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XXXVII ciclo

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In copertina
Utilizzo sostenibile dei sottoprodotti agroindustriali (caffè e agrumi) per migliorare la qualità e la conservazione degli alimenti, promuovendo un approccio eco-friendly nell'industria alimentare

Note biografiche
Miriam Arianna Boninsegna è Dottoranda in Scienze Agrarie, Alimentari e Forestali presso l'Università Mediterranea di Reggio Calabria. Ha conseguito la Laurea Magistrale in Scienze e Tecnologie Alimentari presso lo stesso Ateneo con votazione 110/110 e lode con Encomio. Attualmente, la sua ricerca si concentra sulla valorizzazione dei sottoprodotti agroalimentari, con un focus sull'estrazione di composti bioattivi e il loro utilizzo in applicazioni alimentari. Ha svolto un periodo di ricerca presso l'Università di Zagabria ed ha partecipato a convegni nazionali e internazionali e pubblicato articoli scientifici legati alle tematiche del suo progetto di ricerca.

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BY-PRODUCTS FROM THE AGRI-FOOD INDUSTRIAL SECTOR: RESOURCE OR WASTE?

An eco-friendly utilization to preserve the quality of food

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Abstract

This PhD research is structured around the critical issues the food sector has faced in recent decades. Consumers' priorities have changed considerably in terms of sustainable, beneficial, and natural compounds in food formulations. On the other hand, increased demand of food, due to the world population increase, has led to the production of huge amounts of waste and by-products along the food chain that are causing worrying environmental pollution. In this era of challenges and opportunities, food by-products could be the winning key to meeting consumer and environmental needs.

The PhD project aimed to give new life to by-products derived from coffee and citrus industrial processes already recognized as a source of countless bioactive and functional compounds. The milestone of this research was to highlight the innovative use of agro-industrial by-products to improve food quality and safety and to offer new solutions in the food sector.

In Chapter 2, conventional (maceration) and innovative (ultrasound-assisted) extraction techniques were compared, as well as the variables that support the extraction process (time, temperature, and solvent ratio) to determine the optimal condition to recover bioactive compounds from coffee roasting by-product (coffee silverskin). The experimental data showed that the best coffee silverskin extract (CSE) was obtained by maceration with a hydroalcoholic solvent of ethanol-water (30:70), for 60 minutes at 60 °C. CSE showed a higher content of phenolic compounds (44.15 mg GAE g^{-1}), flavonoids (32.36 mg ECE g^{-1}), chlorogenic acid (3.34 mg g^{-1}) and caffeic acid (1.37 mg g^{-1}) to which was associated a high

antioxidant activity for the DPPH (33.62 μM TEg^{-1}) and ABTS (98.13 μM TEg^{-1}) assays.

In Chapter 3, different percentages of CSE (5% and 10%) were used in the formulation of dipping treatments to delay the quality decay (browning, loss of firmness, loss of aroma, and valuable chemical compounds) of fresh-cut fennel during 14 days of storage at 4°C. The use of CSE in the dipping of fresh-cut fennel processing permitted an increase in the phenolic acids (chlorogenic and caffeic acids) for up to 14 days with good sensory acceptability and physico-chemical and microbiological characteristics, compared to fennel subjected to conventional dipping treatments (ascorbic acid).

In Chapter 4, the influence of different concentrations of CSE (1%, 2%, and 4%) was evaluated on chemical, physical, microbiological, structural, and sensory gummy candies characteristics for 120 storage days. The results up to 120 days of storage revealed the higher quality of gummy candies enriched with 1%, 2%, and 4% CSE not only for their bioactive content compounds but also for their antioxidant activity. Moreover, all the candies enriched showed better physical and sensory characteristics compared to the control taste.

In Chapter 5, the by-products of the lemons process (Lemon pomace) are tested to encourage the transition to the circular economy of the citrus industry. In particular, the aim was to test the efficiency of antioxidant extracts obtained from lemon pomace (LPE) for the formulation of edible coatings alginate-based. The enriched edible coating formulated were applied on a new form of ready-to-eat citrus fruits that were ready-to-eat Clementine segments. As clementine segments were a new food not yet treated in the present scientific literature, the experimental

plan included a preliminary step (section 5.1) to test the efficiency of edible coatings replacing the conventional storage conditions (MAP, modified atmosphere packaging) in the storage of ready-to-eat citrus fruits. In section 5.1 it was found that edible alginate-based coatings can improve the preservation of ready-to-eat clementines up to 21 days of storage at 4°C and under constant lighting while maintaining a good level of nutritional compounds (flavonoids and ascorbic acid) as well as firmness and sensory attributes better than normal atmosphere and MAP. In section 5.2 LPE at different percentages was evaluated as an antioxidant and antimicrobial agent to be inserted into edible coatings alginate-based. The synergistic action between the alginate-based coating and LPE allowed us to preserve the chemical, physical, microbiological, and safety parameters by revealing a dose-dependent effect for organic acids, flavonoids, and microbial growth during the 21 days of storage at 4 °C.

The results obtained in this research indicated that coffee and citrus fruit by-products are valuable sources of bioactive compounds that improve the chemical, physical, sensory, and microbiological characteristics of various food categories. The eco-friendly use of by-products, as proposed in this study, can be considered a starting point for further research focused to converting food by-products, from problems to resources, in the food sector.

Sommario

Questa ricerca di Dottorato si articola intorno alle criticità che il settore alimentare ha dovuto affrontare negli ultimi decenni. Le priorità dei consumatori si sono spostate verso alimenti formulati con composti sostenibili, benefici e naturali. Inoltre, l'aumento della domanda di cibo, dovuto all'aumento della popolazione mondiale, ha portato alla produzione di enormi quantità di rifiuti e sottoprodotti alimentari ai quali è connesso un preoccupante aumento di inquinamento ambientale. In quest'era di sfide e opportunità, i sottoprodotti alimentari potrebbero essere la chiave vincente per soddisfare le esigenze dei consumatori e dell'ambiente.

L'obiettivo di questo progetto di Dottorato è stato dare nuova vita ai sottoprodotti derivanti da lavorazioni industriali di chicchi di caffè e agrumi, già riconosciuti come fonte di innumerevoli composti bioattivi e funzionali. Il caposaldo di questa ricerca è stato mettere in evidenza come l'uso innovativo dei sottoprodotti agroindustriali può migliorare la qualità e la sicurezza degli alimenti e offrire nuove soluzioni al settore alimentare.

Nel capitolo 2 sono state confrontate le tecniche di estrazione convenzionali (macerazione) e innovative (ultrasuoni assistiti), nonché le variabili che supportano il processo di estrazione (tempo, temperatura e rapporto solvente) al fine di determinare le condizioni ottimali per il recupero di composti bioattivi dal sottoprodotto della tostatura del caffè (*Coffee Silverskin)*. I dati sperimentali mostravano che il miglior estratto coffee silverskin (CSE) era stato ottenuto mediante macerazione, per 60 minuti a 60 °C, con un solvente idroalcolico composto da etanolo-acqua (30:70) . CSE ha mostrato un contenuto elevato di composti fenolici (44,15 mg GAE g-1), flavonoidi (32,36 mg ECE g-1), acido

clorogenico (3,34 mg g-1) e acido caffeico (1,37 mg g-1) a cui è stata associata spiccata attività antiossidante per i saggi DPPH (33,62 μM TEg-1) e l'ABTS (98,13 μM TEg-1).

Nel capitolo 3, differenti percentuali di CSE (5% e 10%) sono state utilizzate per la formulazione di trattamenti di immersione atti a rallentare il decadimento qualitativo ad esso associato (doratura, perdita di compattezza, perdita di aroma e composti chimici preziosi) di finocchio di IV gamma durante 14 giorni di conservazione a 4°C. L'immersione dei finocchi nei trattamenti contenenti CSE ha permesso un aumento del contenuto fenolico (acido clorogenico e caffeico) fino a 14 giorni, buona accettabilità sensoriale e caratteristiche chimiche, fisiche e microbiologiche migliori rispetto a finocchi sottoposti a convenzionali trattamenti di dipping (acido ascorbico).

Nel capitolo 4, è stata valutata l'influenza di diverse concentrazioni di CSE (1%, 2% e 4%) sulle caratteristiche chimiche, fisiche, microbiologiche, strutturali e sensoriali di caramelle gommose per 120 giorni di stoccaggio. I risultati fino a 120 giorni di conservazione hanno rivelato la maggiore qualità delle caramelle gommose arricchite con l'1%, 2% e 4% di CSE non solo per il loro contenuto di composti bioattivi ma anche per la loro attività antiossidante. Inoltre, tutte le caramelle arricchite con CSE hanno mostrato migliori caratteristiche fisiche e sensoriali rispetto al test di controllo.

Nel capitolo 5, i sottoprodotti del processo di trasformazione industriale dei limoni (pastazzo di limone) sono testati per favorire la transizione verso l'economia circolare del settore agrumario. In particolare, l'obiettivo era quello di testare l'efficacia degli estratti antiossidanti ottenuti da pastazzo di limone (LPE) per la

formulazione di rivestimenti edibili a base di alginato di sodio. I rivestimenti edibili arricchiti sono stati applicati su una nuova forma di agrumi pronti al consumo ovvero spicchi di Clementine *ready-to-eat*. Poiché gli spicchi di clementine erano un nuovo alimento, non ancora trattato in letteratura scientifica attuale, il piano sperimentale comprendeva una fase preliminare (sezione 5.1) per testare l'efficienza dei rivestimenti edibili come sostituti di condizioni di stoccaggio convenzionali (MAP, confezionamento in atmosfera modificata). Nella sezione 5.1 è stato rilevato che i rivestimenti edibili a base di alginato miglioravano la conservazione degli spicchi di clementine *ready-to-eat* fino a 21 giorni di conservazione a 4 °C e sotto illuminazione costante, mantenendo un buon livello di composti nutrizionali (flavonoidi e acido ascorbico), *firmness* e attributi sensoriali in maniera più efficiente rispetto al confezionamento in atmosfera normale e alla MAP.

Nella sezione 5.2 è stato testato l'aggiunta di LPE, a diverse percentuali, come agente antiossidante e antimicrobico in rivestimenti edibili a base di alginato di sodio. L'azione sinergica tra il rivestimento edibile a base alginato e l'LPE ha permesso di preservare i parametri chimici, fisici, microbiologici e sensoriali rivelando un effetto dose-dipendente per acidi organici, flavonoidi e crescita microbica durante i 21 giorni di conservazione a 4 °C.

I risultati ottenuti in questo lavoro di ricerca indicavano che i sottoprodotti derivanti da lavorazioni industriali di caffè e agrumi, sono preziosi per l'ottenimento di composti bioattivi, atti a migliorare le caratteristiche chimiche, fisiche, sensoriali e microbiologiche di varie categorie di alimenti. L'uso sostenibile dei sottoprodotti, come proposto in questo studio, può essere considerato un punto

di partenza per ulteriori ricerche volte sulla conversione dei sottoprodotti alimentari, da problemi a risorse, nel settore alimentare.

KEYWORDS

Food by-products; Coffee by-products; Lemon by-products; Bioactive Compounds; Food Applications; Enrichments; Dipping Treatments; Edible Coating; high-value foods.

Chapter 1 Introduction

1.1 Agri-food industrial By-products

In recent years, the food sector has encountered critical situations linked to a multitude of circumstances among others the growth of the world's population, extreme climatic events (Galanakis et al., 2023), global wars, and pandemics (Galanakis et al., 2020; Galanakis et al., 2021) but also a drastic change in food preferences and lifestyle by final consumers (Tilman et al., 2011). The food sector is therefore facing an unusual atmosphere characterized by new and difficult challenges concerning meeting the demand for food of the growing world-changed population while preserving reduced food resources available (Galanakis 2024) through lowering food waste production during operations ranging from the farm, passing to processing, storage, distribution, and sale up to the consumer table. In addition, the increase in the world population has also led to increased demand for food products, which is associated with significant environmental pollution, strongly in contradiction with the needs of modern society and consumers in terms of sustainability of production to achieve the 17 universal objectives (Sustainable Development Goals, SDGs) provided by the "Agenda 2030" (United Nation, 2015; Varzakas & Smaoui, 2024)

In this scenario, characterized by critical points, innovation, exciting challenges, and endless opportunities, food by-products can be inserted as a key solution. While food by-products are considered a serious problem for environmental sustainability regarding safe disposal, they could be converted back into a resource for the food sector.

Every year, at a global scale, huge quantities of by-products are generated through food processing operations, especially from vegetable processing. These could be used as livestock feed, organic fertilizer, and, in a minority, to produce biogas (Caruso et al., 2019; Dhungana et al., 2022). However, these uses are limited and sometimes costly for the industries (transport costs, digester costs, soil incompatibility). Due to the aforementioned problems, in most cases the by-products resulting from the industrial process of fruit and vegetables were conveyed to landfills or disposed of irrationally and dangerously into the environment causing serious pollution damage related to groundwater contamination, soil microflora alteration, and greenhouse gas production (Eckhardt et al., 2022: Khedkar & Singh 2018; Rashwan et al., 2023). However, the potential pollutants of by-products could be significantly reduced if they were reintroduced into the food sector and used in food applications (Caponio et al., 2022; Marcillo-Parra et al., 2021).

The by-products derived from the food industrial process of vegetables and fruits (peels, seeds, shells, pomace, and leaves) have already been recognized as a valuable source of many bioactive compounds (Table 1) such as phenols, peptides, carotenoids, anthocyanins, fatty acids, fibers, and enzymes (Yadav et al., 2024; Caponio et al., 2022).

This precious chemical composition makes them interesting also from the point of view of health as a source of compounds useful to prevent diseases caused by oxidative stress (cancer, diabetes, Alzheimer’s, Parkinson's), stimulate the immune system, fight off infections, and improve intestinal mobility (Sorrenti et al., 2023). In today's global scenario, sustainable utilization of agri-food wastes and/or by-products to produce value-added food products can provide opportunities

for earning additional income for the agrifood industrial sector, meet the consumer demands in terms of natural compounds used in food applications, and encourage the rapid transition from linear to a circular economy (Othman et al., 2020) in line with society's overall goal of "zero waste" strategies (Hailemariam & Erdiaw-Kwasie, 2022; Debnath & Sarkar, 2023).

Table 1 Principal bioactive compounds bioactive compounds in fruit, vegetable, and cereal by-products (Kumar et al., 2017).

S. no.	Source	Residue	Bioactive components
Fruits			
1.	Apple	Peel and pomace	Epicatechin, catechins, anthocyanins, quercitin glycosides, chlorogenic acid, hydroxycinnamates, phloretin glycosides, procyanidins
2.	Avocado	Peel and seeds	Epicatechin, catechin, gallic acid, chlorogenic acid, cyanidin 3-glucoside, homogentisic acid
3.	Banana	Peel	Gallocatechin, anthocyanins, delphindin, cyaniding, catecholamine
4.	Citrus fruits	Peel	Hesperidin, naringin, eriocitrin, narirutin
5.	Grapes	Seed and skin	Coumaric acid, caffeic acid, ferulic acid, chlorogenic acid, cinnamic acid, neochlorogenic acid, *p*-hydroxybenzoic acid, protocatechuic acid, vanillic acid, gallic acid, proanthocyanidins, quercetin 3-*o*-gluuronide, quercetin, resvaratrol
6.	Guava	Skin and seeds	Catechin, cyanidin 3-glucoside, galangin, gallic acid, homogentisic acid, kaempferol
7.	Litchi	Pericarp, seeds	Cyanidin-3-glucoside, cyanidin-3-rutonoside, malvidin-3-glucoside, gallic acid, epicatechin-3-gallate
8.	Mango	Kernel	Gallic acid, ellagic acid, gallates, gallotannins, condensed tannins
9.	Palm	By-products of palm oil milling	Tocopherols, tocotrienols, sterols, and squalene, phenolic antioxidants
10.	Pomegranate	Peel and pericarp	Gallic acid, cyanidin-3,5-diglucoside, cyanidin-3-diglucoside, delphinidin-3,5-diglucoside
Vegetables			
11.	Carrot	Peel	Phenols, beta-carotene
12.	Cucumber	Peel	Chlorophyll, pheophytin, phellandrene, caryophyllene
13.	Potato	Peel	Gallicacid, caffeic acid vanillic acid
14.	Tomato	Skin and pomace	Carotenoids
Cereal crops			
15.	Barley	Bran	β-Glucan
16.	Rice	bran	γ-Oryzanol, bran oil
17.	Wheat	Bran and germs	Phenolic acids, antioxidants

1.2 Extraction of bioactive compounds

The vastness of the food sector determines the production of countless by-products resulting from the processing of various animal and vegetable raw materials as shown in Figure 1.3 (Pérez-Marroquín et al., 2023). To promote the reuse of bioactive compounds in the food sector, it is therefore imperative to know the raw material thoroughly to identify the appropriate conditions to be put in place for the recovery of bioactive compounds with a view to subsequent food use. Finding a balance between all the factors that contribute to the success of the extraction is not an easy operation and is not closely tied to the matrix on which one operates. The percentages shown in Figure 1 suggest that a large proportion of by-products are derived from vegetable processing and a negligible part from meat and fish (Pérez-Marroquín et al., 2023).

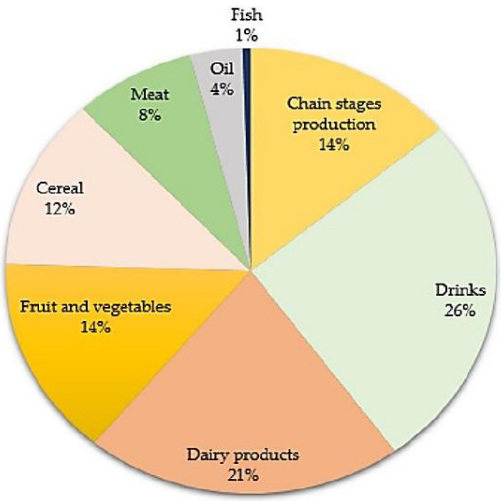

Figure 1 Subdivision (percentage) of by-products generated by food processing (Pérez-Marroquín et al., 2023).

The importance of extraction systems for recovering antioxidant compounds from food by-products is a crucial issue in developing sustainable approaches in the

food industry. Antioxidant compounds, such as polyphenols and flavonoids, are valuable constituents for their bioactive properties, including protection against lipid oxidation and improvement of human health. Recovering them from food by-products enhances by-products and helps reduce by-products promoting the circular economy of the food sector.

Considering the chemical complexity of the food by-product matrices and the vastness of the bioactive target compounds to be recovered, there is undoubtedly no standardized method to use, but different extraction methods and variables must be tested to determine the by-product's response to the extraction process (Sorrenti et al., 2023). The choice of method depends on the equilibrium between efficiency, extract quality, environmental impact, and production costs.

In the modern scenario, different extraction approaches could be used in order to optimize the recovery of bioactive compounds from food by-products. Solid-liquid extraction (Soxhlet, maceration), ultrasound, microwave as well as supercritical fluid, subcritical water extraction, and enzymes were used in order to identify the optimal extraction process for different target compounds (Kumar et al., 2017).

Figure 2 summarizes the main extraction methods used today and the affinity between the extraction method used, and the bioactive compounds recovered.

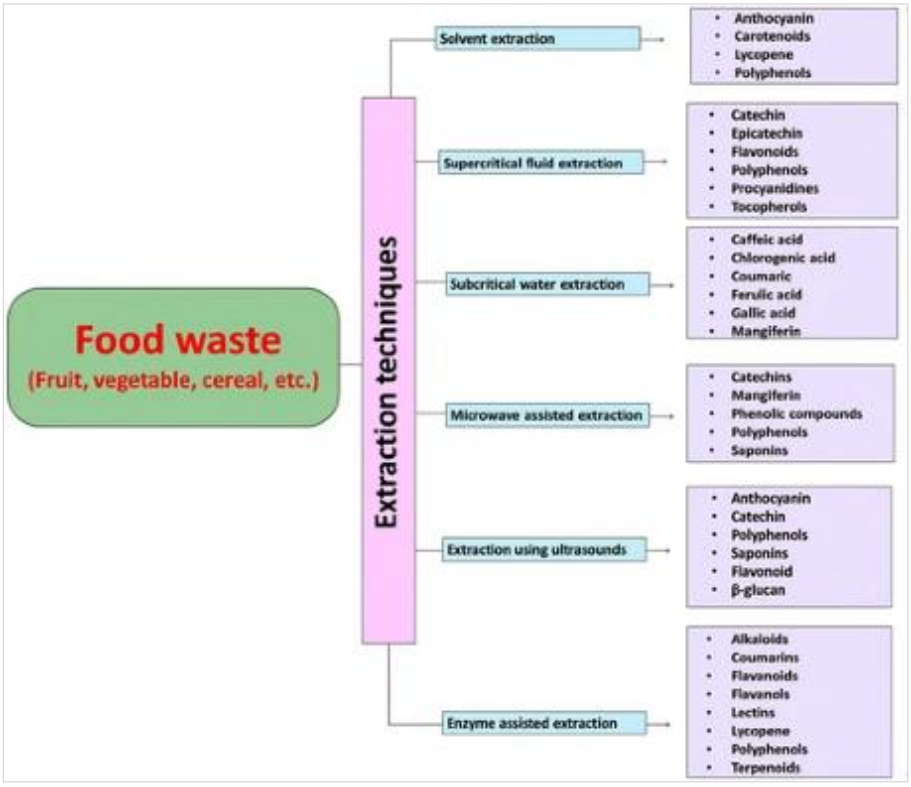

Figure 2 Main extraction approaches used for the recovery of bioactive compounds from food waste and by-products (Kumar et al., 2017)

However, it is not enough to find the extraction method since other variables such as time, temperature solvent, and others contribute to the extraction process's qualitative and quantitative success. To better explain the critical issues related to the extraction process and its correlation with the by-products matrix, we can compare two types of extraction widely used in the food sector: conventional by maceration and innovative by using waves with a frequency above 20 kHz (ultrasound assisted).

