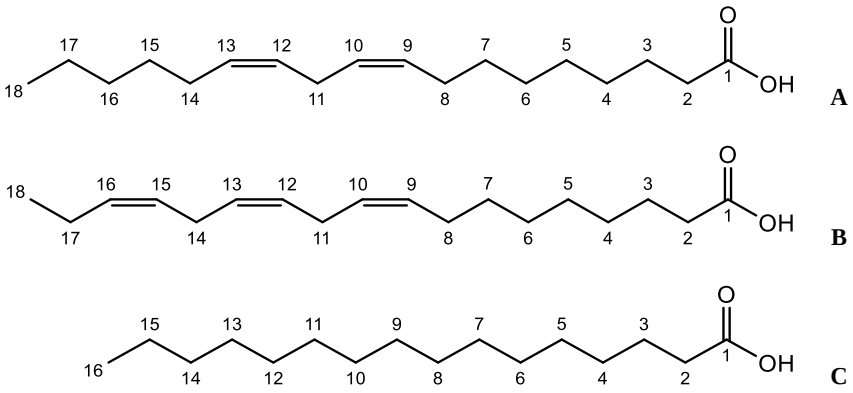


Figure 24 Chemical structure of (**A**) linoleic acid, (**B**) α-linolenic acid, and (**C**) palmitic acid.

4.3. Overall quality changes during shelf-life

A LDA was performed to assess the contribution of quality attributes to the overall variation observed among smoothie samples. The variables pH, TSS, saturation index (*C**), hue angle (h°), fructose, glucose, sucrose, oxalic acid, malic acid, and citric acid were considered simultaneously in the analysis, and the groups were defined considering as variables i) the 12 smoothie samples, ii) the addition of extract (regardless of the storage time), and iii) the storage time (regardless of the formulation). In the first case, three discriminant functions were defined by the model, the first justifying 91.8% of the total variance and separating the samples in the biplot based on the sucrose content, while the second function justified 6.1% and the separation was due to the citric and oxalic acids (**Figure 25A**). Thus, the biplot show up the lower sucrose levels in the samples from day 60 (**Figure 20C**) and the higher citric and oxalic acid levels in the extract-added sample

from day 7 (E7) (**Figure 22A** and **C**), compared to those from day 0, whose markers appears overlapping as there do not present significant differences. However, this analysis did not demonstrate the suitability of quince peel extract for preserving quality attributes.