Nowadays different authors indicate the extraction assisted by the use of ultrasound is a winning, green, and innovative choice for the recovery of bioactive compounds with appreciable antioxidant activity from different by-products such as grapes, oranges, passion fruits, olives, and other fruits and vegetables (Linares

& Rojas, 2022; Kumar eta al., 2021). The sound waves generated by the ultrasound system (frequency greater than 20 kHz) and the establishment of cavitation phenomena related to them lead to rapid cell lysis and improve the diffusion of solvent in the matrix improving the mass transfer and reducing the time and solvent used during the process (More et al., 2022). However, various criticalities linked to variables used during the process (amplitude, frequency temperature, time, solvent/ raw material ratio) determine the result in producing extracts with poor antioxidant activity and damage to the target compounds recovered (Kumar et al., 2021). It is clear from these considerations that the well-known strengths of this method (speed of execution, low solvent use, and high extraction yields) often do not correspond to reality and require the use of equipment that is sometimes expensive (ultrasonic generating instruments). Studies conducted on the recovery of bioactive compounds from by-products of bergamot (Gattuso et al., 2023), apples (Pando et al., 2024), pears (Ferreira et al., 2023), and grapes (Rayaroth et al., 2016) by comparison of ultrasound and maceration using the same binomial time-temperature, provided concrete scientific evidence on the best performance of maceration to ultrasound extraction processes. Furthermore, considering that the mentioned studies were conducted using different extraction systems but the same working conditions (time, temperature, solvent, etc.) the sustainability and speed assumed for "innovative and sustainable" ultrasound-assisted extraction is lost. The discrepancies found between the studies mentioned above are that during the extraction process, it is necessary to consider the stereochemistry of the target compounds, their affinity with the extraction solvent, thermal sensitivity, and sensitivity to oxidation (Murthy et al., 2012; Galanaki et al., 2013; Baiano, et al.,

2023). In addition, the mechanical stresses that some "innovative" methods cause may hinder the extraction yield, unlike the slow diffusion exerted by maceration which can instead promote the integrity of the recovered substances and consequently a better yield from a qualitative and quantitative point of view (Galanakis et al.,2013).

Identifying the right conditions to recover bioactive compounds from food by-products plays a key role in their subsequent use as food additives or for the formulation of value-added foods.

These reasons motivated the realization of this Ph.D. research around the context of vegetable by-products to offer a new perspective regarding the sustainable reuse of food by-products from coffee roasting and citrus processing, which also represent among the principal processed raw materials worldwide to produce various drinks and food formulations.

1.3 Coffee by-products

Coffee silverskin is a unique by-product obtained from the process of roasting green coffee beans. Its disposal of the environment causes a serious pollution problem, due to high organic load. For these reasons in recent decades, many authors have focused on finding new scenarios to breathe new life into it Already known as a source of compounds with strong antioxidant activity and healthy properties (Figure 3) (Regazzoni et al., 2016; Eckhardt et al., 2022; Machado et al., 2023; Mesías et al., 2014; Castaldo et al., 2020), Coffee silverskin can be then considered an interesting by-product to investigate for food applications (Ballesteros et al., 2016; Brandão, et al., 2021; Nolasco et al.,2022). Bertolino et al., (2019) reported therefore that extracts obtained from silverskin can

be considered a safe ingredient, free of toxic substances such as ochratoxin A (OTA), pesticides, and polycyclic aromatic hydrocarbons (PAHs). Moreover, coffee silverskin has already been widely invoked as a functional ingredient in various food categories such as drinks (Martinez-Saez, et al., 2014), cookies (Goceman et al., 2019), bread (Pourfarzad, et al., 2013), sausage (Thangavelu et al., 2022) and gummy (Boninsegna et al., 2024) to encourage its reuse and valorization in the food sector.

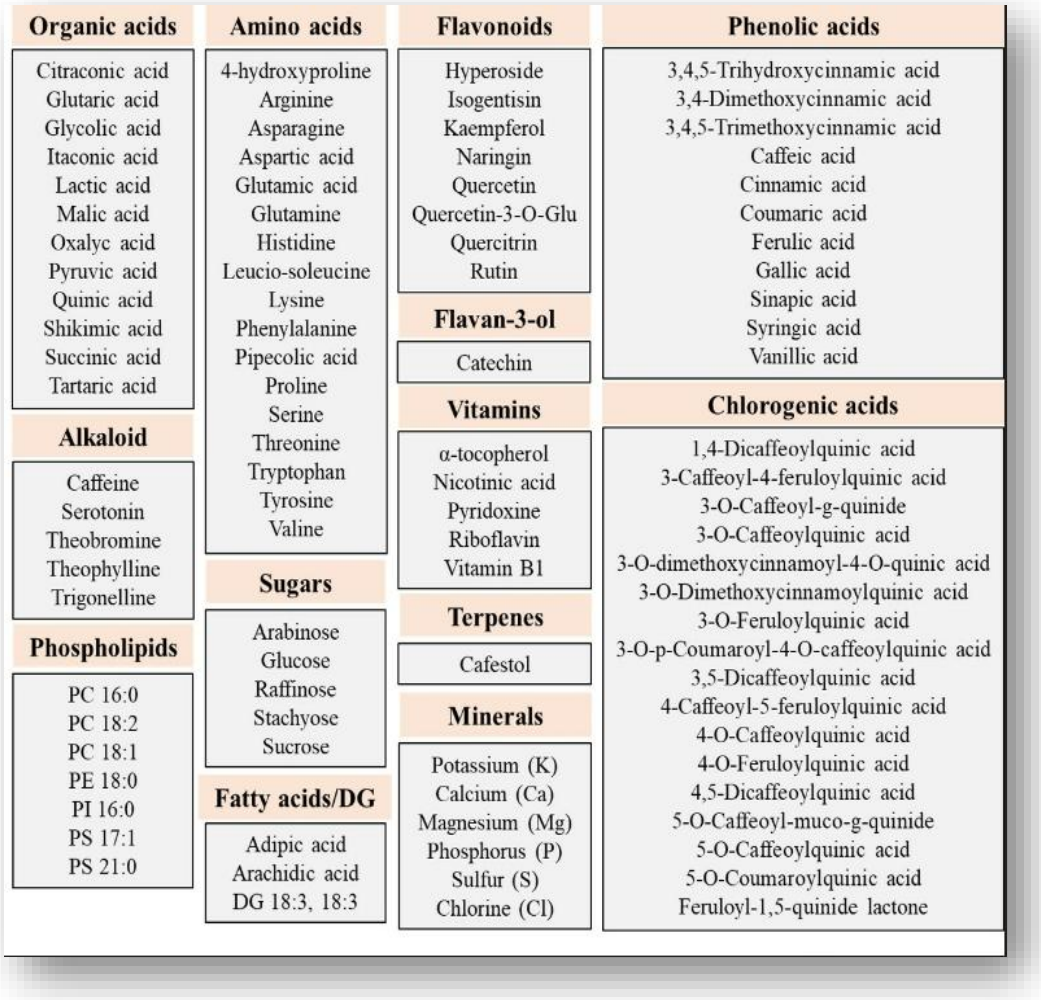

Figure 3 Main bioactive compounds characterizing the chemical composition of coffee by-products (Bojórquez-Quintal et al., 2024)

1.4 Citrus by-product

Citrus fruits are among the most widespread crops in the world (FAO - Food and Agriculture Organization of the United Nations, 2020) used to produce a wide range of foods that consequently generate an abundant number of by-products (Suri et al., 2022). The solid residue derived from the citrus process including albedo, seeds, and pulp is commonly called "*pastazzo*" or citrus pomace (Caballero et al., 2021; Gattuso et al., 2023). Following the above considerations for by-products of the coffee roasting industry, also the irrational disposal of the feed is found to be polluting for the aquifers, soil, and the production of greenhouse gases (Sandoval Herazo et al., 2024). Nowadays, citrus by-products are used as soil conditioning, compost, and animal feed (Zema et al., 2018). However, their use in recent decades is gaining ground in the pharmaceutical and food sectors (D'Amore et al., 2024). The interest of the food industry is due to the valuable chemical composition (Figure 4), in particular phenols, among which the class of flavonoids are the most representative (Kaur et al, 2023). Recognized for its countless antimicrobial, antioxidant, and health-promoting properties, citrus pomace in powder or extract was already widely used in food systems as enrichment or substitute for synthetic additives to preserve the shelf life of a wide range of foods to encourage the revalorization and reuse in the food sector (Panwar et al., 2021; Andrade et al., 2022).

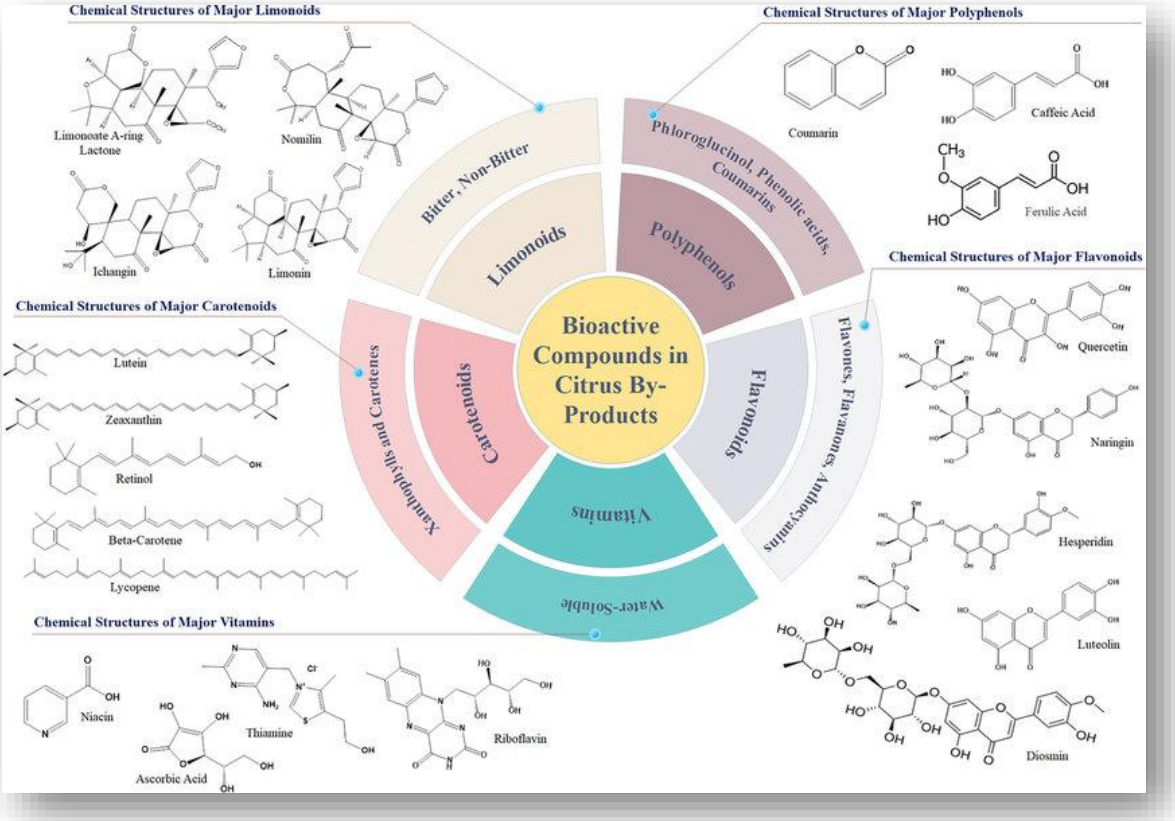

Figure 4 Main bioactive compounds characterizing the chemical composition of citrus by-products (Kaur et al., 2023)

1.5. Objectives and organization of the research

The main aim of this Ph.D. research was to find a synergy between the issues set out in sections 1.1-1.4 to give new life to coffee and citrus by-products within the food sector while respecting the sustainability of production and reduction of waste along the food chain.

The milestone of this work was to highlight the innovative use of agro-industrial by-products to improve food quality and safety and to offer new solutions following environmental challenges and changing consumer preferences that are straining the modern food sector.

The study was divided into successive steps to pursue the objective.

In ***Chapter 2***, green extraction techniques were tested thoroughly using food-grade solvents (ethanol and water) to promote the recovery of bioactive compounds from coffee silverskin. Thus, the best extract obtained in terms of bioactive compounds and antioxidant activity was used in food applications to improve the chemical, physical, sensory, and microbiological characteristics of ready-to-eat fennel (***Chapter 3***) and gummy candies (***Chapter 4***) during storage.

In Chapter 5, the by-products of the lemons process are tested to encourage the transition to the circular economy of the citrus industry. In particular, the aim was to test the efficiency of antioxidant extracts obtained from lemon paste in the formulation of edible coatings for ready-to-eat Clementine segments. As clementine segments were a new food not yet treated in literature and not present in the market, the experimental plan included a preliminary step (section 5.1) to study the efficiency of edible coatings replacing the conventional storage conditions (MAP, modified atmosphere packaging). In section 5.2 the lemon pomace extract was evaluated at different percentages as an antioxidant and antimicrobial agent edible coatings formulation.

A schematic representation of the proposed Ph.D. activities is shown in Figure 5.

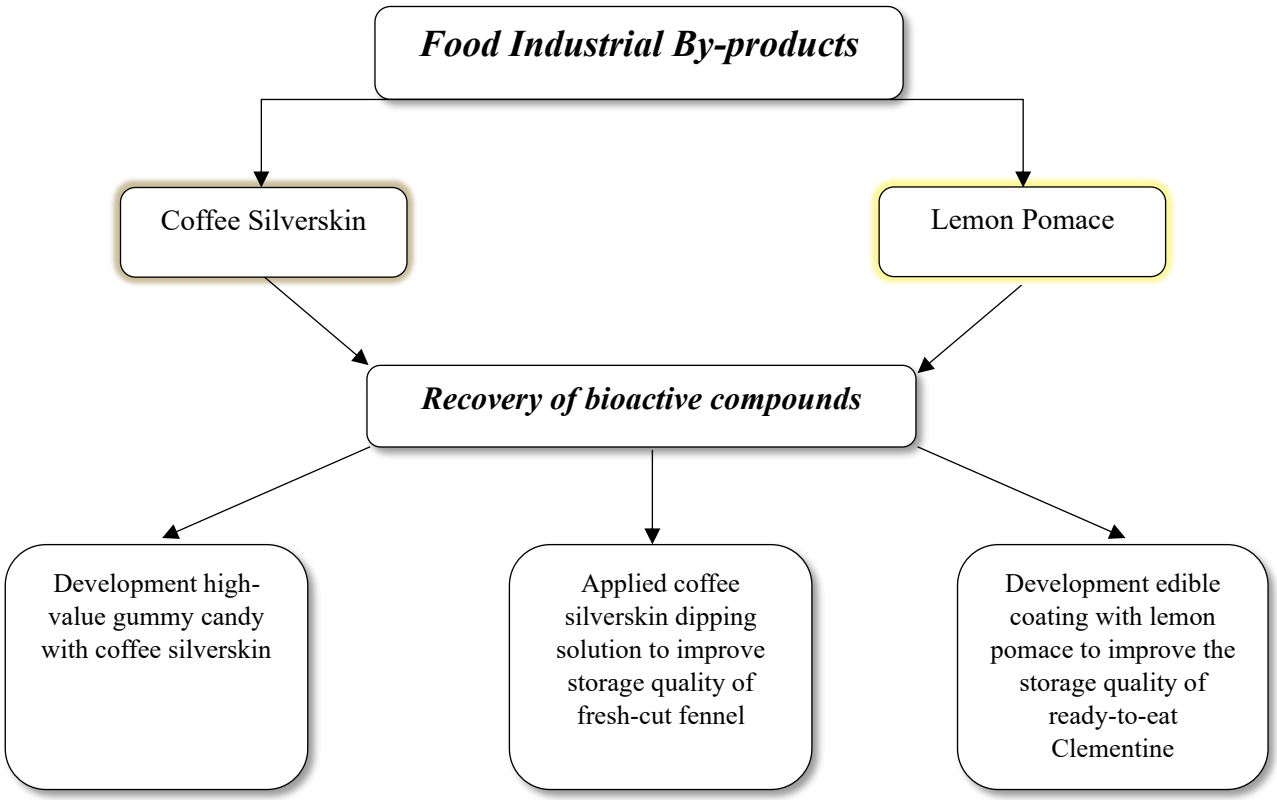

Figure 5 Schematic overview of the Ph.D. activities.

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Chapter 2 Use of food-grade solvents for the sustainable recovery of bioactive compounds from Coffee silverskin

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This work has been submitted to a journal for publication and is currently under review. The personal Author Contributions were regarding Conceptualization, methodology, software, validation, formal analysis, investigation, data curation, and writing—original draft preparation.

Abstract

The extraction of bioactive compounds from food by-products is now a challenge to encourage their reuse in the food sector. This study aimed to optimize the eco-friendly process of extraction of bioactive compounds from the by-product of coffee roasting coffee silverskin (CS) using only food-grade solvents (ethanol and water) with a view to the possible use of the extract in the food sector. Therefore, conventional (maceration) and innovative (ultrasound-assisted) extraction techniques were compared, as were the variables that support the extraction process (time, temperature, and solvent ratio). The data suggested that the process of extraction by maceration favored the recovery of phenolic compounds with chlorogenic and caffeic acids among the most representative. In particular, maceration with a hydroalcoholic solvent of ethanol-water (30:70), carried out for 60 minutes at 60 °C, improved the extraction efficiency of phenolic

compounds (44.15 mg GAE g^{-1}), flavonoids (32.36 mg ECE g^{-1}), chlorogenic acid (3.34 mg g^{-1}) and caffeic acid (1.37 mg g^{-1}) to which was associated a high antioxidant activity for the DPPH (33.62 μM TEg^{-1}) and ABTS (98.13 μM TEg^{-1}). Results were encouraging for future applications of CS extracts in the food sector.

Keywords: Food By-Products; Coffee Silverskin; Extraction Process; Sustainable extraction; Green solvents; Food grade solvents; Bioactive Compounds; Antioxidant activity; Food application.

2.1. Introduction

Coffee is considered one of the world's most appreciated and consumed drinks, with Brazil and Vietnam being the largest producers and exporters of green coffee beans worldwide (Klein et al., 2023; Hoseini et al., 2021). Nowadays, the use in food formulations and the abundant marketing of capsules compatible with espresso coffee machines have caused a higher demand for roasted coffee and, as a result, a greater generation of by-products connected with the roasting process (Freitas et al, 2024; Vimercati et al, 2022).

From the coffee roasting process derives the Coffee silverskin (CS), as the only by-product. It is a thin external layer covering the coffee bean (Behrouzian et al., 2016; Toschi et al., 2014). The safe disposal issues of CS are a serious problem since their organic load and the high content of phenolic acids and caffeine represent a hazard for groundwater contamination, alteration of soil microbiota, and greenhouse gas production (Eckhardt et al., 2022). Therefore, it is necessary to find innovative and eco-friendly strategies for the proper administration of CS.

Different studies reported that CS is a valuable fountainhead of dietary fiber, protein, and polyphenols (especially chlorogenic acids) (Regazzoni et al., 2026; Machado et al., 2023) that exert helpful impact on human health by protecting against oxidative harm, carbonyl stress, accumulation of advanced glycation end-products (AGEs) and prebiotic activity (Fernandez-Gomez et al., 2016; Castaldo et al., 2020). Therefore, CS has already been widely invoked as a functional ingredient in various food categories (Goceman et al., 2019; Pourfarzad et al., 2013; Thangavelu et al., 2022; Martuscelli et al., 2021; Ateş et al., 2019; Boninsegna et al., 2024) to encourage their reuse and valorization in the food sector with a view to a rapid transition towards a circular economy.

The efficient recovery of bioactive compounds from by-products is the preliminary step to obtaining high-value compounds. The extraction process is a critical sequential separation phase in which target compounds migrate from the raw material to the extractor based on their distribution coefficients (Pimentel-Moral et al.,2020; Abbasi-Parizad et al., 2021) where extraction methods and the chemical-physical extraction variables play an important role in optimizing and maximizing the extraction process.

Maceration, in the solid-liquid system, is the conventional extraction technique used for over a century to recover phenolic fractions from plant matrices. Despite the ease and extreme versatility of use, the execution of this type of extraction technique has several critical points closely linked to the demand for huge amounts of solvent and time that can make the execution long and expensive. On the other hand, the maceration extraction technique using passive diffusion has been recognized as an ideal extraction technique to improve the recovery of

bioactive compounds and to preserve the integrity of the recovered compounds because they do not undergo mechanical stress as is the case with other extraction methods (Rifna et al., 2023). Nevertheless, to overcome the critical issues of high solvent quantities and extraction times, research testing the possibility of using innovative injection techniques considers that the recovery efficiency is strongly influenced by the composition of the raw material as well as the variables used during the extraction process (Rifna et al., 2023).

Ultrasound-assisted extraction represented a valid replacement for conventional methods to maximize the yield of extract as well as to reduce the time and solvent used for the extraction. The sound waves generated by ultrasounds (frequency greater than 20 kHz) and the associated phenomena of compression and rarefaction, cause the establishment of cavitation bubbles which, after growing by coalescence, collapse, generating shock waves that cause cell lysis and the fastest diffusion of solvent in the matrix improving the mass transfer (More et al., 2022). However, this method also presents criticalities mainly related to variables used during the process (amplitude, frequency temperature, time, tempering, solvent/ raw material ratio) that lead to unstable cavitation phenomena which cause the development of radical species that result in an accelerated loss of compounds' susceptibility to oxidation (Kumar et al.,2021).

In both extraction methods used, physicochemical variables related to solvent, time, and temperature play a key role in promoting the diffusion rate of bioactive compounds from by-products to solvent (Gil-Martín et al., 2022). The determination of the correct time and temperature in the extraction process allows the preservation of the integrity of the extracted compounds and protects them from

oxidative damage. The temperature allows the viscosity and surface tension of the solvent to be reduced, resulting in an improvement in diffusivity in the matrix and the solvation of the target compounds, while the proper extraction time avoids prolonged exposure of phenolic compounds to oxygen and/or light that aids their degradation process. At the same time, the polarity of the extraction solvent allows for maximization of the recovery of target compounds by affinity (Gullón et al., 2020). Aliphatic alcohols (methanol and ethanol) as well as polar organic solvents are generally used for the recovery of phenolic compounds since they are predominantly polar. However, given the complexity of the chemical composition of food by-products, often aliphatic alcohols and organic polar solvents are mixed with different portions of water to maximize the extraction yield. In this context, the use of GRAS (Generally Recognized as Safe) and food-grade solvents as hydroalcoholic mixtures consisting of ethanol and water is a winning choice in the view of green extraction and to produce extracts that can be used for food applications.

The main purpose of this study was to identify an eco-friendly and easily replicable extraction technique in industrial realities for the improved recovery of bioactive compounds from CS with a view to future use of the extract obtained in food applications. To achieve this purpose, this study was tested the influence of i) Food-grade extraction solvents (ethanol-water alone or in the mixture); ii) extraction methods (maceration and ultrasound-assisted); extraction variables (temperature and time of extraction). The best extract was chosen considering the total content of bioactive compounds (polyphenols, flavonoids, and tannins), quantification of chlorogenic acid and caffeic acid (the most abundant phenolic

acids in CS), antioxidant activity (ABTS and DPPH) as well as time, temperature and quantity of individual solvent used.

The evidence provided by this study could be a starting point for the direct use of CS extracts in the food industry to formulate fortified/enriched foods or food treatments to improve and preserve food storage quality.

2.2. Materials and Methods

2.2.1. Raw Material

Coffee Silverskin (CS) came from a local roasting industry (Caffè Mauro S.p.A., Italy) and was the result of the roasting process of Coffea Arabica and Coffea canephora var. Robusta mixed in the 50:50 ratio. After reception at the FoodTec laboratory of the Mediterranean University of Reggio Calabria (Italy), CS was dried for 2 hours at 50 °C (moisture content 10%), ground, homogenized, and used in various tests provided by the experimental plan.

Figure 1. Coffee silverskin powder (right) and Coffee silverskin extract (left)

2.2.2. Experimental plan

The experimental plan was divided into two stages (Figure 2). The first phase focused on identifying the best method (conventional and innovative) and variable (time and temperature) to maximize the extraction process. In the second phase, the best conditions were used to test the influence of different solvents to promote the recovery of antioxidant compounds from CS. Food-grade and green solvents (ethanol and water) were used in all tests performed and the raw material/ solvent ratio was fixed at 1:10.

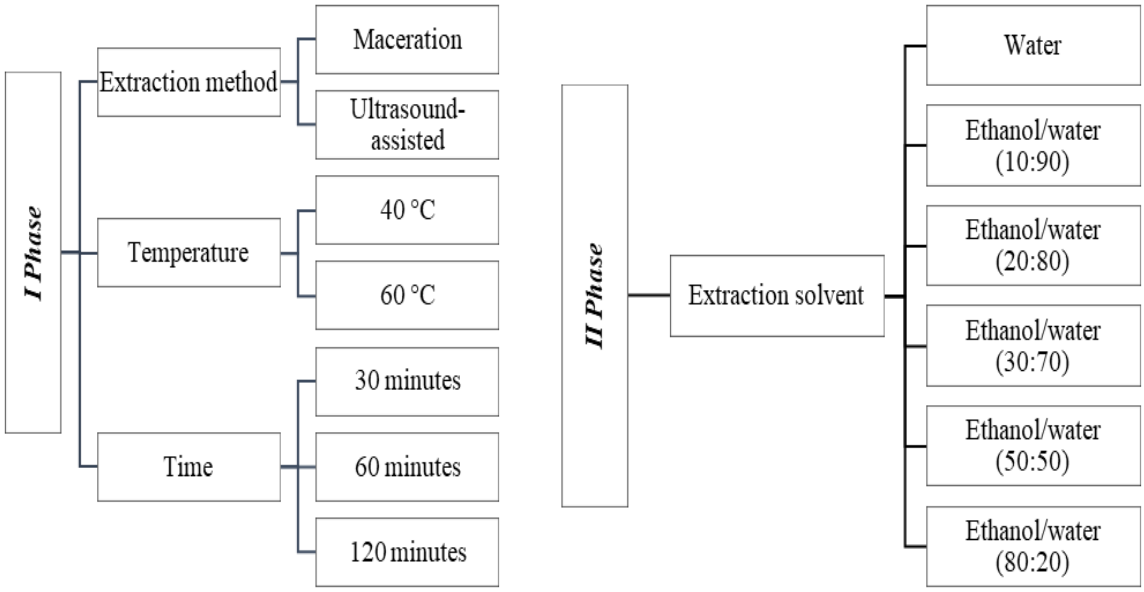


Figure 2. Schematic representation of the experimental design

2.2.2.1. I phase: Identification of the Optimal Condition to Recover Bioactive Compounds from CS

The first phase of this study focused on comparing conventional (maceration) and innovative (ultrasound-assisted) extraction techniques using the variables that most affect the diffusion rate of compounds in the solvent: time and temperature. Three times (30, 60, and 120 minutes) and two temperatures (40 and 60 °C) were used. Temperatures and times were decided based on previous studies on the extraction of bioactive compounds from food by-products.

Regarding extraction solvent, in this phase the mixture of food-grade solvents concerning ethanol and water (1:1) was used since previous studies have already indicated it was appropriate for the recovery of antioxidant compounds from different substrates and extraction methods, as shown in Table 1.

The solid-liquid extractions were performed by mixing a proper quantity of raw material and solvent on a heating plate under constant stirring and ultrasound-assisted extraction was carried out with an ultrasound bath (Flac Instrument, Treviglio, Italy). The different extraction times and temperatures tested are shown in figure 1, the amplitude was set at 59 kHz while the power was 80%.

Later, the obtained extracts were centrifuged (NF 1200 R, Nuve, Ankara, Turkey) at 6000 rpm for 10 min and 20 °C, filtered through a Buchner funnel, and stored at −21 °C up to the time of chemical analysis.

All extracts were prepared in triplicate, and each analysis was performed in triplicate.

Table 1. Use of ethanol-water (1:1) hydroalcoholic mixture for recovery of bioactive compounds from different by-products

By-product	***Solvent***	***Extraction methods***	***References***
Lemon	Ethanol 50%	Maceration	Imeneo et al. 2022
Bergamot	Ethanol 50%	Maceration	Gattuso et al. 2023
Olive leaf	Ethanol 50%	Ultrasound	Şahin et al. 2013
Pineapple skins	Ethanol 50%	Microwave	Nor et al. 2017
Chestnut shell	Ethanol 50%	Maceration	Vázquez et al. 2008
Aloe vera Rind	Ethanol 50%	Ultrasound	Ioannou et al. 2024
Grape	Ethanol 50%	Maceration	Lafka et al. 2007
Passion fruit	Ethanol 50%	Maceration	Sai-Ut et al. 2023

2.2.2.2. II phase: test the effect of food-grade solvents on the recovery of bioactive compounds from CS

The best extraction conditions selected in the preliminary phase (method, time, and temperature) were used to test the impact of the polarity of solvent on the recovery of antioxidant compounds from CS. In this phase, six different mixtures of food-grade solvents ethanol/ water including, 0:100, 10:90, 20:80, 30:70, 50:50, and 80:20 (v/v) were tested. The extractions were carried out by mixing CS powder and solvents (1:10 row material/solvent ratio) with constant stirring at 60 °C for 60 minutes. The extracts produced were centrifuged (6000 rpm, 10 min,20 °C), filtered through a Buchner funnel, and stored at −21 °C.

2.2.3.Total phenolics content (TPC), Total tannins content (TTC), and Total flavonoids content (TFC)

TPC was quantified by the method proposed by Alves et al. (2010) with some modifications. Briefly, 0.3 mL of properly diluted extract was mixed with 2.5 mL of the Folin-Ciocalteu (1:10) reagent (Sigma Chemical Co., USA) and 2 mL of 7.5% (w/v) Na_2CO_3. The reaction mixture was incubated for 15 minutes at 45 °C and cooled to room temperature (30 min), before reading the absorbance at 765 nm by double-beam ultraviolet-visible spectrophotometer (Perkin-Elmer UV- Vis λ2, Waltham, Massachusetts, USA). The mixture without the sample was used as blank. The quantification was carried out by calibration curve of gallic acid (2-10 mg L^{-1}) and expressed as mg of gallic acid equivalents (GAE) 100 g^{-1} of coffee silverskin (CS) dry weight (d.w.).

TTC and TFC were determined according to Costa et al. (2014). For TTC, 0.5 mL of the properly diluted sample was mixed with 2.5 mL of the Folin-Ciocalteu reagent (1:10), and 2 mL 7.5% (w/v) Na_2CO_3 was included after 2 minutes. The absorbance was read after 2 hours of incubation (dark at room temperature at 725 nm against a blank (reaction mixture without sample) and the results were expressed as mg of tannic acid equivalents (TAE) 100 g^{-1} CS d.w. by a calibration curve of tannic acid (1-20 mg L^{-1}). Regarding TFC, 1 mL of extract, 4 mL of distilled water, and 0.3 mL of 25% (w/v) $NaNO_2$ were mixed and incubated for 5 minutes in a test tube (10 mL). 0.3 mL of 10% (w/v) $AlCl_3$ was then added, and after 1 min at room temperature, also 2 mL NaOH (4% w/v) was included in the mixture, which was finally made up to volume with ultrapure water. The reaction was carried out at dark and room temperature for 10 minutes, then the absorbance

of samples was recorded at 510 nm versus a blank (without the sample) and the results were expressed as mg of epicatechin equivalents (ECE) 100 g $^{-1}$ of CS d.w.

2.2.4. Quantification of Chlorogenic and Caffeic Acids

Individual concentrations of chlorogenic and caffeic acids in coffee silverskin extracts were quantified following Brzezińska et al. (2023) with some modifications. 5 µL of properly diluted samples were injected into the UHPLC PLATIN blue system (Knauer, Berlin, Germany) equipped with a binary pump, coupled with a PDA-1 (Photo Diode Array Detector) PLATINblue (Knauer, Germany), Knauer blue orchid C18 column (1.8 mm, 100 × 2 mm). Chlorogenic acids were detected at 330 nm while caffeic acid was at 280 nm using formic acid 0.1 % (A) and methanol (B) as elution solvents. The chromatographic separation was conducted at 30 °C with the conditions reported in Table 2. The quantification of chlorogenic and caffeic acids was determined using external standards (Merck, Darmstadt, Germany), and the results were expressed as mg g^{-1} of CS d.w.

Table 2. Elution program used to detect Chlorogenic and Caffeic acid in Coffee Silverskin

Time (minutes)	***Eluent A (%)***	***Eluent B (%)***	***Flow rate (mL/min)***
Initial	98.00	2.00	0.40
3.00	80.00	20.00	0.40
9.00	50.00	50.00	0.40
14.00	50.00	50.00	0.40
16.00	80.00	20.00	0.40
18.00	95.00	5.00	0.40
20.00	95.00	5.00	0.40

2.2.5. Antioxidant activity of coffee silverskin (DPPH and ABTS assays)

The antioxidant activity of the extract was determined by a multitarget approach using the DPPH and ABTS assays.

The DPPH assay was performed as described by Vimercati et al. (2022) with some modifications. An adequately diluted extract (40 µL) was mixed with 2960 µL of a methanolic solution 6 x 10^{-5} M of DPPH (2,2-Diphenyl-1-picrilidrazil) for 30 minutes in the dark at room temperature. The absorbance was measured at 515 nm against methanol with a spectrophotometer (Perkin-Elmer UV- Vis λ2, Waltham, Massachusetts, USA).

The modified protocol proposed by Blige et al. (2022) was used for testing the capacity of CS extract to react with the radical cation, 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) ($ABTS^{+}$), in lipophilic and hydrophilic environments. The ABTS solution and 40 µL of properly diluted extracts were mixed with 2960 µL of ABTS + solution. After 6 min of reaction in the dark at room temperature, the absorbance was read at 734 nm. Ethanol was used as a blank.

The results of the antioxidant assay (ABTS and DPPH) were expressed as µM TE (Trolox Equivalents) g^{-1} of CS, compared with a Trolox (Sigma Chemical Co., USA) calibration curve (2-30 µM L^{-1}).

2.2.6. Microbiological Analysis

The microbiological analyses were performed to evaluate the contamination of coffee silver skin extracts by the procedure described by Nolasco et al. (2022). First, the serial dilutions of CSE were prepared by mixing proper aliquots of CSE with a Ringer solution in a ratio of 1:1, 1:10, 1:100, and 1:1000. Then the dilutions

were inoculated in a selective microbiological culture medium consisting of Plate Count Agar (PCA) and Rose Bengal Chloramphenicol (DRBC) for de-tecting the presence of total bacterial count (TBC), and yeasts and moulds (Y&M) respectively. The enumerations were carried out after incubating at 25 °C of 48 h for TBC and 120 h for Y&M [18,38]. The results were quantified as Log10 CFU mL-1 of CSE.

2.2.7 Statistical analysis

Statistical analyses were carried out through the use of SPSS Software (Version 15.0, SPSS Inc., Chicago, IL, USA), Tukey's post hoc test at $p<0.05$ were used for the Multivariate and One-way analysis of variance (MAVOVA and ANOVA). All analyses are performed in triplicate and expressed as a means ± standard deviation of experimental data.

2.3. Results and discussion

2.3.1. Chemical results of I stage: Total phenolics content (TPC), Total tannins content (TTC) and Total flavonoids content (TFC), Chlorogenic acid, Caffeic acids and Antioxidant Activity

The results reported in Table 3 suggest that TPC, TFC TTC, and antioxidant activity from ABTS and DPPH assays of the extracts obtained by maceration (CSE 1-6), were significant ($p < 0.01$) higher than those detected in the extracts obtained by ultrasound-assisted extraction (CSE 7-12).

Table 3. Chemical Characterization of Coffee Silverskin

Extraction variables				Parameters				
Extraction method	Temperature (°C)	Time (min)	Sample number	TPC (mg GAE 100 g^{-1})	TTC (mg TAE 100 g^{-1})	TFC (mg ECE 100 g^{-1})	DPPH (μM TE g^{-1})	ABTS (μM TE g^{-1})
Maceration	40	30	CSE1	515.02±19.12^{cd}	3.65±0.01^{cde}	106.81±0.35^{b}	16.5±0.79^{bc}	66.92±3.15^{abc}
		60	CSE2	656.14±38.54^{c}	3.22±0.99^{e}	101.48±2.38^{bc}	16.64±0.78^{bc}	69.95±5.37^{ab}
		120	CSE3	546.04±23.29^{cd}	4.90±0.19^{b}	125.16±7.79^{a}	15.91±0.66^{bc}	67.42±5.91^{abc}
	60	30	CSE4	1586.41±25.51^{a}	4.95±0.24^{ab}	111.39±0.57^{b}	19.97±1.31^{ab}	69.46±0.49^{ab}
		60	CSE5	1678.83±25.54^{a}	5.55±0.83^{a}	131.33±0.57^{a}	23.35±0.04 ^{a}	79.63±4.12^{a}
		120	CSE6	1167.61±41.65^{b}	2.52±0.01^{f}	101.45±6.80^{bc}	18.36±2.83^{bc}	70.72±1.36^{ab}
Ultrasound	40	30	CSE7	564.42±5.92^{cd}	4.05±0.12^{cd}	91.75±1.33^{cd}	14.67±0.75^{c}	57.82±1.89 ^{bc}
		60	CSE8	522.94±64.35^{d}	3.48±0.22^{de}	70.06±0.21^{ef}	16.72±2.01^{bc}	63.36±3.22^{abc}
		120	CSE9	593.33±19.67^{cd}	4.14±0.23^{c}	84.02±3.56^{d}	15.41±0.38^{bc}	59.92±2.05^{bc}
	60	30	CSE10	404.36±19.65^{ef}	2.20±0.26^{f}	80.67±1.58^{e}	19.61±0.78^{bc}	67.76±7.51^{abc}
		60	CSE11	404.85±8.12^{ef}	2.46±0.11^{f}	64.06±1.67^{f}	17.19±1.76^{bc}	59.92±1.42^{bc}
		120	CSE12	386.94±4.99^{f}	2.45±0.12^{f}	84.15±0.54^{d}	18.65±0.73^{abc}	53.66±3.21^{c}
Sign.				**	**	**	**	*

Different letters in the column are significantly different by Tukey's post hoc test. Abbreviation: Sign., significance; ns, not significant; **Significance at $p < 0.01$; *Significance at $p < 0.05$.

Although the ultrasound-assisted extraction technique is considered efficient for the recovery of valuable compounds from many by-products, in the case of Coffee silverskin its efficiency was significantly lower compared to maceration extraction. The results obtained of the I stage of CS extraction agree with previous studies on bioactive compounds recovery from bergamot (Gattuso et al., 2023), apples (Pando Bedriñana et al., 2024), pears (Ferreira et al., 2023), and grapes (Rayaroth et al., 2016) by-products. The drastic loss of antioxidant compounds during the ultrasound-assisted extraction process was due to the synergistic action among sound wave frequency, the chemical structure of compounds, time, temperature, and solvent, promoting an unstable cavitation phenomena generation

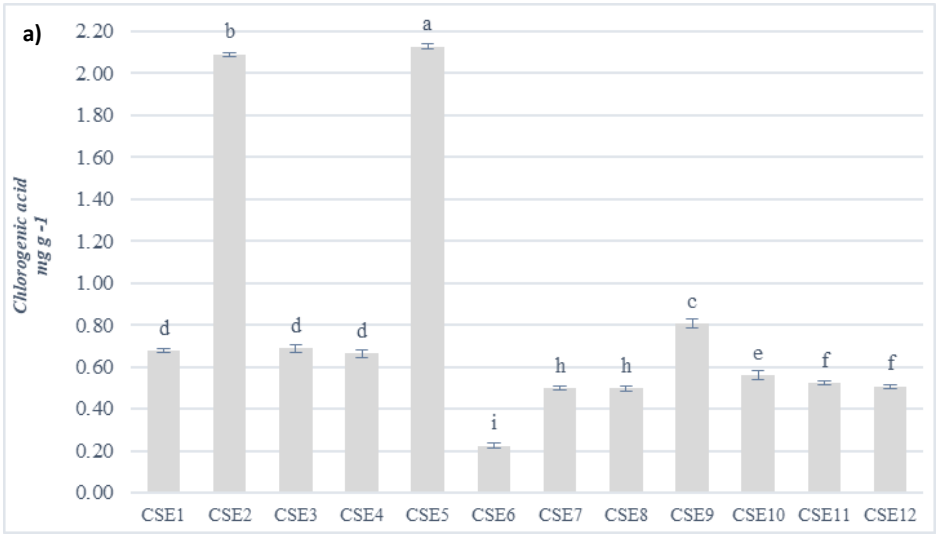
and hence leading to the oxidation of sensitive compounds, as already observed by Rayaroth, et al. (2016). The process of unstable cavitation is a burdensome phenomenon observed especially using hydroalcoholic solvents resulting in the generation of unstable bubbles that collapse to favor the generation of the hydrogen ions (H^+) and hydroxyl radical (OH^+), which can mediate solvent and solute decomposition, polymerization and degradation of polyphenols (Masuda et al., 2015). This decrease is even more severe if high temperatures are reached and the extraction is prolonged for a long time as observed in our study (Table 3).

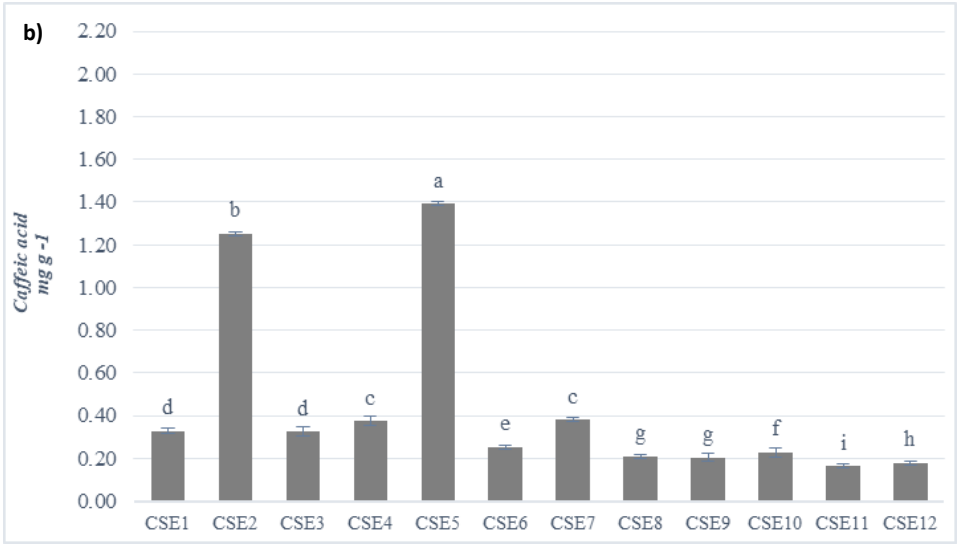
Besides the extraction method, the tests conducted in stage I also hinted that temperature and time play a key role in the extraction efficiency. The increase from 40 to 60 °C significantly improved ($p < 0.01$) the extraction efficiency, assisted by the rise in the extraction time from 30 to 60 minutes (Table 3). In contrast, long extraction times from 60 to 120 minutes adversely affected the extraction efficiency in all samples, due to oxidation and hydrolysis phenomena of antioxidant compounds, as already reported by Garcia-Salas et al. (2010), Carrera et al. (2012) and Baiano et al. (2023).

Regarding the quantification of chlorogenic and caffeic acids, the most representative phenolic acids in coffee silverskin, as reported in Figure 2. The extraction by maceration denoted a significant ($p < 0.01$) higher recovery efficiency in CS2 (1.25 mg g^{-1} caffeic acid and 2.08 mg g^{-1} chlorogenic acid) and CS5 samples (1.39 mg g^{-1} caffeic acid and 2.14 mg g^{-1} chlorogenic acid). In contrast, all other extractions showed a significant decrease consistently with the other detected parameters. Chlorogenic acid and Caffeic acid are often more effectively extracted by maceration than ultrasound for several chemical and mechanical reasons linked

to the nature of the molecules themselves and the structure of the raw material. In maceration extraction, the slow diffusion of chlorogenic and caffeic acid in the solvent preserves their integrity while the ultrasound-induced cavitation generates overheating and a physical force responsible for causing substantial degradation, as just reported by Guglielmetti et al. (2017).

Figure 1. Quantification of Chlorogenic acid (a) and Caffeic acid (b) in CS.





Different letters are significantly different assessed by Tukey's post hoc test ($p < 0.05$). For the sample name abbreviations see Table 3.

The multivariate analysis (Table 4) evidenced the significant effect of extraction variables investigated on the recovery of bioactive compounds. Only the time of extraction (and its single combination with the other variables) did not affect the antioxidant capacity of the samples.

Table 4. Multivariate statistical analysis of different extraction methods

Extraction Variables	*Parameter*						
	TPC	TTC	TFC	ABTS	DPPH	Caffeic Acid	Chlorogenic Acid
Extraction Method	**	**	**	**	*	**	**
Temperature	**	**	**	n.s	**	**	**
Time	**	*	*	n.s.	n.s	**	**
Extraction Method*Temperature	**	*	**	n.s.	n.s.	**	**
Extraction Method * Time	**	**	**	n.s.	n.s.	**	**
Temperature*Time	**	*	*	n.s.	n.s.	**	**
Extraction Method*Temperature*Time	**	**	**	*	*	**	**

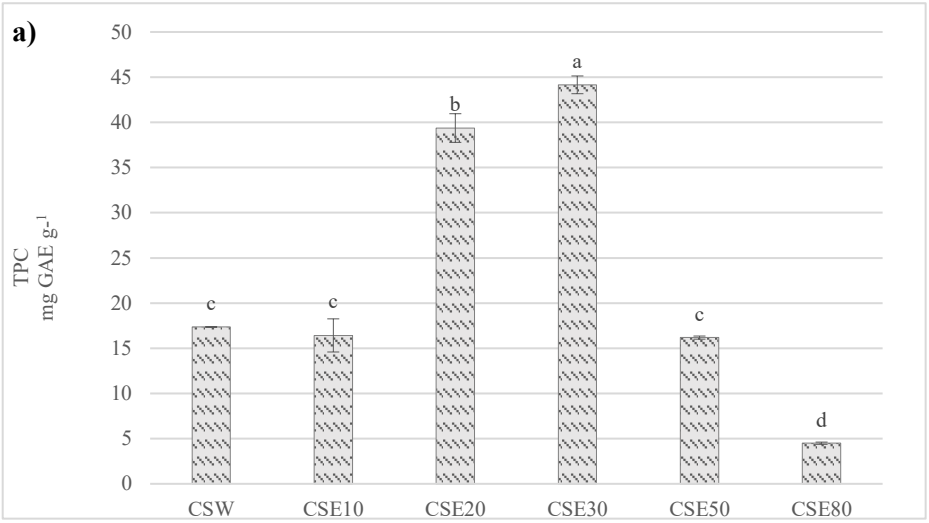
Abbreviations: ns, not significant; **Significance at p <0.01; *Significance at p <0.05 by Tukey's post hoc test.