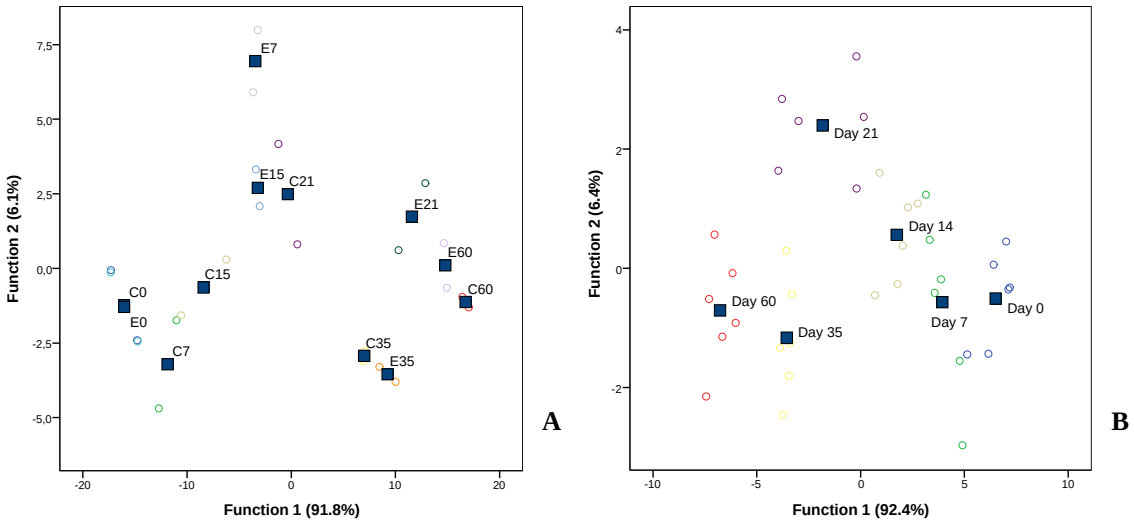


Figure 25 Spatial distribution of markers set by the canonical discriminant function coefficients when considering (**A**) the 12 smoothie samples and (**A**) storage time regardless of the formulation. C – control smoothie; E – extract-added smoothie. The numbers stand for the days of storage.

The distribution of markers in the biplot as a function of storage time, regardless of the addition of extract to the smoothie, is shown in **Figure 25B**. In this case, sucrose also accounted for 92.4% of the variance in function 1. Function 2 accounted for the remaining 6.4% of the variance, which was associated with malic acid. As illustrated and shown in **Figure 22B**, the levels of this organic acid were higher after 21 days of storage.

When evaluating the impact of adding quince peel bioactive extract to the smoothie (regardless of storage time), it was concluded that the main differences were related to the hue angle (h°) and the fructose and oxalic acid contents.

5. CONCLUSIONS AND FUTURE STUDIES

This master's work successfully investigated the suitability of a natural bioactive extract from quince peel for preserving quality attributes of a fruit and vegetable smoothie during refrigerated storage for 60 days. The investigation started with the production and characterization of the extract composition in malic acid and phenolic compounds and *in vitro* antioxidant activity. The hydroethanolic extract was particularly rich in malic acid, flavonols (*O*-glycosylated quercetin derivatives), flavan-3-ols (β-type (epi)catechin trimers), and caffeoylquinic acids, and inhibited in some extent the formation of TBARS and the oxidative hemolysis. These findings, together with previous results on the extract's antimicrobial effects, highlight its potential application as a natural preservative in food and beverage formulations.

The prepared fruit and vegetable smoothie was particularly rich in carbohydrates (including the soluble sugars fructose, glucose, and sucrose) and also contained interesting levels of mineral elements (including K, Mg, Mn, and Cu) and total dietary fiber. These constituents did not change during refrigerated storage. The pH of the smoothie samples was lower after 60 days of storage. Interestingly, the control smoothie underwent the greatest color changes compared to the initial sample during the first 14 days, while the color of the extract-added smoothie was better preserved during the same period of time and the greatest difference was observed only after 60 days. The contents of the reducing sugars fructose and glucose increased during storage, while the sucrose content decreased during this time, which could be related to enzymatic or microbial activity. However, the total soluble sugar content was relatively stable. Regarding organic acids, oxalic, malic, and citric acids were detected in the smoothie samples, as well as trace amounts of succinic and fumaric acids. The citric acid content was higher in the extract-added smoothie at day 7 (in agreement with the increase in TSS), while successive increases occurred in the control formulation after 14 and 21 days. At the end of the storage time, the percentage of SFA (including C16:0, C18:0, and C22:0) and MUFA (C18:1*n*9c) was lower in the smoothie with added extract compared to the initial samples, while the percentage of PUFA (C18:2*n*6c and C18:3*n*3) was higher. Hence, these results highlighted a potential positive effect of the extract used as a natural antioxidant. According to an LDA, sucrose was the smoothie constituent most affected by storage time (regardless of the formulation), while the addition of extract (regardless of storage time) mainly impacted the hue angle (h°) and the fructose and oxalic acid contents.

Overall, this comprehensive study contributed to increasing knowledge about the preservative potential of quince peel extract in a fruit and vegetable smoothie and provided valuable information on the impact of refrigerated storage on the quality attributes of the prepared beverage. The findings serve as a scientific basis for the development and formulation of functional and nutritionally rich beverages, meeting consumers' "clean label" preferences and health-conscious choices.