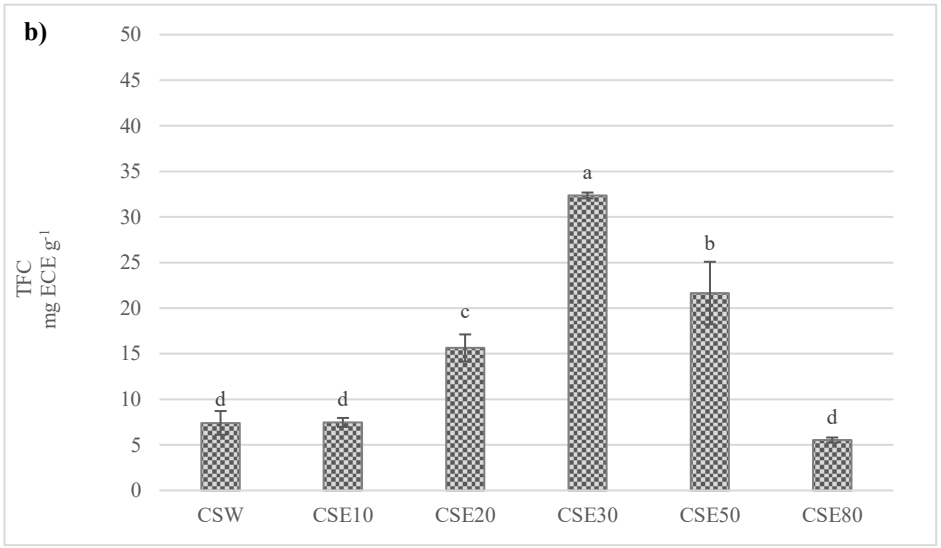
The overall results obtained in stage I suggested that optimal conditions for the maximum recovery of bioactive compounds with high antioxidant activity from CS were a temperature-controlled maceration extraction at 60 °C for 60 minutes.

2.3.2. Chemical results of II stage: Total phenolics content (TPC), Total tannins content (TTC) and Total flavonoids content (TFC), Chlorogenic acid, Caffeic acids and Antioxidant Activity

The observed results in total phenolic, flavonoid, and tannin content suggested that despite the promising extraction yields obtained in stage I using

a hydroalcoholic mixture (ethanol: water 50:50) (table 3), the decrement of ethanol percentage in the mixture ranged from 20%-30%, (CS20 and CS30 samples) involved a significant ($p < 0.01$) increase of bioactive (of 55%) The mixture with a higher ethanol concentration ranged from 50-80%(CS50 and CS80 samples) led a drastic reduction of these valuable compounds. (Figure 2).





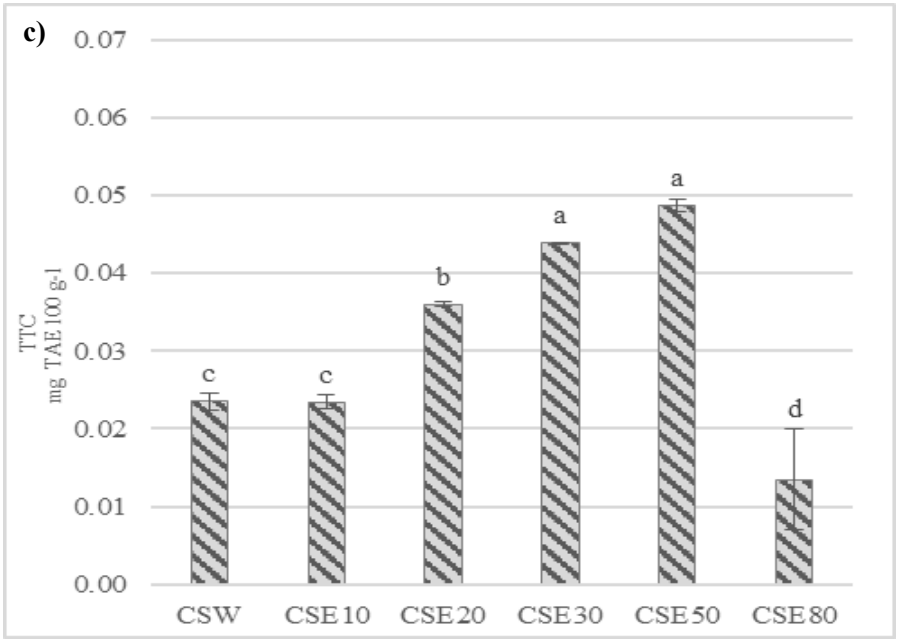


Figure 2. Quantification of Total Phenolics Content (TPC) (a), Total Flavonoids Content (TFC) (b), and Total Tannin Content (TTC) (c). Different letters are significantly different by Tukey's post hoc test. Abbreviations: CSW (H2O solvent), CSE20 (EtOH 10% solvent), CSE30 (EtOH 30% solvent), CSE 50 (EtOH 50% solvent), CSE 80 (EtOH 80% solvent).

The highest TPC was recorded in the sample CSE30 with values of 44.15 mg GAE g^{-1}, followed by CSE20 with values of 39.37 mg GAE g^{-1}. However, experimental data for TFC and TTC showed that the sample CS50 had a significantly higher ($p<0.01$) content of these compounds than the sample CSE20 while the value recorded for the sample CSE30 remained higher than the others. These different recovery trends of the analyzed compounds were related to the complex and varied stereochemistry of the molecules as well as the influence of the solvent in assisting the recovery of different compounds present in CS such as caffeic acid, chlorogenic acids, quercetin, and epicatechin (Silva et al., 2021; Nzekoue et al., 2020). The results of this study showed that hydroalcoholic solutions with 20-50% ethanol (CSE20, CSE30, and CSE50) were particularly

effective in extracting the phenolic compounds present in the CS, favoring a more complete recovery of antioxidants than the use of ethanol or water alone or in the mixture at 10% and 80% (CSW, CSE10, and CSE80). Specifically, the hydroalcoholic mixture consisting of ethanol-water (30:70) (CSE 30) increased the diffusion rate and the speed of solution of the compounds in the solvent more consistently than other more expensive and difficult-to-apply extraction systems such as solid-state fermentation with Penicillium purpurogenum (Palomino Garcia et al., 2015), supercritical (Narita et al., 2012) and ultrasound extraction (Wen et al., 2019).

The influence of the extraction solvent on the main phenolic compounds present in CS was also clearer by the quantification of chlorogenic and caffeic acid performed by the UHPLC system (Figure 3).

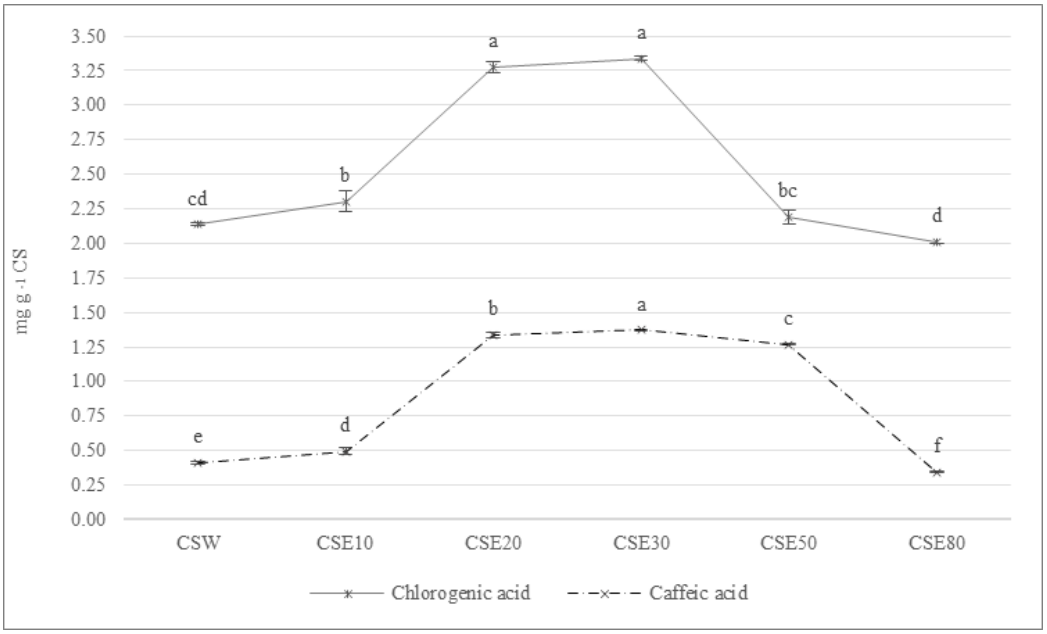


Figure 3. Quantification of Chlorogenic acid and Caffeic acid. Different letters are significantly different by Tukey's post hoc test. The sample name abbreviations are reported in Figure 2.

The chlorogenic and caffeic acid recovery efficiency was directly proportional to the increase in ethanol concentration up to 30% and inversely proportional from 50% to 80% (Figure 3). Consistent with other studies focused on the recovery of phenolic compounds from vegetable matrices, moderate concentrations of ethanol allow to create an ideal extraction environment, by promoting the equilibrium of the solvent polarity and consequently optimizing the rate of diffusion of the compounds in it. The peculiar chemical structure of phenolic compounds including an aromatic ring (no polar) linked to one or more hydroxyl groups (polar) determines the change in polarity of the compounds themselves as already reported by Galanakis et al. (2013). The affinity of the extraction solvent (intermolecular forces that occur between phenolic compounds and the solvents) to the stereochemistry (polar and non-polar groups) of phenols leads to a higher recovery efficiency. Hydroalcoholic solutions allow both polar and apolar compounds to be extracted efficiently, thanks to the balance between water (polar) and ethanol (less polar) (Galanakis et al. 2013); the aqueous fraction allowed the solubility of the polar groups present within the molecules while ethanol eased the extraction of the less polar groups.

These conditions were decisive in the extraction carried out with hydroalcoholic mixtures of 50% ethanol (CSE50) where it was noted that, despite maintenance of high values of caffeic acid, the chlorogenic acid values differed significantly ($p < 0.01$) from samples obtained with hydroalcoholic mixtures composed of 20% and 30 % ethanol, samples CSE20 and CSE30 respectively. Consistent with the results obtained for TPC (figure 2), the qualification of Chlorogenic and Caffeic acids results suggest that the optimization

of the extraction efficiency was found for the CS30 sample with values of 3.34 mg g^{-1} and 1.37 mg g^{-1} for chlorogenic and caffeic acid respectively. These results were higher than those obtained using another no-food grade solvent such as methanol, methanol/water mixtures (50:50) (Zeing et al., 2020), and isopropanol 60% (Murthy at al., 2012).

The ABTS and DPPH assay were used to obtain more accurate and representative results for the overall antioxidant efficacy of CS extract. DPPH mainly measures electron transfer whereas ABTS measures electron and hydrogen atom transfer. Therefore, the combined use of these assays allows for the covering of different types of antioxidant mechanisms that are useful for detecting a wider range of antioxidants, including hydrophilic and lipophilic molecules.

Table 4. Antioxidant activity of different CS extract samples

Samples	DPPH (µM TE g^{-1})	ABTS (µM TE g^{-1})
CSW	18.31±1.95 ^{c}	71.87±9.89 ^{c}
CSE10	27.47±1.51 ^{b}	92.12±0.52 ^{b}
CSE20	26.11±0.62 ^{b}	99.79±0.49 ^{a}
CSE30	33.62±1.49 ^{a}	98.13±2.99 ^{a}
CSE50	25.03±0.13 ^{b}	78.23±3.10 ^{c}
Sign.	**	**

Abbreviations: ns, not significant; **Significance at p <0.01; *Significance at p <0.05 by Tukey's post hoc test. The sample name abbreviations are reported in Figure 2.

Increasing levels of antioxidant activity for DPPH and ABTS were found in all extracts obtained using a hydroalcoholic mixture (from 10 to 30% ethanol) with ABTS assay more efficient than DPPH assay. Polyphenols, phenolic acids, and melanoidins are the most reactive compounds in coffee by-products, being

particularly efficient in eliminating the free radical ABTS, thanks to their chemical structure that promotes the stabilization of the radicals (Arya et al., 2021). In particular, the combination of hydroalcoholic solvents allows for improved recovery of these compounds, since water and ethanol can optimize the extraction of both polar and semi-polar molecules. This experimental data agreed with previous results obtained by Ballesteros et al. (2014) whereby it was shown that the antioxidant compounds in CS are more soluble in less polar organic solvents of water such as hydroalcoholic mixtures. Indeed, by observing the experimental data it is possible to see that the antioxidant activity is less expressed after extraction with high concentration of water or ethanol. The experimental data obtained in this study denoted that CS possessed an antioxidant activity comparable to a lot of fresh fruit (Almeida et al., 2011) and higher than other by-products (Zeng et al., 2023) of the food industry already recognized as a source of antioxidant compounds. This evidence suggests that CS may be used in the food sector as a substitute for natural anti-irritants or for the formulation of high-value-added foods.

Regarding the microbiological analysis in both Stage I and Stage II, no evidence of microbial presence was found (data not shown): it is plausible that the high temperature of the roasting process, the low moisture content of CS (less than 10%) (Toschi et al., 2014), the storage condition of row material (Martuscelli et al., 2021) and the extraction conditions allow inhibition of microbial growth.

4. Conclusions

The results of this work can be useful to exploit an industrial by-product through adequate extraction for the recovery of compounds with high added value. The maceration proposed in this study could be considered a sustainable and low-cost bioactive extraction from coffee silverskin replicable in industrial realities. In addition, the use of hydroalcoholic solvents consisting of only 30% ethanol could promote the use of the extract, as obtained, in the food sector without having to resort to difficult and expensive instruments for solvent removal.

In conclusion, with the increasing demand for functional foods and the preference for natural additives in processed foods, coffee silverskin offers important application prospects in food processing.

The results are the first point of a more thorough study to understand the best conditions for the use of this by-product and how to develop new formulations in which the coffee silverskin could play a positive role in quality control and stability, in compliance with sustainable food production.

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Chapter 3 Use of Coffee roasting by-products (*Coffee Silverskin*) as a natural preservative for fresh-cut fennel slices

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This work is being revised by the authors and will be submitted to a journal for publication.

Abstract

Coffee roasting by-product, coffee silverskin, represents a serious problem of environmental pollution, but it is also an interesting source of chemical compounds, that can be recovered and used in the food industry to improve the physical, chemical, and sensory properties of a wide range of food products. This study aimed to evaluate the effect of the coffee silverskin extract (CSE) used to preserve the storage and the qualitative decay (browning, loss of firmness, loss of aroma, and valuable chemical compounds) of fresh-cut fennel during 14 days of storage at 4°C. The experimental plan evaluated two dipping solutions with coffee silverskin extract (5% and 10%)compared with a conventional dipping in 2% ascorbic acid and a control dipping in water. The use of coffee silverskin in the dipping of fresh-cut fennel processing permitted an increase in the phenolic (chlorogenic and caffeic acids) content for up to 14 days with good sensory acceptability and physico-chemical and microbiological characteristics.

Keywords: Agri-food waste; Coffee silverskin; fresh-cut fennel slices; storage quality.

3.1 Introduction

Modern society faces a serious problem related to the sustainable management of by-products from food processing. It is imperative to find quick and effective solutions to encourage their re-use in the view of a rapid transition towards an economy that complies with the goals proposed in "2030 Agenda" to encourage zero-waste production as well as the green economy (O. D. D. S. 2015; Gustavsson et al. 2011). Similarly, today consumers have increased the demand for natural additives to preserve the physico-chemical characteristics of foods as "natural" and "sustainable". These reasons lead scientific research towards the recovery of bioactive compounds from industrial by-products and their use in food applications such as perishable vegetables to prevent the rapid loss of quality during storage (Jiang et al., 2021; Chen et al., 2024).

Coffee silverskin (CS) is the exclusive by-product of the coffee roasting process (Behrouzian et al., 2016) whose production increased exponentially in modern times due to a substantial increase in demand for coffee and all the commercial products associated with it (Boninsegna et al., 2024). The disposal issues of CS constitute a profound environmental trouble since its chemical composition could be harmful to groundwater, soil microflora, and greenhouse gas production (Eckhardt et al., 2022). However, several studies have indicated CS as the source of countless bioactive compounds with strong antioxidant activity, with chlorogenic and caffeic acid among the most representative ones (Lober et al., 2022; Nolasco et al., 2022; Zengin et al. 2020; Arya et al. 2021). The bioactive compounds present in CS were characterized by complex and varied chemical structures (Wen et al. 2019; Costa et al. 2014) therefore their recovery was a critical operation that

required many attempts to identify the right balance between the method used and the extraction variables (Galanakis et al. 2013). The bioactive compounds present in CS are recognized to be able to improve the characteristics of foods and make, at the same time, beneficial effects on the health of the final consumer (Bertolino et al., 2019; Vimercati et al. 2022). Therefore, CS powder and CS extracts have already been used for the formulation of various enriched foods, such as drinks (Martinez-Saez et al., 2014), cakes (Ateş et al., 2019), cookies (Gocmen et al., 2019), yogurt (Bertolino et al., 2019), candies (Boninsegna et al., 2024), sausages (Thangavelu et al., 2022). In addition, was already reported the safety of CS since they present no carcinogen risk and very low or no presence of toxic substances such as ochratoxin A (OTA), pesticides, and polycyclic aromatic hydrocarbons (IPA) (Bertolino et al. 2019). However, the effect of CS compounds on the physical, sensory and microbiological characteristics of highly perishable food products, such as fresh-cut vegetables is still poorly studied.

Fennel (*Foeniculum vulgare Mill. subsp. vulgare*) is a winter vegetable, typical of the temperate Mediterranean regions among which Italia is the largest producers in Europe (Capotoro et al., 2018. In the markets, the fennel is generally sold in the whole form ("*grumulo*"), because the proposal as fresh-cut fennel is prevented by the characteristic rapid browning after cutting, due to the polyphenoloxidase enzyme activity (Escalona et al. 2005), the loss of chlorophylls and b (Adams et al., 2010; Zhang et al., 2022) and the rapid dehydration of tissues (Castro-Ibáñez et al., 2017; Saini et al., 2017). In addition, the minimal processes to which fresh-cut vegetables are subjected expose them to a greater risk of spoilage micro-organisms proliferation causing rapid tissue deterioration and health dangers

for the consumers (Wang, L., & Teplitski, 2023). The visual appearance, firmness and typical freshness taste are the main discriminants for consumer acceptability of fresh vegetables, in particular fennel. Several chemical compounds are reported to have inhibitory effects on enzymatic browning, such as ascorbic and citric acid used alone or in combination (Suttirak et al. 2010). Capotorto et al. (2018) and Albenzio et al. indicated that the process of browning fresh fennel can be slowed down by dipping it in a solution of citric acid and ethanol. Valentino et al. (2023) reported that also the application of active coating based on sodium caseinate prevent the faster decay of fresh cut fennel slices up to 15 days of storage. Regarding the typical freshness aroma, the principal cause was indicating the changes in organic acid content during storage, normal process that is speeded up in ready-to-eat vegetables due to stress resulting from cutting and the physiological responses associated with it (high respiration rate, weight loss, decreased sugar (Escalona et al., 2005). Nevertheless, the formulation with extracts derived from food by-products, such as the CSE extract proposed in this study, it has not yet been proven as a sustainable and innovative solution to encourage their reuse and replacement with synthetic additives and more complex storage techniques that are expensive and costly to implement.

The main aim of this study was to test the effect of Coffee silverskin extract diluted in the dipping step of fresh-cut fennel processing. Specifically, its efficiency was evaluated in preserving the chemical, physical, microbiological and sensory characteristics of fennel slices.

The results of this study could encourage the valorisation of coffee silverskin, with the obtaining of a new natural additive for food processing, determining also a new life for coffee roasting industry by-products.

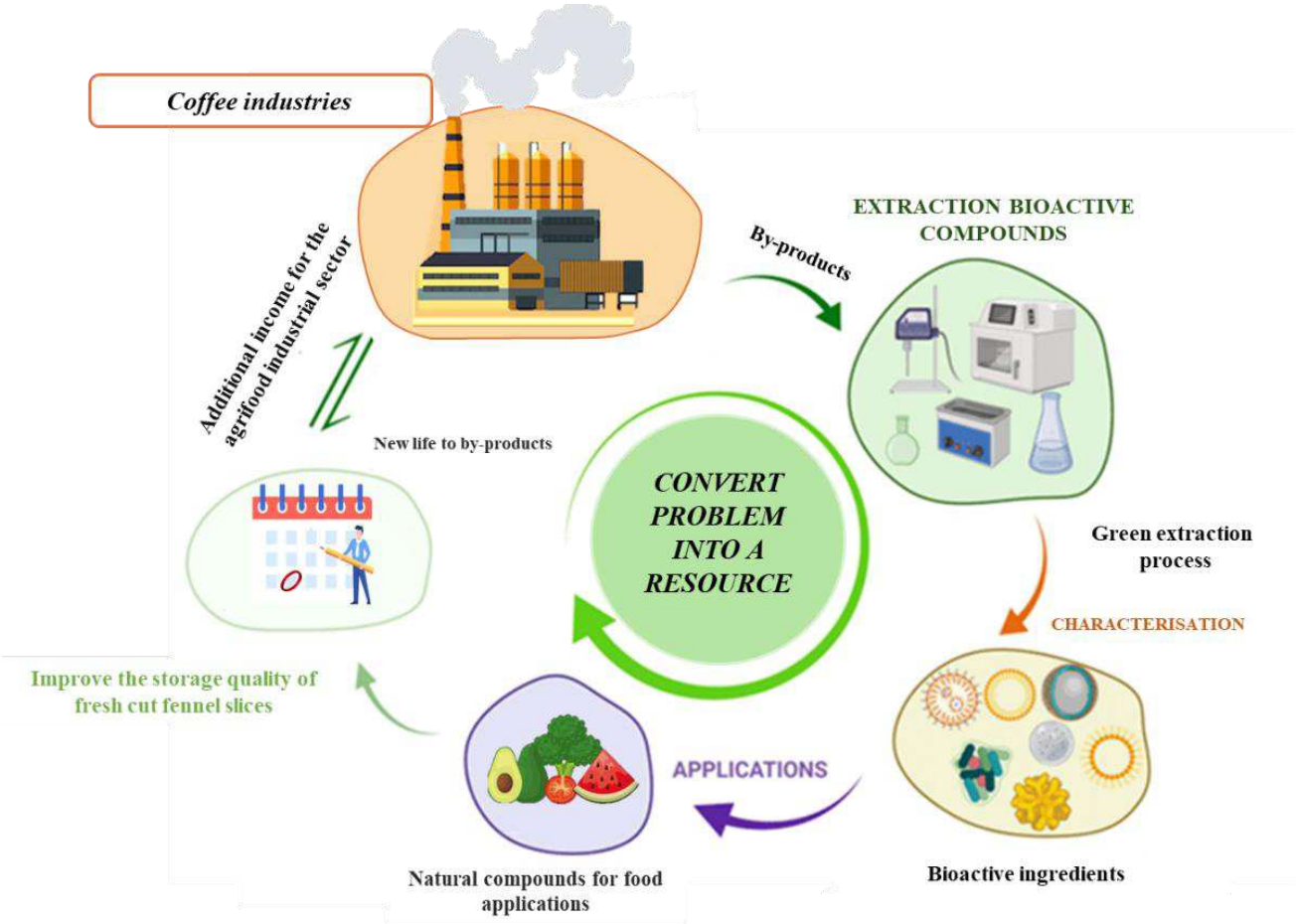


Figure 1. Graphical representation of the principal aims of this study

3.2 Material and Methods

3.2.1 Extraction of bioactive compounds from coffee silverskin

Coffee silverskin (CS) was supplied by the Mauro S.p.A. Coffee industry (Reggio Calabria, Italy) as a blend of Coffea Arabica and Coffea Canephora (variety Robusta) 50:50. CS was transferred to laboratory of Food Technologies of University "Mediterranea" of Reggio Calabria (Italy) and then submitted to the

preliminary treatments: dehydration at 50 °C until 10 %of moisture, grinding and homogenization. The extraction of bioactive compounds was performed at 60 °C for 60 minutes by maceration of the CS sample with a hydroalcoholic mixture (water:ethanol, 70:30, solvent:sample 10:1) The obtained extract (CSE) was thus centrifuged(NF 1200 R, Nuve, Ankara, Turkey) (6000 rpm, 10 min, 20 °C), filtered on paper (0.45 mm) and stored at -21 °C

The chemical analyses of coffee silverskin extract were conducted in triplicate and expressed as a means ± standard deviation.

3.2.1.1. Chemical characterization of CSE

The total content of phenolic compounds (TPC) was detected by a Folin–Ciocalteu method reported by Boninsegna et al. (2024). In a test tube, 2.5 mL of Folin-Ciocalteu reagent (10% v/v), 0.3 mL of appropriately diluted (1:20) CSE, and 2 mL of Na_2CO_3 solution (7.5% w/v) were mixed in a test tube for 15 minutes at 45 °C. The mixture was let react for 30 minutes at room temperature before reading the absorbance by a double-beam UV spectrophotometer(Perkin-Elmer UV- Vis λ2, Waltham, Massachusetts, USA) at 765 nm. The reaction mixture without a sample was used as blank. Results were expressed as mg of gallic acid equivalents (GAE) L^{-1} of CSE using a calibration curve of gallic acid (2-10 mg L^{-1}).

Total tannin contents (TTC) and total flavonoid contents (TFC) were determined according to Costa et al. (2014). The reaction mixture to quantify the TTC was prepared by mixing 2.5 mL of Folin-Ciocalteu reagent (10% v/v), 0.5 mL of CSE, and 2 mL of Na_2CO_3 solution (7.5% w/v) then, after the incubation of 2 h (dark room), the absorbance was recorded at 725 nm. For TFC, in a graduate test

tube, 0.5 mL of diluted CSE (1:1) was mixed with 0.3 mL of 25% (w/v) $NaNO_2$, and after 10 minutes of incubation 0.3 mL of 10% (w/v) $AlCl_3$. Then, 2 mL NaOH 1M was inserted, and the reaction mixture was brought to a known volume of 10 mL with distilled water. The absorbance was recorded at 510 nm.

The results of TTC were expressed as mg of Tannic acid equivalents (GAE) L^{-1} of CSE using the calibration curve of Tannic acid (1-20 mg L^{-1}), while TFC was expressed as mg of epicatechin equivalents (ECE) L^{-1} of CSE by the use of calibration curve of epicatechin (1-200 mg L^{-1}).

The Quantification of Chlorogenic and Caffeic Acids was performed according to an opportunely modified procedure proposed by Brzezińska et al. (2023). Briefly, 5 μL of diluted (1:10) CSE were injected into the UHPLC PLATIN blue system (Knauer, Berlin, Germany) equipped with a Knauer blue orchid C18 column (1.8 mm, 100 × 2 mm)and a binary pump, coupled with a PDA-1 (Photo Diode Array Detector) PLATINblue (Knauer, Germany) 0.1 % Formic acid (A) and methanol (B) were the elution solvents and the chromatographic separation was conducted at 30 °C at the conditions reported in Table 1. Caffeic and chlorogenic acid were detected respectively at 280 and 330 nm. Their quantification was determined by external standards, and the results were expressed as mg L^{-1} of CSE.

Time (minutes)	*Eluent A (%)*	*Eluent B (%)*	*Flow rate (mL/min)*
Initial	98.00	2.00	0.40
3.00	80.00	20.00	0.40
9.00	50.00	50.00	0.40
14.00	50.00	50.00	0.40
16.00	80.00	20.00	0.40
18.00	95.00	5.00	0.40
20.00	95.00	5.00	0.40

Table 1. Elution program used to detect Chlorogenic and Caffeic acid in Coffee Silverskin

The multitarget approach consisting of the simultaneous use of the DPPH, ABTS, and FRAP assays was used in order to determine the total antioxidant activity of CSE extract. Considering the complexity of the analysed matrix and the different mechanisms of action of the antioxidant compounds present in it, the use of DPPH, ABTS and FRAP allowed to cover mechanisms of action linked to the ability to donate hydrogen atoms, electrons and metal reduction thus restitution a complete measure of the antioxidant activity of CSE.

The ABTS, DPPH and FRAP tests were performed using the methodology proposed by Boninsegna et al. (2024). The results were quantified using a Trolox calibration curve (2-30 mM L^{-1}) and expressed as mM equivalent Trolox (TE) L^{-1} of CSE.

The presence of total bacteria (CBT), yeasts, and molds (L&M) in CSE was determined by the protocol described by Nolasco et al. (2022) and expressed as Log10 colony-forming units (CFUs) mL^{-1} of CSE.

3.2.2 Fresh-cut fennel processing

The fennel (*Foeniculum vulgare Mill. subsp. vulgare*) was supplied from a local market farm situated in Reggio Calabria (Italy), transported to the FoodTec laboratory of the Mediterranea University of Reggio Calabria and inspected to selected for the absence of defects and similar size. The whole fennels were then washed and sanitized under a jet of cold water for 2 minutes and then immersion in sodium hypochlorite solution (100 ppm) for 2 minutes. Then the whole fennels were dried on steel grids for 15 minutes at room temperature.

Subsequently, the fennels were trimmed and cut into slices (1 cm thickness) perpendicularly to the longitudinal axis with appropriately sharpened tools to prevent damage to tissues (Capotorto et al., 2017).

The fennel slices were submitted for 5 min to three different dipping treatments: 2% (v/v) ascorbic acid (AA), 5% (v/v) CSE (CS5), and 10% (v/v) CSE (CS10). Fennel slices dipped in water were used as a control test (CTR).

The obtained samples were then dried (15 min), packaged (200 g) in a PP tray covered with a polyethylene terephthalate/polypropylene (PET/PP) film by heat-sealing machine (VGP 25n, ORVED, Italy) and stored at 4 °C for 14 days.

Sensory analyses were conducted at the beginning and the end of storage.

Chemical and physical analyses were carried out immediately after preparation (time 0 of storage) and after 3, 7, and 14 days of storage.

Figure 2 Fennel slices dipped in CSE treatment and packaged in a PP tray

3.2.3 Qualitative analyses of fresh-cut fennels

3.2.3.1 Sensory analysis

The sensory analysis was conducted by a group of 10 panelists comprised between 21 and 42 years old and with previous experience in the sensory analysis on different food products.

Fresh-cut fennel was evaluated for visual, olfactory and structural attributes as reported by Capotorto et al., (2017) while the combination of visual, olfactory and structural attributes decreed the score related to the total acceptability of fennel samples (overall acceptability). The panelists used a hedonistic scale from 0 to 9 to determine the score. The limit acceptability was fixed at 5 score. The descriptions of attributes and scores are reported in Table 2.

Table 2 Description of attributes and corresponding scores used for sensory analysis of fresh-cut fennel slices.

Attribute	*Description attribute*	*Description score*
Visual		
Appearance	Evaluation related to the perception of fresh and freshly cut vegetables: Intensity of white and brightness, draining of surfaces, sheath and stem intact and free from defects.	0 Total absence of required visual attributes (not acceptable); 5 slight perceptions of (acceptability limit); 9 Presence of visual attributes like as fresh vegetable perception (excellent acceptability).
Browning	Browning on cutting surface, sheath, and stem	0 No browning (excellent acceptability); 5 Modest browning (acceptable limit); 9 Severe browning (not acceptable).
Olfactory		
Aroma	The aroma of freshly cut fennel associated with fresh and herbaceous	0 No presence (not acceptable); 5 Moderate Presence (limit acceptability); 9 Intense aromas (excellent acceptability)
Structural		
Crunchiness	Sensory attributes associated with firmness, fractureability, and density	0 No crunchy (not acceptable); 5 Moderate crunchy (limit acceptability); 9 Intense crunchy (excellent acceptability)
Dehydration	Dehydration of tissues	0 No Dehydration (excellent acceptability); 5 Moderate Dehydration (limit acceptability); 9 Severe Dehydration (not acceptable)

3.2.3.2 Colour and Textural analysis of ready-to-eat fennel slices

The surface colour parameters were determined by tristimulus colorimeter (Minolta CM-700d Spectrophotometer, Konica Minolta, Inc., Sakai, Osaka, Japan) with D65 illuminant using the CIE L* a* b* system reference, where L* was the lightness (0 black and 100 white), a*was red (positive value) or green (negative value) intensity and b* was the yellow (positive value) or blue (negative value) intensity of samples

on the corresponding axis. The measurements were conducted at two points, for each fennel segment on ten segments for each group.

The colour differences (ΔE) after 14 days of storage were estimated by using the equation 1.

Eq. 1

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

The structure analysis was performed using a TA-XT Plus Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK). The hardness of the sample was evaluated by penetration test, following the method suggested by Rizzo et al. (2010) with some modifications. A test was performed by compression, using a 2 mm cylindrical probe with the pre-test speed of 1.00 mm/S, test speed 2.00 mm/S, post-test speed of 10.00 mm/S, distance of 4.0 mm, and trigger force of 5.0 g.

Crispiness of fennel samples was measured by the Volodkevich shear test. The use of a Volodkevich Bite Jaws consent to determine the consistency and crunchiness of samples to be assessed by simulating the action of the front incisors when biting the food. Therefore, previous studies suggest that the obtained data could return an instrumental evaluation comparable to sensorial analysis (Varela er al., 2007; Alvarez et al. 2020). The following variables were used: test-speed 2.00 mm/S and strain 50%. Hardness and Crispness were expressed as force (N) required to cause deformation and subsequent fracture of the sample.

Data acquisition and curve integration were carried out using Exponent software 6.1.4.0 (Stable Micro Systems Ltd., Godalming, UK).

3.2.3.4. Total Soluble solids, pH, moisture and weight loss of fresh-cut fennel samples

The fresh-cut fennel (20 g) were homogenized with an Ultraturrax (T 25 digital, IKA, Staufen, Germany), centrifuged, and filtered by paper filter (0,45 μm filter). Total Soluble solids (TSS, Bx°), and pH determinations were conducted on the obtained juices, following the method suggested by Escalona et al. (2005), respectively by a digital refractometer (Atago Co, Tokyo, Japan) and a pH meter (pH 4, pH 7; Crison Basic 20, Spain) equipped with an ion-selective electrode.

Moisture content was estimated by placing 5 g of fennel sample inside a thermal balance (Sartorius Moisture Analyzer MA37). The results were expressed on a percentage basis by using the Equation 2 (AOAC 1994):

Eq. 2

$$\text{U.R. \%} = \frac{(W_0 - W_1)}{W_0} * 100$$

Where W_0 was the initial weight of the sample and W_1 was the final weight of the sample.

The weight loss was calculated as the difference between the initial and final weights of the fennel samples at different times of storage , by the AOAC standard method (1984), using the following equation 3:

Eq. 3

$$\text{Weight loss (\%)} = \left(\frac{Wi - Wt}{Wi} \right) \times 100$$

where W_i was the initial weight and W_t was the weight at time t.

3.2.3.5. Quantification of Malic, Oxalic and Citric acid

Oxalic, malic, and citric acids were detected by the experimental protocol described by Boninsegna et al (2024).20 µL of fennel juice obtained as reported in 2.3.4 paragraph, was injected in Knauer HPLC Smartline Pump 1000, equipped with a Knauer Smartline UV Detector 2600 and SYNERGY HYDRO-RP (250 mm × 4.6 mm i.d., 4 µm). Potassium phosphate 20 mM at pH 2.9 min was used in isocratic conditions at 22 °C at a flow rate of 0.7 mL. Ascorbic acid was recorded at 254 nm and other organic acids at 210 nm, with results expressed as mg of acid 100 g^{-1}.

3.2.3.6. Quantification of Chlorogenic and Caffeic Acids

10g of fennel samples were homogenized by Ultraturrax with 20 mL of the hydroalcoholic mixture (methanol: water 80:20), under the ice. Then, the mixture was centrifuged at 8000 rpm (NF 1200R, Nüve, Ankara, Turkey) for 10 minutes at -2 °C, and filtered by a paper filter (0,45 µm). The obtained extract (5 µL) was submitted with some modifications to the chromatographic analysis described by Brzezińska et al. (2023) for the determination of chlorogenic and caffeic acid concentrations (mg g^{-1}).

3.2.3.6 Microbiological analysis of ready-to-eat fennel slices

The microbial analysis was carried out according to Boninsegna et al. (2024). The microbiological suspensions were prepared homogenizing 2 g of each sample with 20 mL of Ringer solution by Stomacher (BagMixer® 400 P, Interscience, France) for 2 minutes. Subsequently, the obtained homogenates were serially diluted. Total aerobic mesophilic bacteria (CBT), Yeast and molds, Escherichia coli

e Listeria monocytogenes were isolated using ready-to-use chromogenic plates (Compact Dry, R-Biopharm AG, Darmstadt, Germany).

3.2.4. Statistical analysis

All chemical determinations were expressed as a means ± standard deviation of three replicates. Textural determinations were expressed as means ± standard deviation of twenty measurements for each replicate (twelve) of fennel samples. The analysis of variance (one-way ANOVA) was conducted by applying the *post-hoc* Tukey test at $p < 0.05$ (SPSS software, version 15).

3.3 Results

3.3.1 Coffee Silverskin Chemical Characterisation

The CSE exhibited a high content of antioxidant compounds related to phenolic (TPC), flavonoid (TFC), and tannin (TTC) content (Table 3). The microbiological analysis of CSE did not evidence bacterial, yeast, and mould contamination (data not shown). From these results, CSE was considered useful as a food ingredient in the formulation of dipping solutions for fresh-cut fennel processing.

Table 3 Chemical Characterization of Coffee Silverskin Extract (CSE)

Total bioactive compounds		
TPC (mg GAE 100 mL^{-1})	TFC (mg ECE 100 mL^{-1})	TTC (mg TA 100 mL^{-1})
234.74	18.07	2.94
Phenolic Acids (mg 100 mL^{-1})		
Chlorogenic acid		Caffeic Acid
33.99		15.21
Antioxidant Assays (mmolTE 100mL^{-1})		
ABTS	DPPH	FRAP
33.42	21.99	5.75

3.3.2 Quality Evaluations of Fresh-cut Fennel Slices

3.3.2.1. Sensory analysis

Positive sensory evaluation is crucial to ensuring the success of marketable and consumer acceptability of ready-to-eat fruit and vegetables. The sensory evaluations obtained in this study, for the different types of treatment tried, are illustrated in Table 4.

Table 4 Sensory evaluation of fresh-cut fennel samples

	Days	***CTR***	***AA***	***CS5***	***CS10***	***Sign.***
Appearance	0	6.50± 0.55b	7.50± 0.84ab	7.33±0.82 ab	7.67±0.52 a	*
	14	4.83±0.41b	6.50± 0.55a	6.33±0.52a	6.17±0.41a	**
Browning	0	3.17±0.75a	1.83±0.75b	1.50±0.55b	1.67±0.52b	*
	14	7.17±0.75a	4.67±0.52c	5.00±0.63bc	5.67±0.52b	**
Aroma	0	7.50±0.55	8.00± 0.89	8.00±0.63	7.83±0.75	n.s.
	14	5.33±0.52b	6.50± 0.55a	6.83±0.41a	6.17±0.41a	**
Crunchiness	0	7.5± 0.84	7.83±0.41	7.67±0.52	7.83±0.75	n.s.
	14	4.67±0.52b	5.83±0.75a	6.33±0.52a	5.83±0.41a	**
Dehydration	0	2.50± 0.55a	1.83±0.41ab	1.67±0.52b	1.83±0.41ab	*
	14	7.00±0.63a	6.17±0.41ab	5.33±0.52b	5.50±0.55b	**
Overall	0	7.50±0.55	8.00±0.63	7.83±0.75	7.67±0.82	n.s.
acceptability	14	4.83±0.41b	6.17±0.52a	6.50±0.55a	6.67±0.41a	**

Small letters within a row show significant differences as assessed by Tukey's *post hoc* test. Abbreviations: **, significance at $p < 0.01$; *, significance at $p < 0.05$; n.s., not significant

At the beginning of storage (Table 4 and Figure x), the fennel slices dipped with different pretreatments arose better than no treated samples with high score obtained for CS10, followed by CS5 and AA, while the CTR sample showed a significantly lower score (p> 0.05), these trends were same for appearance, browning and dehydration parameters. In contrast, the aroma as well as the crispness did not show significant differences between treatments, indicating that initially all samples retain these characteristics unchanged.

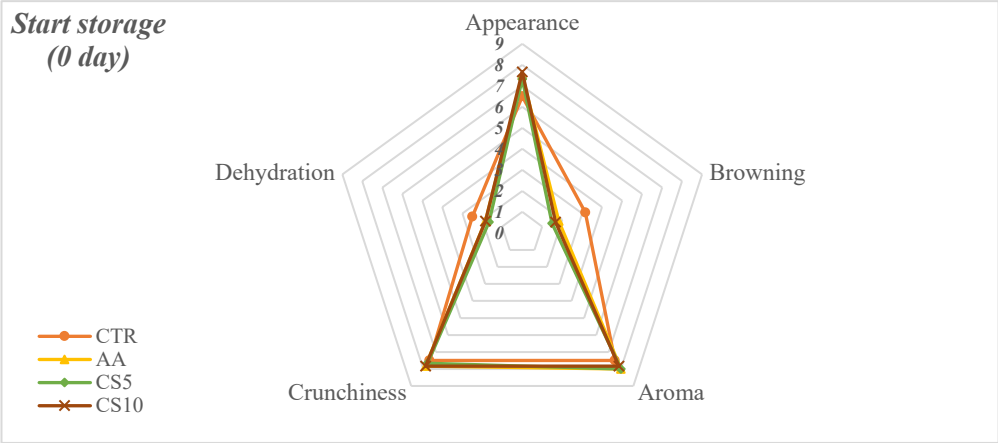


Figure 3 Spider plot of the means scores obtained for the individual sensory attributes at the beginning storage time of the fresh-cut fennel slices

At the end of storage (14 days) (Figure4 and Table 4), there was a general deterioration in sensory quality, with more marked differences between treatments. In particular, the appearance worsened in all samples, but CS10 maintained the best score, followed by CS5 and AA. The other parameters related to browning, aroma, crunchiness, and dehydration also showed a similar trend, suggesting a dependence between the variation of sensory characteristics over time and the treatment applied. The CTR sample showed significantly more marked deterioration than samples dipped in another different pretreatment.

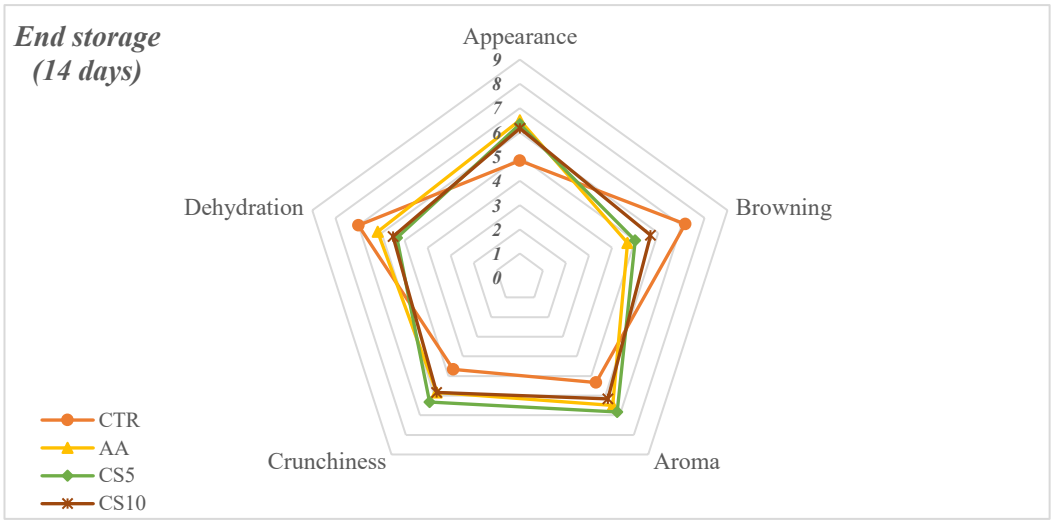


Figure 4 Spider plot of the means scores obtained for the individual sensory attributes at the end storage time of the fresh-cut fennel slices

Overall acceptability scores (Table 4 and Figure 5) followed individual sensory trends. Initially, quality was uniform across treatments, but a decline occurred over time. CS5 and CS10 maintained better appearance, crispness, and aroma, outperforming AA, while CTR showed significant deterioration, falling below the acceptability threshold (score 5).