The results of this work made it possible to outline future studies, such as:

- to test other concentrations of quince peel extract in the smoothie, as well as refined extracts to be more effective;
- to evaluate the effectiveness of the bioactive extract in other smoothie and food formulations (with less added bioactive ingredients);
- to carry out sensory analyzes to evaluate the acceptability of the smoothie by consumers;
- to evaluate the microbiological quality of the smoothies with added extract during refrigerated storage;
- to evaluate the impact of non-thermal pasteurization methods, such as high pressure and pulsed electric fields, in order retain thermolabile vitamins;
- to assess the suitability of hyperbaric storage as a sustainable alternative to cold storage;
- to conduct long-term shelf-life studies to evaluate the stability of the smoothie and efficacy of the extract;
- to investigate the potential impact of the smoothie formulations on human health, considering parameters such as antioxidant status and inflammation markers, among other indicators;
- to evaluate the sustainability of the smoothie formulations, considering factors such as ingredient sourcing, production processes, and packaging materials to align with eco-friendly practices.

By delving into these future studies, it will be possible to improve understanding of the potential of quince peel extract in smoothie formulations, providing a comprehensive framework for optimizing both the preservative efficacy and overall smoothie quality.

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ATTACHMENTS

Table A1 Analytical parameters of the chromatography analysis.

Standard	Calibration curve	Linear working range	R^2	LOD	LOQ
Chlorogenic acid	$y = 168823x - 161172$	2.5–80 µg/mL	0.9999	0.20 µg/mL	0.68 µg/mL
p-Coumaric acid	$y = 466578x + 527324$	2.5–80 µg/mL	0.9987	0.68 µg/mL	1.61 µg/mL
(+)-Catechin	$y = 84950x - 23200$	2.5–80 µg/mL	1	0.17 µg/mL	0.68 µg/mL
Quercetin-3-*O*-(6-acetylglucoside)	$y = 34843x - 160173$	25–800 µg/mL	0.9998	0.21 µg/mL	0.71 µg/mL
Oxalic acid	$y = 3\times10^6x + 103{,}773$	0.01–1.25 mg/mL	0.9995	0.02 mg/mL	0.07 mg/mL
Malic acid	$y = 325{,}181x + 26{,}173$	0.08–10 mg/mL	0.9999	0.07 mg/mL	0.21 mg/mL
Citric acid	$y = 414{,}016x + 38{,}156$	0.04–5 mg/mL	0.9994	0.09 mg/mL	0.29 mg/mL
D(–)-Fructose	$y = 1.04x$	0.375–24 mg/mL	0.9999		
D(+)-Glucose anhydrous	$y = 0.935x$	0.375–24 mg/mL	0.9991		
D(+)-Sucrose	$y = 1.087x$	0.375–24 mg/mL	0.9999		
Ca	$y = 0.0938x + 0{,}0043$	0.25–5 ppm	0.9993		
Fe	$y = -0.0045x^2 + 0.1074x + 0.0048$	0.25–5 ppm	0.9997		
K	$y = -5563.2x^2 + 16183x + 34.586$	0.25–5 ppm	0.9999		
Na	$y = -6130.7x^2 + 14460x + 39.736$	0.125–2.5 ppm	0.9997		
Mg	$y = -0.0105x^2 + 0{,}3291x + 0.0003$	0.0625–1.25 ppm	1		
Zn	$y = -0.014x^2 + 0.2638x + 0.0019$	0.0625–1.25 ppm	0.9999		
Cu	$y = -0.0088x^2 + 0.1969x + 0.0003$	10–40 ppb	1		
Mn	$y = -0.0053x^2 + 0.1216x + 0.0041$	10–40 ppb	0.9998		

LOD: limit of detection; LOQ: limit of quantification.