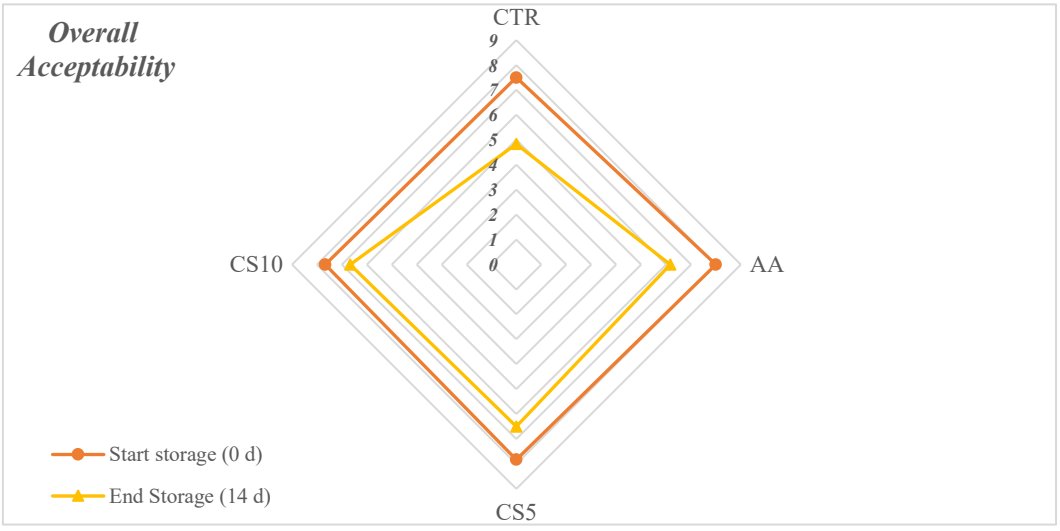


Figure 5 Spider plot of the means scores obtained for the individual sensory attributes at the beginning storage time of the fresh-cut fennel slices

3.3.2.2. Colour and textural analysis

In previous studies, the variation of the L*, a*, and b* parameters was used to estimate the enzymatic browning and consumer acceptability of fresh-cut vegetables after the cutting operations (Barrett et al., 2010).

L* values for all monitoring times, reveal that there was increasing loss in the CTR sample during storage while the maintenance of values was found in the CS5 sample where no significant ($p< 0.05$) difference was revealed up to 14 days of storage.

As shown in Table 5, CS5 and CS10 after the dipping treatment (0 days of storage) showed an increase of a* value than CTR and AA, due to the pigmentation of the extract obtained from coffee by-products. However, a gradual increase in the a* value, from negative to positive values was detected in CTR and AA, and not in CSE and CS10, indicating the colour change toward browning during storage. b* values did not show significant variations during the whole storage period in all the fresh-cut fennel samples.

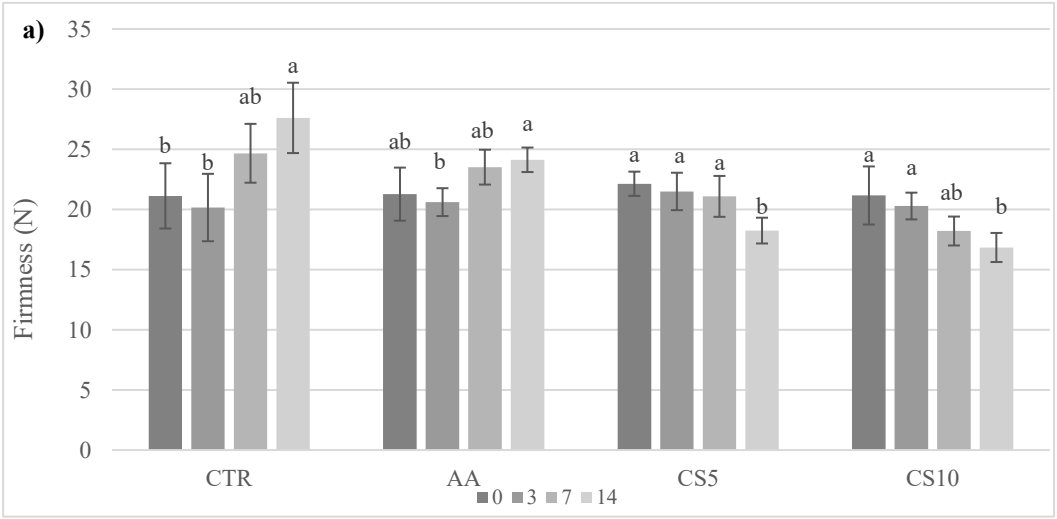
From ΔE value elaboration, it was also confirmed that the most evident chromatic changes during storage were observed on CTR and AA, in the trend of CTR > AA > CS5 > CS 10 with values from 7.09 to 4.96.

Table 5 Colorimetric values of fresh cut fennel slices during the storage period.

Parameter	Sample	Time (days)				Sign.
		0	3	7	14	
L*	CTR	70.27± 2.10 ^{A}	68.90± 2.69 ^{bAB}	69.65± 1.94 ^{bA}	66.55± 1.41 ^{bB}	*
	AA	70.42± 1.18 ^{B}	73.64± 2.25 ^{aA}	73.08± 2.07 ^{aA}	74.36± 2.49 ^{aA}	*
	CS5	73.84± 4.59	73.48± 2.65 ^{a}	73.94± 2.74 ^{a}	75.80± 3.94 ^{a}	n.s.
	CS10	70.15± 1.96 ^{B}	74.85± 2.25 ^{aA}	74.91± 2.10 ^{aA}	75.27± 2.02 ^{aA}	*
Sign.		n.s.	**	**	**	
a*	CTR	-1.1± 0.06 ^{bC}	-0.57± 0.04 ^{abB}	0.29± 0.04 ^{aA}	0.39± 0.02 ^{aA}	**
	AA	-1.06± 0.05 ^{bB}	-0.91± 0.05 ^{aB}	-0.16± 0.02 ^{bA}	-0.13± 0.07 ^{bA}	**
	CS5	-0.74± 0.04 ^{a}	-0.59± 0.09 ^{ab}	-0.66± 0.05 ^{c}	-0.71± 0.04 ^{c}	n.s.
	CS10	-0.51± 0.04 ^{a}	-0.34± 0.02 ^{b}	-0.38± 0.09 ^{ab}	-0.59± 0.05 ^{c}	n.s.
Sign.		*	*	**	**	
b*	CTR	11.82± 3.37	11.47± 2.90	10.93± 2.33 ^{ab}	12.35± 2.64	n.s.
	AA	11.43± 2.91	10.66± 2.95	10.99± 2.32 ^{a}	12.32± 3.23	n.s.
	CS5	11.79± 2.39	11.66± 2.83	10.84± 2.41^{ab}	10.59± 3.83	n.s.
	CS10	11.61± 2.71	8.24± 4.44	9.00± 1.99 ^{b}	11.62± 3.32	n.s.
Sign.		n.s.	n.s.	*	n.s.	

Small letters within a column and capital letters within a row show significant differences as assessed by Tukey's *post hoc* test. Abbreviations: **, significance at $p < 0.01$; *, significance at $p < 0.05$; n.s., not significant.

Regarding the firmness parameter (Figure 6), significant differences were found in the CTR and AA samples already after 7 days of storage, which showed a progressive hardening. CS5 and CS10 maintained instead in the same period their textural properties and tended to be less firm (<20N) at the end of storage (Fig. 5 a). All the samples did not differ from each other during storage for the crispiness parameter (Fig. 6 b).



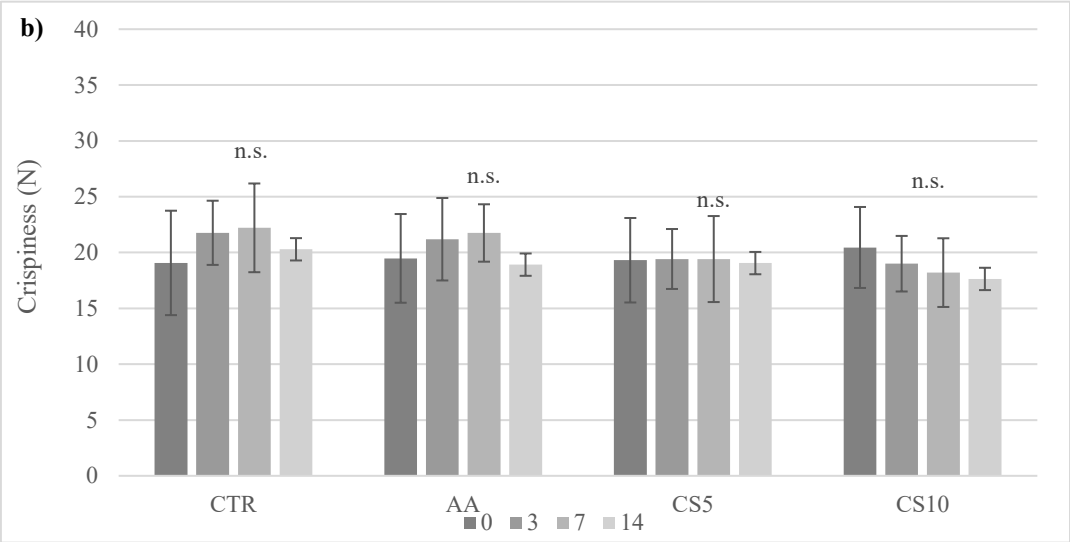


Figure 6 Textural parameter of fresh cut fennel slices during the storage period for firmness (a) and Crispiness (b).Small letters show significant differences as assessed by Tukey's *post hoc* test.

3.2.3.4. Total Soluble solids, pH, moisture and weight loss of fresh-cut fennel samples

Immediately after immersion in dipping solutions, a decrease in pH was found in sample AA (5.93) while CTR, CS5, and CS10 did not show differences with values around 6.1 (Figure 7 a). These experimental data showed the pH value dependence with the immersion solution used. However, during the storage of fresh-cut fennel slices, no significant variations were found up to 7 days of storage while a rise in pH value was found after 14 days of storage, index of qualitative decay of fresh-cut vegetable analysed linked to the loss of organic acids characterizing the chemical composition of fennel. Total soluble solids in samples treated with dipping were higher than CTR samples at the start of storage and then tended to decrease up to 14 days, particularly in CS5 and CS10.(Figure 7 b).

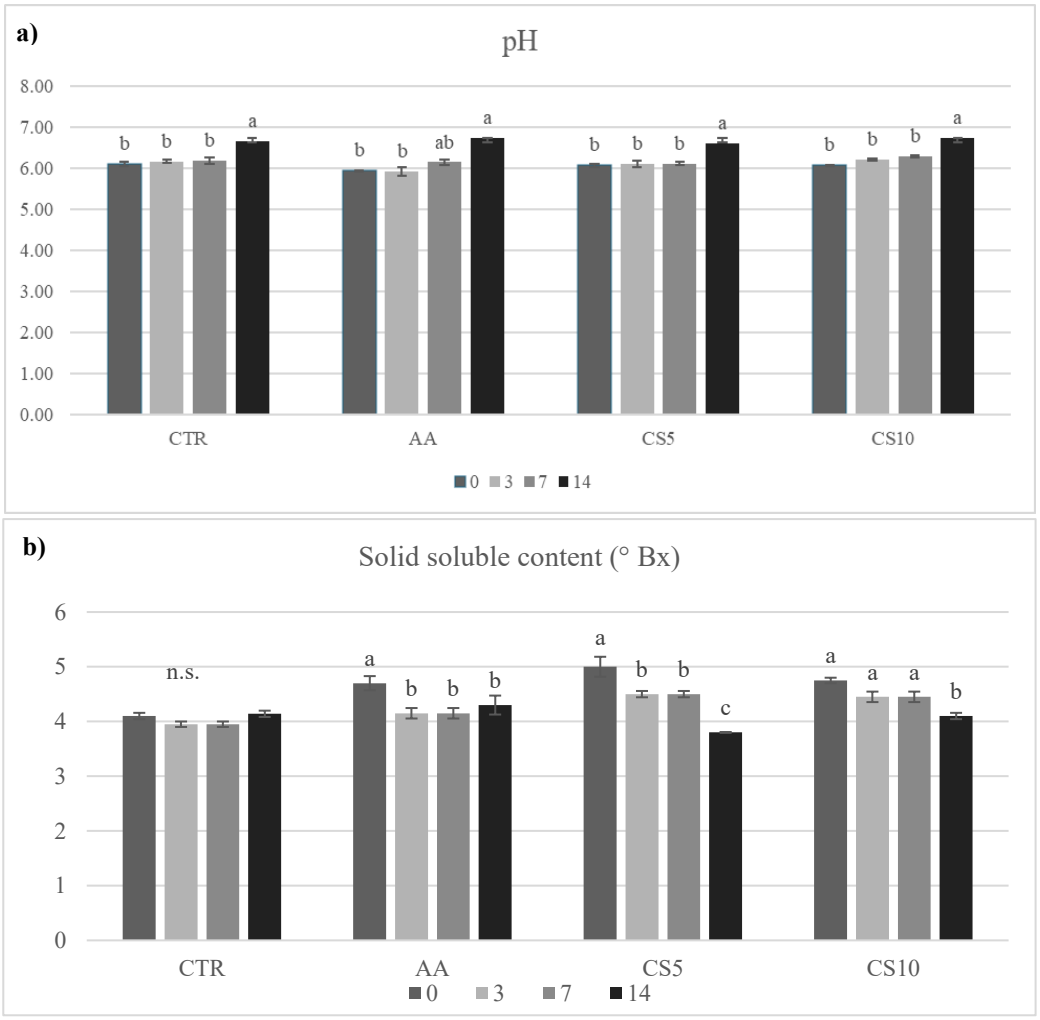


Figure 7 pH values (a) and solid soluble content (b) of fresh-cut fennel slices during the storage period. Small letters show significant differences as assessed by Tukey's *post hoc* test.

The moisture content of samples tended to decrease particularly in the CTR sample (from 96.98 to 92 58 during storage), while it maintained similar percentages in AA, CS5 and CS10 (about 95.90) (data not shown).

During storage, a gradual weight loss was found in all samples (Figure 8) with higher development in CTR than in the other samples.

This phenomenon is mainly due to a slowdown of the metabolic activities discharged by dipping treatments. In particular, a lower weight loss was found in

CS10, followed by CS5 and AA, suggesting a greater maintenance of sensory and structural characteristics, as confirmed by the previous analyses.

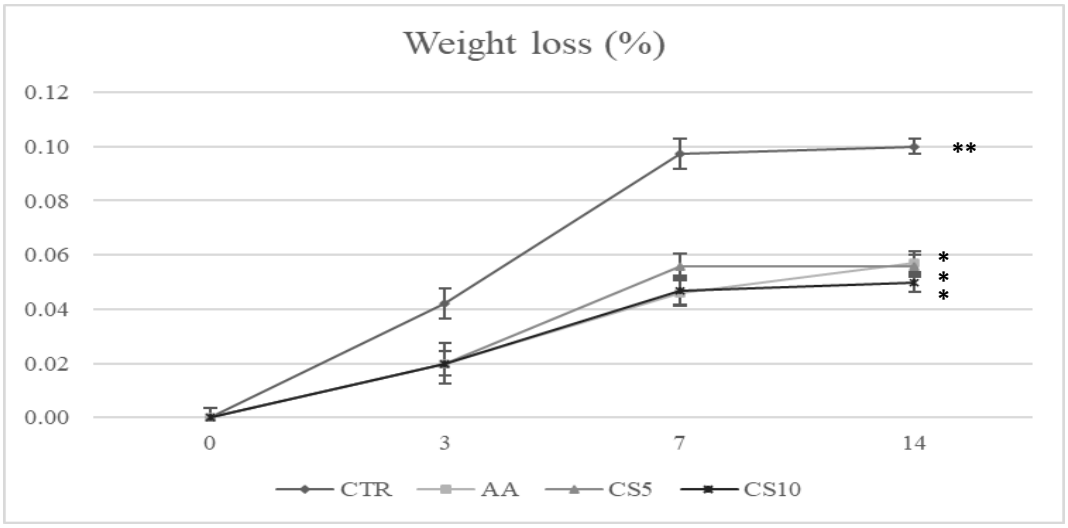


Figure 8 Weight loss of fresh cut fennel slices during the storage period. Abbreviations: **, significance at $p < 0.01$; *, significance at $p < 0.05$; n.s., not significant assessed by Tukey's *post hoc* test.

3.2.3.5. Quantification of Malic, Oxalic and Citric acid

The results shown in Table 6 suggested a marked dependence between the quantity and trends of organic acids and the dipping treatment to which the fennel slices were subjected. A significant dose-dependent effect ($p< 0.05$) was also recorded for fennel slices dipped in both CSE concentrations. A general increase was found at the beginning of storage for oxalic and malic acids following the trend CS10>CS5>AA>CTR while the citric acid in AA samples was similar to the CTR sample and significantly lower ($p < 0.01$) than the CS5 and CS10 samples. These trends were maintained during storage, up to 21 days, with levels of malic and oxalic acid remaining consistent, although with some fluctuations, while for citric acid a more marked variation was observed during storage, especially in the CTR and AA samples. The trends observed in this study suggested a different sensitivity

in the response of organic acid content and their maintenance over time to the treatment used. According to previous studies, the trends followed by organic acids were closely linked to the chemical composition of dipping treatments used as well as to intermolecular interactions and enzymatic reactions which occur during the storage of ready-to-eat vegetables (Galani et al., 2017; Erbaş 2023).

Table 6 Trends of Malic, Oxalic and Citric Acids during the storage of fresh-cut fennel slices.

Parameter	***Sample***	***Time (days)***				***Sign.***
		0	3	7	14	
Malic acid (mg 100g-^{1})	CTR	165.11±0.91 ^{Bb}	174.88±1.88 ^{bA}	165.08±0.94 ^{Bb}	152.34±0.56 ^{Cc}	**
	AA	166.55±1.37 ^{bB}	182.76±0.94 ^{Aa}	171.47±1.88 ^{Bb}	171.02±2.62 ^{bB}	**
	CS5	199.39±3.60 ^{Aa}	183.92±1.30 ^{aB}	182.84±3.38 ^{aB}	178.92±1.00 ^{aB}	**
	CS10	192.13±3.14 ^{a}	180.14±0.21 ^{a}	180.98±1.64 ^{a}	179.32±0.94 ^{a}	**
	Sign	**	**	**	**	
Oxalic acid (mg 100g-^{1})	CTR	150.28±1.82 ^{Bab}	140.51±0.04 ^{Cb}	169.3±4.92 ^{abA}	164.84±9.14 ^{Ba}	*
	AA	156.13±7.42 ^{ab}	147.03±5.36 ^{c}	145.58±7.17 ^{c}	138.00±3.84 ^{c}	n.s.
	CS5	160.15±3.77 ^{ab}	158.88±2.02 ^{b}	155.57±3.76 ^{bc}	161.22±1.23 ^{b}	n.s.
	CS10	187.44±13.62 ^{a}	174.14±0.93 ^{a}	179.58±0.45 ^{a}	192.9±0.38 ^{a}	n.s.
	Sign	*	**	**	**	
Citric acid (mg 100g^{-1})	CTR	165.34±2.11 ^{Ba}	162.9±3.28 ^{aA}	147.22±0.16 ^{Bb}	117.59±4.17 ^{Ac}	**
	AA	146.6±8.28 ^{Ba}	122.6±6.16 ^{Bab}	101.85±0.41 ^{cB}	103.47±7.84 ^{aB}	**
	CS5	186.57±1.95 ^{aA}	181.97±5.77 ^{Aa}	164.27±0.94 ^{abB}	160.29±0.24 ^{bB}	**
	CS10	195.24±5.63 ^{Aa}	180.69±5.28 ^{aB}	187.81±14.83 ^{aB}	173.57±3.87 ^{bB}	n.s.
	Sign	**	**	**	**	

Small letters within a column and capital letters within a row show significant differences as assessed by Tukey's *post hoc* test. Abbreviations: **, significance at $p < 0.01$; *, significance at $p < 0.05$; n.s., not significant.

3.2.3.5. Quantification of Chlorogenic and Caffeic Acids

The trend of chlorogenic and caffeic acid showed that the dipping treatment with 5-10% CSE significantly increased ($p < 0.01$) their presence in slices of fresh-cut fennel and this tendency was found throughout the storage (Figure 8). In particular, CS10 showed the highest content of these phenolic acids up to 14 days of storage.

The observed general reduction of chlorogenic acid was due to physiological processes mediated by key enzymes such as Polyphenoloxidsase (PPO) (Chen et al., 2020). However, the high amounts of chlorogenic and caffeic acid during storage, suggested that immersion in CSE may lead to a significant increase in these valuable compounds involved not only in preventing the rapid decay of vegetables and fruits but also in processes that lead to a positive effect on the health of consumers.

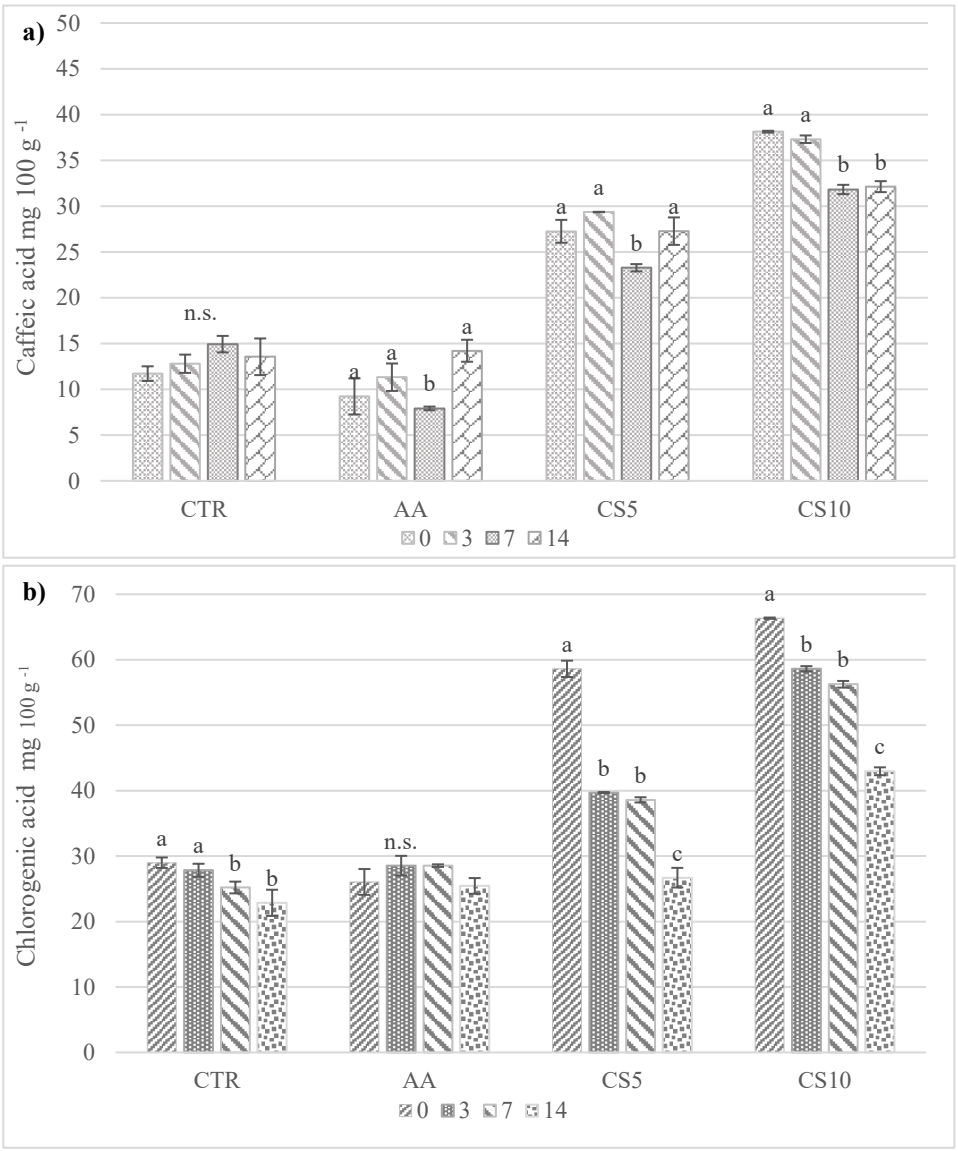


Figure 8 Trends of Caffeic (a) and Chlorogeic (b) Acids during the storage of fresh-cut fennel slices. Small letters show significant differences as assessed by Tukey's *post hoc* test.