Nutrition Calculator and Recipe Builder

Summary

Recipe name	Smoothie
Number of servings	1
Final weight, g ⓘ	100

Ingredient	Amount	Calories
Orange juice, raw	49 g	22
Apples, without skin, raw	17 g	8.2
Carrots, raw	17 g	7
Blueberries, raw	12 g	6.8
Beets, raw	5 g	2.2

Smoothie

Nutrition Facts

Portion Size **100 g**

Amount Per Portion

Calories **46**

% Daily Value *

Total Fat 0.2g	**0 %**
Sodium 16mg	**1 %**
Total Carbohydrate 11g	**4 %**
Dietary Fiber 1.2g	**4 %**
Sugar 8.2g	
Protein 0.7g	**1 %**
Vitamin D 0mcg	0 %
Calcium 13mg	1 %
Iron 0.2mg	1 %
Potassium 193mg	4 %

* The % Daily Value (DV) tells you how much a nutrient in a serving of food contribute to a daily diet. 2000 calories a day is used for general nutrition advice.

Smoothie nutrition facts and analysis per 100.00 g

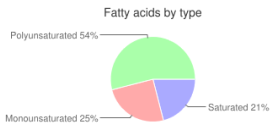
Vitamins

Nutrient	Amount	DV
Vitamin A, RAE	147.65 mcg	16 %
Carotene, alpha	594.03 mcg	
Carotene, beta	1432.35 mcg	
Cryptoxanthin, beta	85.02 mcg	
Lutein + zeaxanthin	112.53 mcg	
Lycopene	0.17 mcg	
Retinol	0 mcg	
Thiamin [Vitamin B1]	0.064 mg	5 %
Riboflavin [Vitamin B2]	0.037 mg	3 %
Niacin [Vitamin B3]	0.445 mg	3 %
Pantothenic acid [Vitamin B5]	0.174 mg	3 %
Vitamin B6	0.058 mg	3 %
Vitamin B12 [Cobalamin]	0 mcg	0 %
Vitamin B12, added	0 mcg	
Folate, DFE [Vitamin B9]	24.1 mcg	6 %
Folate, food	24.1 mcg	
Folic acid	0 mcg	
Vitamin C [Ascorbic acid]	27.6 mg	31 %
Vitamin D	0 mcg	0 %
Vitamin E (alpha-tocopherol)	0.21 mg	1 %
Vitamin E, added	0 mg	
Tocopherol, alpha	0.21 mg	
Tocopherol, beta	0 mg	
Tocopherol, delta	0 mg	
Tocopherol, gamma	0.04 mg	
Tocotrienol, alpha	0 mg	
Tocotrienol, beta	0 mg	
Tocotrienol, delta	0 mg	
Tocotrienol, gamma	0.01 mg	
Vitamin K	4.6 mcg	4 %

Carbohydrates

Nutrient	Amount	DV
Carbohydrate	11.12 g	4 %
Fiber	1.23 g	4 %
Starch	0.24 g	
Sugars	8.19 g	
Fructose	1.72 g	
Galactose	0 g	
Glucose	1.24 g	
Lactose	0 g	
Maltose	0 g	
Sucrose	0.76 g	
Net carbs	9.89 g	

Fats and Fatty Acids



Nutrient	Amount	DV
Fat	0.21 g	0 %
Saturated fatty acids	0.025 g	0 %
Butanoic acid	0 g	
Decanoic acid	0 g	
Docosanoic acid	0 g	
Dodecanoic acid	0 g	
Eicosanoic acid	0 g	
Heptadecanoic acid	0 g	
Hexadecanoic acid	0.021 g	
Hexanoic acid	0 g	
Octadecanoic acid	0.001 g	
Octanoic acid	0 g	

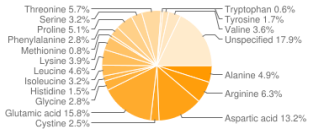
Vitamin K1 [Phylloquinone]	4.6 mcg	
Dihydrophylloquinone	0 mcg	
Betaine	6.5 mg	
Choline	6.1 mg	1 %

Minerals

Nutrient	Amount	DV
Calcium	13.37 mg	1 %
Copper	0.05 mg	6 %
Fluoride	0.5 mcg	
Iron	0.23 mg	1 %
Magnesium	9.98 mg	2 %
Manganese	0.093 mg	4 %
Phosphorus	19.59 mg	2 %
Potassium	193.19 mg	4 %
Selenium	0.12 mcg	0 %
Sodium	16.24 mg	1 %
Zinc	0.11 mg	1 %