3.2.3.6 Microbiological analysis

During the 14 days of storage, all samples showed a gradual growth of CBT. At end-of-shelf life the lowest values were obtained from CS10, followed by CS5. This suggests a dose-dependent CSE effect. Regarding yeasts, no statistical differences were found up to 14 days of storage; these microorganisms increased during storage in all samples. No moulds were detected in all samples analysed until the end of storage. In all samples, *Listeria Monocytogenes* and *E. coli*, were not detected (data not shown).

Table 7 CBT and Yeast during the storage of fresh-cut fennel slices (Log10 CFU g^{-1})

Parameter	***Sample***	***Time of storage***				***Sign.***
		0	3	7	14	
CBT	CTR	3.11 ± 0.02^{D}	4.83 ± 0.11^{C}	5.98 ± 0.18^{Ba}	7.01 ± 0.02^{Aa}	**
	AA	2.99 ± 0.16^{D}	4.79 ± 0.08^{C}	5.28 ± 0.03^{Ba}	6.49 ± 0.03^{Aab}	**
	CS5	3.04 ± 0.12^{D}	4.77 ± 0.21^{C}	5.33 ± 0.09^{Ba}	6.36 ± 0.08^{Ab}	**
	CS10	2.95 ± 0.07^{C}	5.04 ± 0.22^{B}	4.84 ± 0.12^{Bb}	6.37 ± 0.11^{Ab}	**
Sign.		n.s.	n.s.	**	*	
Yeast	CTR	0.00 ± 0.00^{C}	1.79 ± 0.17^{B}	1.89 ± 0.17^{B}	2.84 ± 0.34^{A}	**
	AA	0.00 ± 0.00^{C}	1.95 ± 0.24^{B}	1.86 ± 0.28^{B}	2.85 ± 0.21^{A}	**
	CS5	0.00 ± 0.00^{C}	1.70 ± 0.01^{B}	1.96 ± 0.24^{B}	2.20 ± 0.71^{A}	**
	CS10	0.00 ± 0.00^{B}	1.80 ± 0.17^{A}	1.86 ± 0.28^{A}	2.35 ± 0.92^{A}	**
Sign.		n.s.	n.s.	n.s.	n.s.	

Small letters within a column and capital letters within a row show significant differences as assessed by Tukey's *post hoc* test. Abbreviations: **, significance at $p < 0.01$; *, significance at $p < 0.05$; n.s., not significant

3.4. Discussions

The antioxidant extract obtained from CS showed high levels of bioactive compounds linked to total content in polyphenols, flavonoids, and tannins and high antioxidant activity for the DPPH, ABTS, and FRAP assays. The stereochemistry of the compounds in CS as well as the slow diffusion that takes place during maceration are the basis for obtaining high yields of extraction of valuable compounds with a high antioxidant activity (Nzekoue et al. 2020), this is aided by

a suitable extraction temperature which allows the diffusion rate of compounds in the solvent to be increased without damaging them (Rifna et al. 2023; More et al. 2022). In particular, the synergy between the extraction method, the variables used during the process (temperature and time), and the extraction solvent (30% EtOH) allowed the recovery of valuable compounds such as chlorogenic acid and caffeic acid which have proved to be indispensable in supporting the quality characteristics of fresh-cut fennel slices up to 14 days of storage at 4 °C.

The effects of dipping solutions with CSE and the interactions between the compounds in it to counteract the rapid decay of fennels were particularly appreciable concerning the preservation of the quality index related to sensorial and physical-chemical aspects.

The results of the sensory analysis showed that immediately after treatment no statistically significant differences in overall acceptability were found. These results were to be considered satisfactory as they suggested that CSE does not alter the sensory characteristics of fresh-cut fennel. However, during preservation, a gradual decay of sensory parameters was recorded for all samples with a marked deterioration for CTR followed by AA and better maintenance of all sensory attributes found in CS5 and CS10 (Table 4 Figure 3-5). In particular, the score of overall acceptability recorded at the end of storage revealed that CS5 and CS10 samples differed significantly than CTR and slice samples treated with conventional dipping of ascorbic acid (AA) for better maintenance of appearance (browning), freshness, aroma and a lower dehydration of the tissues. The sensory stability over time was therefore significantly dependent on the treatment and, in the case of CSE samples, the higher scores compared to the other samples tested

were due to the synergy between a higher concentration of organic acids (Galani et al., 2017; Erbaş 2023) and the action of chlorogenic acid (Cheng et al., 2020) that contributed to the maintenance of aroma and to counteract browning respectively. According, the sensory evaluations were congruent with the instrumental results of chemical-physical analyses.

Regarding the colour, a prolonged reduction of lightness (L*) was observed in the storage time of CTR samples while the treated fennels were similar to each other, suggesting the dependence between L* and the applied dipping. During storage, a gradual increase of a* parameter, index of browning, was observed in CTR and AA fennels, while in CS5 and CS10 no significant differences were found. In according, ΔE value, used to compare the instrumental colour data with the visual acceptability of the consumer, confirms the assessments obtained in the sensory analysis by browning and appearance of CS5 and CS10 samples, revealing values ≤ 5, which agrees with Mokrzycki & Tatol, M. (2011) indicated a non-perception of browning by the human eye. The anti-browning effect of CSE dipping solutions was due both to the hydroalcoholic nature of the extract (Capotorto et al.2018), and the high concentration of chlorogenic acid which led to a rearrangement of the PPO secondary structure and a significant decrease in browning and oxidation products up to 14 days of storage (Cheng et al., 2020). Indeed, the presence of CSE in treated fennel slices was clear by quantifying chlorogenic and caffeic acid, the phenolic acids predominating in the coffee roasting by-products (Boninsegna et al., 2024). Immediately after treatment, the CS5 and CS10 samples contained levels significantly higher than CTR and AA showing clear dose-dependent treatment effects (Figure 8).

The firmness parameter (Figure 5), significant differences were found in the treated samples after 7 days of storage. CTR and AA showed progressive hardening due to progressive moisture loss and weight loss (Figure 8), probably in combination also with the action of lignification by the PAL phenylalanine ammoniumlyase enzyme. In contraposition, in CS5 and CS10 samples, better maintenance of firmness was observed for up to 7 days, after which the natural enzymatic activity resulting from cutting operations linked to metabolic stress and their decompartmentalization and action on the cell wall led to a decrease in this parameter (Degl'innocenti et al. 2005). These results also suggested a dependence between the treatment and the type of metabolic response to which the fruit and vegetables go after being subjected to cutting, denoting the activation of several enzymatic pools, already reported by Chen et al. (2024) and Asrey et al. (2024)

The analysis carried out by the variation of total soluble solids, and pH (Figure 7) also confirmed the slowing down of metabolic activity and the better maintenance of treated fresh-cut fennel slices, with the values recorded in thew within the range 4.0-8.0 and 5.5-6.5 respectively, consistent with previous studies concerning the chemical quality of fennel (Rubio et al., 2024). The values of pH increase and decrease in SST are attributable to metabolic activities that are against vegetables subjected to cutting operations and their variations are directly proportional to the intense metabolic activity causing the rapid deterioration (Degl'Innocenti et al., 2005). The best maintenance in these ranges, found for CS samples followed by AA, suggested better maintenance of freshness and initial quality than the untreated sample (CTR).

The results in Table 6 showed an increase in the concentration of the oxalic, malic, and citric acids in the fennel slices treated with CSE and a better retention of these over the storage time. The AA sample showed better retention of malic and oxalic acids and a drastic reduction in citric acid compared to CS5, CS10, and CTR samples. Erbaş (2023), Gong et al., (2022) and Galani et al., (2017) reported that the major causes of the variable tendency of organic acids during the storage of fruit and vegetables were attributed to the degradation of precursor compounds, oxidative stress, stress responses, interaction with micro-organisms and storage conditions. In this study, the trends of the organic acids supported the sensory and physical analyses in explaining the better maintenance of parameters considered in samples CS5 and CS10 since were closely related to the better sensory attribute's perception (Escalona et al. 2006) as well as the better maintenance of textural and colour parameters (Suttirak, W., & Manurakchinakorn, S., 2010).

The experimental data obtained in this study for the quantification of phenolic acids most present in CSE, caffeic and chlorogenic, showed a dose-dependent increase and better retention in fennel slices treated with dipping solution CSE than others (Figure 8). These trends helped to explain the anti-browning, sensorial, and textural response of fennel slices treated with CSE since the Chlorogenic acid and caffeic acid are powerful antioxidants that can delay enzymatic browning and sensory degradation, protecting the cell structure and help to preserve colour and aroma (Cheng et al., 2020, Lattanzio V., 2003). On the other hand, they suggested that a dipping treatment as proposed in this study would allow enriching the chemical composition of fresh-cut fennel slices with valuable compounds allied to human health (protecting oxidative harm, carbonyl stress, accumulation of

advanced glycation end products (AGEs) and prebiotic activity) (Mesías et al.2014; Fernandez-Gomez et al. 2016; Castaldo et al., 2020).

Finally, the microbiological counts in Table 7 reveal that the dipping treatments lead to a significant effect on mesophilic bacteria grown (CBT) from the 7 days of storage. In addition, no molds and bacteria pathogenic to humans (*Listeria Monocytogenes* and *E. Coli*) were detected during the entire storage period of fresh-cut fennel slices. Regarding yeasts, no statistical differences were found up to 14 days of storage; these microorganisms increased during storage in all samples. The results suggested that all fennel slices treated with dipping solutions (AA, CS5, and CS10) were microbiologically safe for up to 14 days of storage with better results obtained for CS5 and CS10. The slowing of microbial growth by ascorbic acid has already been extensively documented in previous studies (Moreira et al. 2006; Capotorto et al.2018; Przekwa et al. 2020) while the effect on microbial flora by CSE, recorded in this study, has not yet been investigated and can be due to the synergy of nature the hydroalcoholic nature of extract (Plaskova et al. 2023) and their chemical composition (Inácio et al. 2023; Ziemah et al 2024; Chaves-Ulate et al. 2024). The biocidal and fungicidal activity of organic acids (malic and citric acid) and phenolic acids (chlorogenic and caffeic) has already been documented for gram-positive bacteria, gram-negative bacteria, and various moulds such as *Pseudomonas* spp., *Escherichia Coli, Salmonella* spp., *Listeria* spp., *Candida* spp., and *Aspergillus* spp. (Castro Diaz et al., 2025; Kabir et al., 2014; Feng et al., 2010; Karpiński & Ożarowski 2024). The action of these molecules is mainly due to chelation mechanisms of metals, interaction with the cell membrane, and alteration of osmotic equilibrium that directly interfere with microbial metabolism (Kabir et

al., 2014; Feng et al., 2010). The fennel slices immersed in CSE showed a significantly higher content of organic acids and phenolic acids than the other tests, therefore the enrichment in their chemical composition and the synergic action of these compounds were decisive in maintaining the proliferation of micro-organisms, at levels deemed safe for the consumer (Mir et al., 2018), up to 14 days of storage.

3.5 Conclusion

In this study, the dipping solution with 5-10% CSE led to a slowing down of metabolic activity and the maintenance of chemical, physical, and sensorial characteristics, also confirmed by the analysis carried out by the variation of total soluble solids, pH, and weight loss.

The use of CSE as proposed in this study, was a simple and ecofriendly strategy for the upgrading of food by-products derived from the coffee roasting process, giving it new life in the food sector.

The results showed that dipping treatments with CSE lead to an increase in valuable substances such as organic acids (oxalic, phenolic, and malic) and phenolic acids (caffeic and chlorogenic) directly involved in the slowing down of metabolic processes and complex reactions underlying better maintenance of the total acceptability of fresh-cut fennel slices. These results are encouraging and represent a first step in the potential use of coffee silverskin hydroalcoholic extract in food applications as a potential replacement of synthetic additives in maintaining chemical physical, microbiological, and sensory characteristics, of highly perishable and rapidly browning vegetables. It was also found that the dipping

solutions of CSE allow enrichment in chlorogenic and caffeic acid in the slices of fresh-cut fennel slices, suggesting providing a high value to the food.

Future studies may focus on the in vivo activity of compounds in coffee silverskin as well as their application in other food formulations to prevent rapid quality decay during storage.

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Chapter 4 Sustainable use of coffee roasting by-products: development of high value-added gummy candies

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Abstract
The sustainable utilization of production wastes in the agri-food sector is an increasing challenge. This work aims to evaluate the applicability of coffee silverskin, the main by-product of coffee roasting, in the formulation of gummy candies. Firstly, the experimental plan envisaged the extraction and characterization of bioactive compounds from coffee silverskin. The influence of different concentrations of coffee silverskin extract (1%, 2%, and 4%) was then evaluated on chemical, physical, microbiological, structural, and sensory gummy candies characteristics for 120 storage days. Candies formulated without coffee silverskin extract were used as a control. The results up to 120 days of storage revealed the higher quality of gummy candies enriched with 1%, 2% and 4% coffee silverskin

extract not only for their bioactive content, ranging from 147.9-161.1 mg GAE Kg $^{-1}$ of phenolic compounds but also for their antioxidant activity, with values at the end of storage of 15.06, 30.25, 31.50 and 28.20 μmol TE g^{-1} respectively in control and gummy candies enriched with 1%, 2% and 4% coffee silverskin extract. Moreover, all the candies enriched with silverskin coffee extract showed better physical and sensory characteristics compared to the control taste. The results show that the proposed use of silver coffee skin improves and preserves the quality of gummy candies and then be employed as an ingredient to improve the quality of confectionery products.

Keywords

Agri-food waste; coffee roasting by-product; coffee silverskin; gummy candy.

4.1 Introduction

Large volumes of processing waste are generated along the food chain, not only costly to dispose of but-also produce significant damage to the surroundings when not disposed of appropriately. The reusability of processing waste in multi-hued sectors, such as the food sector, has been viewed as the ideal solution to raise environmentally eco-friendly supply chains and reduce disposal expenses (De Bruno et al., 2022).

In recent times, coffee silverskin (CS) has been pointed out as a source of precious fibers, proteins, polyphenols, and melanoidins, suitable to improve food characteristics and bring salutary benefits to the consumer (Biondić Fučkar, et al.,

2023). In addition, coffee silverskin extracts (CSE) have been described as a safe food element in antecedent studies due to their non-carcinogenicity and low or no existence of toxic substances (ochratoxin A, pesticides, and polycyclic aromatic hydrocarbons) (Bertolino et al., 2019). Potential applications of CS powders and extracts have been reported in cookies as a partial substitute for flour (Goceman et al., 2019) and in chicken meat to prevent oxidation (Martuscelli et al., 2021). However, the influence of coffee silverskin extract on gelatinous matrices, as proposed in this study, has not yet been tested. In addition, due to the growing consumer claim for food products free of synthetic supplements, the development of sweet products based on natural constituents with antioxidant properties could provide a chance to realize new and healthier products for both the consumer and the confectionery industries.

The gummy candies could be considered a composite gel complex boasting gelling agents, sugars, water, and different minor constituents. This peculiar formulation makes them matrices qualified for the inclusion and retention of compounds with high added value (Cano-Lamadrid, et al., 2020).

The work aimed to test the effect of different percentages of CSE (1%, 2%, and 4%), derived by eco-friendly extraction with food-grade solvents, on healthy compounds and quality characteristics of enriched gummy candies, focusing particular attention on common defects found during the storage (crystallization of sugars, loss sensorial characteristics, presence of mold, etc.) that endanger the acceptance and safety of the product itself.

4.2 Materials and Methods

4.2.1 Recovery of antioxidant compounds from coffee silverskin and chemical characterization

Coffee silverskin (CS) was-supplied by a local coffee roaster industry (Caffè Mauro S.p.A.) and was the result of the roast of a commercial coffee blend, comprised of 50% *Coffea Arabica* and 50% *Coffea Canephora* var. Robusta beans.

After receiving, the sample was dehydrated (50 °C for 2 hours) up to 10% of moisture content then it was ground, homogenized, and used to prepare extracts.

The extraction was executed by mixing 2 g of CS powder with 20 mL of hydroalcoholic solvent (EtOH 30%). The solid-liquid extractions were realized on a heating plate (60 °C) with continuity stirring for 60 minutes. Thereafter, the extracts were centrifuged (6000 rpm, 10 min,20 °C) and the supernatant was recuperated, filtered using a Buchner funnel, and stored at −21 °C until further analysis. In triplicate, all determinations were carried out.

The pH and total soluble solids (TSS, °Bx) of CS extract (CSE) were determined using a digitally calibrated pH meter (pH 4, pH 7; Crison Basic 20, Spain) equipped with an ion-selective electrode and a digital refractometer (DBR 047 SALT) respectively.

The total content of phenolic compounds (TPC) was found using the Folin–Ciocalteu colorimetric method according to Alves et al. (2010) with some modifications. Briefly, 0.3 mL of a diluted CSE (1:20) was mixed with 2.5 mL of the Folin-Ciocalteu reagent (10% v/v) and 2 mL of a Na_2CO_3 solution (7.5% w/v). The mixture was incubated for 15 minutes at 45 °C, and after 30 min at room

temperature, absorbance readings at 765 nm were performed, against a reagent blank, using a double-beam UV spectrophotometer (Perkin-Elmer UV- Vis λ2, Waltham, Massachusetts, USA). A calibration gallic acid curve (2-10 mg L^{-1}; R^2 = 0.999) was used and the obtained results were expressed as mg of gallic acid equivalents (GAE) L^{-1} of CSE.

Total flavonoid contents (TFC) were determined according to Costa et al. (2014). The reaction mixture was prepared by adding 1 mL of CSE with 4 mL of distilled water and 300 µL of $NaNO_2$ (25%). After 5 min at room temperature, 300 µL of 10% $AlCl_3$ were added, and after 1 minute 2 mL NaOH (4% m/v) and 2.4 mL of ultrapure water. The absorbance at 510 nm was measured after 10 minutes at room temperature and total flavonoid content was calculated through a calibration epicatechin curve (0-100 mg/L; R^2 = 0.999) and expressed as mg of epicatechin equivalents (ECE)/ Kg of CSE.

The antioxidant activity of the extract was determined by a multitarget approach using the DPPH, ABTS, and FRAP assays. The DPPH assay was performed as described by Vimercati et al. (2022) with some modifications. In brief, 40 µL of CSE (diluted 1:10) were added to 2960 µL of a 6 $x10^{-5}$ M of methanol solution of DPPH and left in darkness for 30 min at room temperature. The absorbance was assessed at 515 nm using a double-beam UV spectrophotometer (Perkin-Elmer UV- Vis λ2, Waltham, Massachusetts, USA) versus a blank (methanol).

For the ABTS assay, the methodology followed the protocol reported by Bilge et al. (2022). The FRAP assay was carried out by the method described by Benzie et al. (1996) with some modifications. Briefly, 3360 µL of the FRAP reagent

(consisting of 25 mL acetate buffer 0.3 M, 2.5 mL 10 mM TPTZ, and 2,5 mL 20 mM solution ferric chloride) was mixed with 40 µL of CSE. The mixture was vortexed and kept in a water bath for 6 minutes at 37 °C in the dark. After, the absorbance was reordered at 595 nm. The results of antioxidant assays were expressed as mM Trolox equivalents L^{-1} of CSE, compared with a Trolox calibration curve.

The total bacterial count bacteria (TBC), yeasts, and molds were detected to evaluate the microbiological contamination of CSE by the procedure described by Nolasco et al. (Nolasco et al., 2022). The sample was serially diluted and, subsequently, 1 mL of each dilution was transferred on to the surfaces of the used plates. TBC was performed by inoculating ready-to-use chromogenic plates (Compact Dry) and incubating them at 25 ± 2 °C for 48 ± 3 h. Dichloran Rose Bengal Chloramphenicol (DRBC) agar base plates were used to enumerate yeasts and molds, and the plates were incubated after solidification at 25 °C for 4–5 days before counting the colonies. The results are reported as Log10 colony-forming units (CFUs) mL^{-1} of CSE.

4.2.2 Gummy Candy Manufacturing

The gummy candies were made as described by Miranda et al. (2020)with some modifications, the ingredients are reported in Table 1. A mixture of apricot juice, sucrose, glucose syrup (40 DE), and citric acid was heated at 85 °C under stirring for complete dissolution. After cooling to approximately 50°C, CSE was added and homogenized for 10 minutes. The pork gelatin sheets (240 °Bloom) were then added to water for 10 minutes, to favor the hydration, and finally, the ~~gelatine~~ gelatin was added to the mixture and homogenized at 50 °C ± 5 °C under stirring

for complete dissolution. The jelly mass was immediately placed in silicone molds and dried in an aseptic environment by using a vertical laminar flow hood (UV lamp 30 W, mod. ASALAIR 1200 FLV, Asal Srl, Milan, Italy) for 72h at 25°C until the candies reached the water activity (a_w) less than 0.70.

Subsequently, the gummy candies were de-molded, packed in waxed paper, and stored in darkness in constant climate chambers (25 °C) for 120 days. Gummy candies samples were identified as follows: CTR (0% CSE), CS1 (1% CSE), CS2 (2% CSE), and CS3 (4% CSE).