Proteins and Aminoacids

Protein by amino acid



Nutrient	Amount	DV
Protein	0.72 g	1 %
Alanine	0.035 g	
Arginine	0.045 g	
Aspartic acid	0.095 g	
Cystine	0.018 g	
Glutamic acid	0.114 g	
Glycine	0.02 g	
Histidine	0.011 g	1 %
Isoleucine	0.023 g	1 %
Leucine	0.033 g	1 %
Lysine	0.028 g	1 %
Methionine	0.006 g	
Phenylalanine	0.02 g	
Proline	0.037 g	
Serine	0.023 g	
Threonine	0.041 g	3 %
Tryptophan	0.004 g	1 %
Tyrosine	0.012 g	
Valine	0.026 g	1 %
Phenylalanine + Tyrosine	0.032 g	1 %
Methionine + Cysteine	0.006 g	0 %

Pentadecanoic acid	0 g
Tetracosanoic acid	0 g
Tetradecanoic acid	0 g
Monounsaturated fatty acids	0.029 g
Docosenoic acid	0 g
Eicosenoic acid	0 g
Heptadecenoic acid	0 g
Hexadecenoic acid	0.002 g
Octadecenoic acid	0.027 g
Pentadecenoic acid	0 g
Tetradecenoic acid	0 g
Polyunsaturated fatty acids	0.064 g
Cis,cis-eicosadienoic n-6 acid	0 g
Docosahexaenoic n-3 acid (DHA)	0 g
Docosapentaenoic n-3 acid (DPA)	0 g
Eicosadienoic acid	0 g
Eicosapentaenoic n-3 acid (EPA)	0 g
Eicosatetraenoic acid	0 g
Octadecadienoic acid	0.05 g
Octadecatetraenoic acid	0 g
Octadecatrienoic acid	0.013 g
Fatty acids, total trans	0 g

Sterols

Nutrient	Amount	DV
Cholesterol	0 mg	0 %
Phytosterols	1.25 mg	

Other

Nutrient	Amount	DV
Alcohol, ethyl	0 g	
Ash	0.47 g	
Caffeine	0 mg	
Theobromine	0 mg	
Water	87.5 g	

Instructions

Add ingredient	Use the search bar above to quickly add ingredients to the recipe. You can specify the item and weight in grams or ounces in the search box, e.g. 'bananas 35 g', 'tomatoes 4 oz'. UPC barcodes are also supported, e.g. '070470496528'. Alternatively you can add an item directly from favorites, recipes, custom foods, food details and search result pages.
Remove ingredient	Click on the 'delete' link in the item row to remove it from the recipe.
Change amount	Click on the amount link to modify the amount used in the recipe.
Recipe name	Click on the pencil icon in the recipe name row, modify the name as desired and hit enter or click anywhere on the page to save changes.
Number of servings	Click on the pencil icon in the number of servings row, modify name as desired and hit enter or click anywhere on the page to save changes.
Final weight	If not specified then the total weight of all ingredients will be used as the final weight of a recipe. To account for water evaporation or adsorption you can directly specify final weight by clicking on the pencil icon in the final weight row.
Save recipe	Click on the 'Save to your recipes' button to save the current recipe to your recipe list.
Share recipe	Click on 'Share by URL' link to the copy URL suitable for sharing this recipe. Sharing by URL feature only exists for recipes that don't depend on other recipes or custom ingredients.

Nutrition facts

Nutrition facts label and detailed nutrient analysis are displayed under each recipe. Hover above or click/tap on a nutrient in order to learn more about it. Hover or click/tap on an amount in order to view how each ingredient contributes to that total nutrient amount for the recipe. If an ingredient does not specify nutrient amount then it won't contribute (or effectively contributes 0) to the nutrient total value.