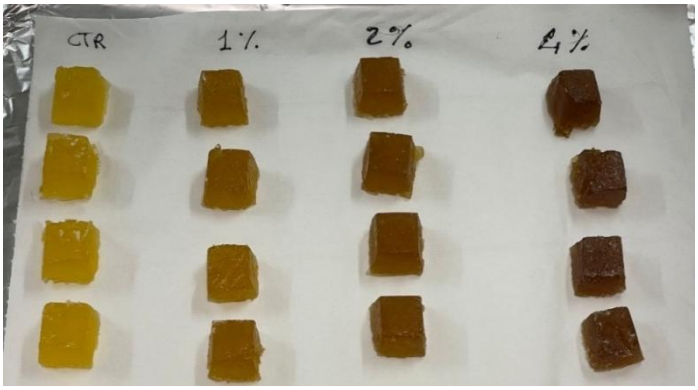


Figure 1 Gummy candies after formulation. (CTR: gummy candies without CSE; CS1: gummy candies with 1% CSE; CS2: gummy candies with 2% CSE; CS3: gummy candies with 4% CSE)

Table 1 Ingredients used for the formulation of gummy candies

Ingredients (g/ 100 g of gummy candies)	**Sample**			
	CTR	**CS1**	**CS2**	**CS3**
Sucrose	31	31	31	31
Glucose syrup	28	28	28	28
Pork gelatin	8	8	8	8
Apricot juice	22	22	22	22
Citric acid	1	1	1	1
Extract coffee silverskin	-	1	2	4
Water	10	9	8	6

4.2.3 Physicochemical and microbial analyses of gummy candies

Physicochemical analyses were performed immediately after candy production (0 days) and, subsequently, after 15, 30, 60 90, and 120 days of storage. The sensory and microbiological analyses were carried out immediately after the formulation and after 120 days.

4.2.3.1 Color

Color analysis was evaluated using a Spectrophotometer using a D65 illuminant (Minolta CM-700 d, Japan), with measurement on twenty-four points for each sample.

The results of the colorimetric measurements were expressed in the CIE L*a*b* scale adopted as standard by the International Commission on Illumination. The total color difference (ΔE) after 120 days of storage was obtained by using the following equation (1):

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

4.2.3.2 Water activity, pH, total soluble solids, moisture content, and microbial analyses

The water activity (a_w), pH, and total soluble solids (TSS) were measured according to Cedeño-Pinos et al. (2020). The moisture content was carried out using gravimetric techniques, according to the Association of Official Analytical Collaboration (AOAC, Official methods of analysis,19th ed., 2012.).

The mold enumeration was performed on Dichloran Rose Bengal Chloramphenicol (DRBC) base plates. Briefly, 10 g of each sample was placed in a sterile bag with 100 ml of Ringer solutions, homogenated with Stomacher

(BagMixer® 400 P, Interscience, France) for 3 minutes. The resulting microbiological suspension was diluted in series then, 1 ml of each dilution was placed on the plates. The enumeration of molds was made after incubation at 25 ºC for 4-5 days. The results were expressed as $\log_{10}$ CFU g^{-1} of gummy candies.

4.2.3.3 Total Phenolics Content and Antioxidant Activity

The samples (6g) were dissolved with 20 ml methanol under agitation for 45 minutes at room temperature, to estimate the total content of phenolic compounds and the antioxidant activity of gummy candies. The mixture was then centrifuged (8000 rpm and 4 ºC for 10 min), the supernatant was recovered, filtered (PTFE 0.45 µm, diameter 15 mm), and frozen at -80 ºC until analysis.

The total content of phenolic compounds (TPC) in gummy candies was determined using the Folin–Ciocalteu with an experimental procedure proposed by Cedeño-Pinos et al. (2020) with modifications. In a 10 ml graduated flask were mixed 1 ml of CSE, 5 ml of distilled water, and 0.8 ml of Folin-Ciocalteu reagent were mixed. After 8 minutes at room temperature and under constant stirring, 1,2 ml of 20 % (v/v) Na2CO3 was added. Then the reaction mixture was completed to a volume of 10 ml with distilled water and incubated in a dark at room temperature for 2 hours. The absorbance was obtained at 760 nm using a spectrophotometer (Perkin-Elmer UV–Vis k2, Waltham, Massachusetts, U.S.). A mixture reaction without a sample was used as a blank. The results were expressed as µg gallic acid equivalents (GAE) g^{-1} of gummy candies using a calibration curve of gallic acid as a standard (R^2 = 0,9996).

Literature shows that the ABTS test is the best way to test the antioxidant activity of gummy candies (Samakradhamrongtha et al., 2021). Then, the antioxidant activity of gummy candies was tested with ABTS assay considering both the matrix of the tested food and the antioxidant activity performed by the compounds present in CSE to extinguish the $ABTS^{+}$ in lipophilic and hydrophilic environments by the procedure described by Re et al. (1999) with some modifications. ABTS radical cation was generated by mixing the 7 mm ABTS stock solution with 2.45 mm potassium persulfate and allowing the mixture to stay in the dark at room temperature for 12-16 hours before use. Then, it was balanced to 30 °C using PBS 0.1 M (pH 7.4) up to an absorbance of 0.70 ± 0.02 to 734 nm. For sample analysis, 100 µL of sample extract and 2900 µL ABTS were mixed and incubated in darkness for 6 min. Sample absorbance was measured at 734 nm against a blank (PBS). A calibration curve was prepared with Trolox, and the results were expressed as µM Trolox Equivalents (TE) g^{-1} of gummy candies.

4.2.3.4 Textural properties of gummy candies

To determine CSE's influence on gummy candies' structure, various parameters were monitored periodically at 0, 15, 30, 60, 90, and 120 days of storage.

The texture was evaluated with two types of tests: texture profile analysis (TPA) and the perforation test following the methods described by Teixeira-Lemos et al. (Teixeira-Lemos et al., 2021).

All tests were conducted by measuring force on compression, using a 50 kg load cell and a trigger force of 0.05 N. All mechanical properties were measured on 7 jellies gummies of each type, at room temperature.

The texture profile analysis (TPA) was conducted with a flat cylindrical probe P/50, the compression distance was 5mm, and the pre-test and post-test speeds were all equal to 0.5mm/s. Two compression cycles were performed with a 5 s interval between them. The texture profile analysis on samples was expressed in terms of: hardness (N) as the force required to compress the sample; springiness (mm) as thickness recovered from the sample between the first and second compression; cohesiveness (no units) as the force due to the interaction among the various ingredients used for the formulation of the sample, and expressed as the ratio between the areas of the curves (force x time) obtained during the first and second compression; chewiness (N) as the energy required to chew food before swallowing, and expressed as the hardness x cohesiveness x springiness; stickiness (N) expressed as the force necessary to resist the separation of two surfaces in contact (caramel and probe).

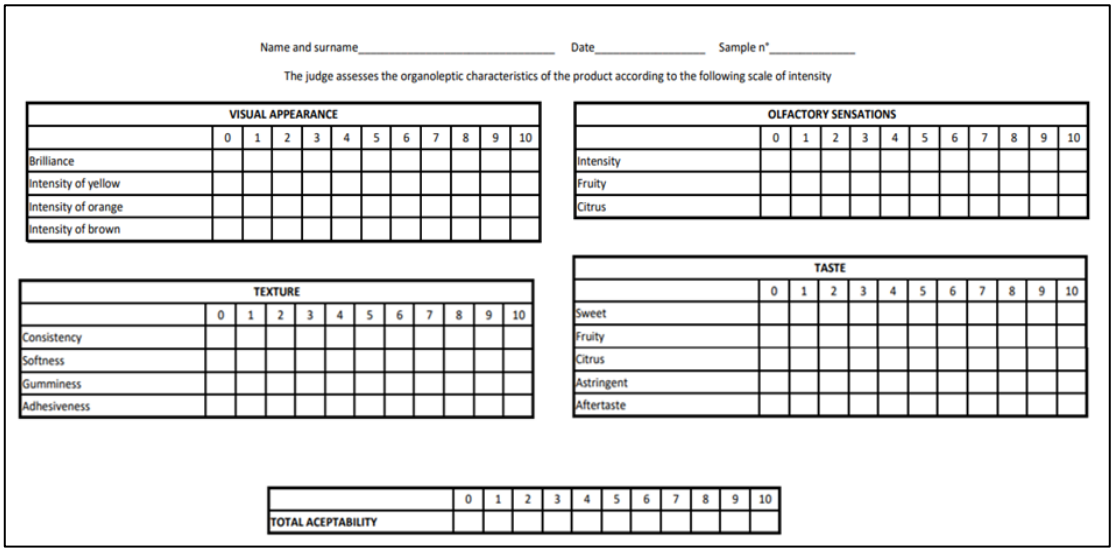
The perforation tests with a probe P/5 with cylindrical termination with a 5 mm diameter. This test utilized a perforation distance of 3mm, pre-test speed of 2.0mm/s, test speed of 1.0mm/s, and post-test speed of 1.0mm/s.

4.2.3.5 Quantitative Descriptive Sensory Analysis of gummy candies

A sensory quantitative sensory descriptive analysis (QDA) was performed by recruiting ten panelists (five men and five women aged between 22 and 45 years) among departmental students and faculty staff of the Mediterranean University of Reggio Calabria with previous experience in sensory analyses. The participants were trained before the sessions to identify, select and quantify the main sensory descriptors for gummy candies (ISO Sensory Analysis, ISO 8586, 2012). Table 2 shows the olfactory and taste descriptors, and the reference used to train the

participants. Visual appearance (brilliance, intensity of yellow, intensity of orange, intensity of brown) and texture descriptors (consistency, softness, gumminess, adhesiveness) were identified, selected and quantified based on the previous experience of the panelist. The quantification of sensorial attributes was carried out using a hedonic scale, from 0 to 10 points, where a score of 0 indicates the absence of the attribute, 10 indicates the total presence of the attribute and 5 score was fixed as the minimum acceptable (Figure 2). In addition, the panelists were asked to give an overall score from 0 to 10 based on the overall judgment of the taste, olfactory, visual and texture attributes considered (total acceptability). Sensory analysis of the gummy candies was conducted in opportune tasting booths (90 cm), equipped with sinks, lighting and shelves for samples. Participants were given water to rinse their palate during the sensory session.

The sensory analysis was carried out on gummy candies samples at the beginning (1 day) and end of storage (120 days). The results were reported as an average of the evaluations.



Name and surname_____ Date_____ Sample n°_____

The judge assesses the organoleptic characteristics of the product according to the following scale of intensity

VISUAL APPEARANCE	0	1	2	3	4	5	6	7	8	9	10
Brilliance											
Intensity of yellow											
Intensity of orange											
Intensity of brown											

OLFACTORY SENSATIONS	0	1	2	3	4	5	6	7	8	9	10
Intensity											
Fruity											
Citrus											

TEXTURE	0	1	2	3	4	5	6	7	8	9	10
Consistency											
Softness											
Gumminess											
Adhesiveness											

TASTE	0	1	2	3	4	5	6	7	8	9	10
Sweet											
Fruity											
Citrus											
Astringent											
Aftertaste											

	0	1	2	3	4	5	6	7	8	9	10
TOTAL ACEPTABILITY											

Figure 2 Gummy candies sensory descriptors list.

Table 2 Olfactory and taste descriptors and corresponding references used to train the panelist.

Descriptor	References
Taste	
Sweet	Sucrose solution (0-10 g L^{-1})
Fruity	Apricot juice solution (0-1000 mL L^{-1})
Citrus	Lemon juice solution (0-1000 mL L^{-1})
Astringent	Acid tannic solution (0-0.1 g L^{-1})
Aftertaste	Time of persistence of taste sensations after swallowing
Olfactory	
Fruity	Apricot aroma (0-20 drops)
Citrus	Lemon aroma (0-20 drops)
Intensity	Intensity perceived by the combination of fruity and citrus descriptors

The reference taste solutions were made with distilled water. The reference of aromas was placed on strips for aromas.

4.2.3.6 Statistical Analysis

The analytical data were reported as means ± standard deviations of replicates. The analysis of variance (one-way ANOVA) was conducted by applying Tukey's *post-hoc* at $p < 0.05$ by Version 20.0 SPSS software (SPSS Inc., Chicago, IL, USA).

4.3 Results and Discussion

4.3.1 Chemical Characterization of Coffee Silverskin Extract

The Total soluble solids (TSS) and pH values of coffee silverskin extract (CSE) were 8.53 and 9.3, respectively (Table 3).

The spectrophotometric analysis showed a high content in phenolics compounds (TPC) (1954.75 ± 3.13 mg GAE L^{-1}), flavonoid compounds (TFC) (1426.21 ± 67.4 mg ECE L^{-1}) and strong antioxidant activity (ABTS 330.16 ± 6.3

mmol Trolox L^{-1}, DPPH 207.08 ± 9.95 mmol Trolox L^{-1} and FRAP 57.78 ± 7.8 mmol Trolox L^{-1}) of CSE (Table 3).

The results obtained agree with previous studies (Dzah et al., 2020; Murthy et al., 2012) and are closely related to the variables used during the extraction process. Indeed, it has been observed that the extractability of CS antioxidant compounds is affected by the polarity of the used solvent and may be related to the fact that many phenolic compounds often have intermediate solubility. However, it should be noted that the chemical profile of CS can be strongly influenced by different variables, including coffee varieties, ~~the~~ production environment, climatic conditions, field treatments, methods of coffee processing, and methods of storage of the by-product (Nolasco et al., 2022).

Finally, the microbiological analysis did not provide evidence of the microbial presence in extracts: it is plausible that the high temperature of the roasting process and the low moisture content limited its microbial load and extended its storage (Thangavelu, et al., 2022; Toschi et al.,2014).

Table 3 Chemical Characterization of of Coffee silverskin extract

Parameter	Results
pH	8.53 ± 0.00
TSS (° Bx)	9.30 ± 0.00
TPC (mg GAE L^{-1})	1954.75 ± 3.13
TFC (mg ECE L^{-1})	1426.21 ± 67.4
DPPH (mmol Trolox L^{-1})	207.08 ± 9.95
ABTS (mmol Trolox L^{-1})	330.16 ± 6.30
FRAP (mmol Trolox L^{-1})	57.78± 7.80

4.3.2 Physico-chemical and microbiological characterization of gummy candies

4.3.2.1 Colour

Colour is an important quality index for all foods and is linked to the overall acceptability of products by the final consumer. Table 4 shows the results relating to the colorimetric measurement of the different formulations of gummy candies, immediately after the preparation (time 0) up to 120 days of storage at 25 °C. The gelling process, shelf-life, and physical-chemical properties of gummy candies could be influenced by the applied extracts, as described by Delgado et al. (2015). Differences in L*, a*, and b* values were observed among CTR and CS1, CS2, and CS3. The addition of CSE has predictably led to a progressive browning (decrease of L*) of the candies and a visible variation of colour from bright orange to orange-brown (decrease of a* and b*). This trend was maintained throughout storage. The significant colour differences were attributable to the presence in CSE of melanoidins, responsible for the brown colour of many foods (Echavarría et al., 2012). Moreover, the liquid nature of the CSE allowed a better dispersion in the gelatinous matrix of candies and, consequently, a stronger variation of colour in all enriched candies than no enriched ones (De Moura et al, 2019).

A significant increase in the L* value was observed in CTR after 60 days of storage, probably due to the crystallization of the sugar that occurred following of moisture adsorption and desorption from the surroundings (Ergun et al., 2010). It was reported already that during the storage of gummy candies, a slow migration of moisture could happen inside the package, causing the lowering of the glass transition temperature, greater mobility of the molecules embedded in the matrix, and, as a result, the crystallization of the sucrose, loss of the aromatic

characteristics, hardening and a change in the colour of the candies (Roe et al., 2005; Normand et al., 2019).

The advantageous impact of CS on the support of colour was too affirmed by the results of overall colour change (ΔE) ranging from 1.65 to 3.61, where the highest value was recorded in CTR and the lowest one in CS2 after 120 days of storage. The results obtained suggest that the presence of 2% and 4% CSE in the formulation does not affect the visual appearance and, consequently, the visual acceptability of the product for 120 days of storage.

Table 4 Colour parameter of gummy candies during the storage period.

Parameter	Sample	Storage time (days)						
		0	**15**	**30**	**60**	**90**	**120**	**Sig.**
Lightness (L*) CIE units	CTR	49.2 ^{aAB}	50.6 ^{aA}	49.1 ^{aAB}	48.7 ^{aAB}	48.2 ^{aB}	50.5 ^{aA}	**
	CS 1	47.4 ^{bAB}	48.3 ^{abA}	47.7 ^{abAB}	48.3 ^{aA}	46.6 ^{aB}	47.9 ^{bAB}	*
	CS 2	45.9 ^{cA}	44.9 ^{bA}	46.4 ^{bA}	46.0 ^{bA}	46.1 ^{aA}	46.3 ^{bA}	ns
	CS 3	46.6 ^{bA}	46.1 ^{bA}	47.2 ^{bA}	45.4 ^{bA}	48.3 ^{aA}	46.6 ^{bA}	ns
Sig.		**	**	**	**	ns	**	
Redness (a*) CIE units	CTR	2.04 ^{bBC}	2.77^{aA}	2.34^{aABC}	1.78 ^{aC}	2.41 ^{aAB}	2.43 ^{aAB}	**
	CS 1	2.49 ^{aA}	2.65 ^{aA}	2.84 ^{aA}	1.44 ^{bB}	1.44 ^{bB}	1.73 ^{bB}	**
	CS 2	1.71 ^{bA}	1.42 ^{bAB}	1.21 ^{bAB}	1.02 ^{cB}	1.01 ^{cB}	1.51 ^{bA}	**
	CS 3	0.87 ^{cAB}	0.99 ^{bA}	0.81 ^{bAB}	0.59 ^{dB}	0.75 ^{cAB}	0.71 ^{cAB}	**
Sig.		**	**	**	**	**	**	
Yellowness (b*) CIE units	CTR	6.49 ^{aA}	6.67 ^{aA}	6.32 ^{aA}	5.07 ^{aB}	5.85 ^{aAB}	4.99 ^{aB}	**
	CS 1	5.58 ^{bAB}	5.54 ^{bABC}	6.06 ^{aB}	4.90 ^{aBCD}	4.47 ^{bCD}	3.86 ^{bD}	**
	CS 2	3.71 ^{cA}	3.84 ^{cA}	3.71 ^{bA}	3.40 ^{bAB}	3.79 ^{cA}	2.82 ^{cB}	**
	CS 3	3.04 ^{cBC}	3.88 ^{cA}	3.64 ^{bAB}	2.69 ^{cCD}	3.55 ^{cAB}	2.35 ^{cD}	**
Sig.		**	**	**	**	**	**	

Small letters within a column and capital letters within a row show significant differences as assessed by Tukey's *post hoc* test. Abbreviations: **, significance at $p < 0.01$; *, significance at $p < 0.05$; n.s., not significant.

4.3.2.2 Water activity, Moisture content, pH, Total soluble solids, and mold count of gummy candies

The gummy candies are characterized by a colloidal system containing gelling agents, sugars, water, and other minor constituents. For this reason, these foods are highly hygroscopic and present serious difficulties in drying, stabilization, and storage (Dewi et al., 2018). Typically, all gummy candies are affected by a progressive loss of moisture during storage that can be attributed to many factors, such as formulation ingredients, storage temperature, and packaging materials (Delgrado et al., 2015). In addition, due to the hygroscopic characteristics, continuous migration of moisture between the candy and the surrounding environment may cause chemical-physical, sensory, and microbiological variations. In gummy candies, the ideal moisture content value to allow long storage and preserve the physicochemical characteristics over time should be between 8% and 22% (Ergun et al.,2010).

In this study, enrichment with liquid CSE influenced the moisture content as well as the stabilization time of all treated candies. In fact, in the first storage period (up to 15 days) a faster water loss in CS1, CS2, and CS3 was observed compared to CTR. Probably, the interaction between the basic ingredients (used to formulate candies) and CS determined a change of structure in gummy candies and, consequentially, the delay of stabilization. A low and smooth significant decrease of moisture occurred (from 15 to 120 days). The great loss of moisture recorded in the enriched candies was due to the hydroalcoholic nature of the extract and the progressive increase of its concentration in the candy formulation. Matulyte and colleagues (Matulyte et al., 2021) found that the use of ethanolic extracts in gummy

candies formulation affected the moisture loss more than other samples because alcoholic and hydroalcoholic extracts evaporate faster than other ones.

Water activity (a_w) is essential to maintain the chemical and storage stability of food products and is closely related to food microbiological safety. As shown in Table 5, differences among enriched (CS1, CS2, and CS3) and not enriched (CTR) candies were found throughout the whole storage period also for this parameter. A_w was affected by the hygroscopicity and intermolecular interactions encouraged by the presence of additional solutes in CS1, CS2, and CS3. The a_w values of all gummy candies decreased from 0.66 to 0.58 in CTR, from 0.66 to 0.55 in CS1, and from 0.65 to 0.64 in CS2 up to the end of storage. In CS3 the values remained constant. The a_w results found in this study ensure the safety and physicochemical quality of the final product and agree with previous studies where the value of a_w for jellies and candies should be between 0.55 and 0.75 (Busserie et al., 1985).

Contrary to the trend shown of moisture and a_w total soluble solids increased significantly during the storage: this is related to both progressive dehydration of food and to chemical changes at the expense of sugars present in the complex matrix of gummy candies with a progressive polysaccharides' conversion into monosaccharides and oligosaccharides during their shelf-life, confirmed by literature (Dar et al., 2011).

All quality parameters' observed trends (a_w, moisture, and total soluble solids) suggested the need for more barricaded packaging to better protect the candies from dehydration and, consequently, from all the related changes over time (Ergun et al., 2010).

Moreover, significant differences in pH were found throughout the storage time among the candies: greater in CS1, CS2, and CS3 than in CTR and caused by the original characteristics of the used extract (pH = 8.9).

The formulation of gummy candies has ensured food safety by avoiding the proliferation of mold during storage (data not shown): it is due to the maintenance of a_w, pH, and moisture values below the threshold limits, as reported in previous studies (Matulyte et al., 2021).

Table 5 Moisture content, Water activity, pH, total ~~Solid~~ soluble solids, and mold of gummy candies.

Parameter	Sample	Storage time (days)						
		0	**15**	**30**	**60**	**90**	**120**	**Sig.**
Moisture (g/100 g)	CTRL	19.87 ^{aA}	19.41 ^{aA}	19.61 ^{aA}	19.80 ^{aA}	16.62 ^{bB}	17.80 ^{aB}	**
	CS 1	19.97 ^{aA}	18.30 ^{bBC}	18.75 ^{aB}	17.51 ^{bC}	20.21 ^{aA}	16.82 ^{bD}	**
	CS 2	18.50 ^{bA}	16.10 ^{cB}	16.52 ^{bB}	15.40 ^{dC}	14.95 ^{cC}	16.53 ^{bB}	**
	CS 3	18.30 ^{bA}	15.90 ^{cBC}	15.37 ^{bC}	16.13 ^{cB}	13.63 ^{dD}	13.52 ^{cD}	**
Sign		**	**	**	**	**	**	
Water activity (a_w)	CTRL	0.65 ^{bC}	0.68 ^{aA}	0.67 ^{cB}	0.67 ^{aB}	0.61 ^{bD}	0.58 ^{cE}	**
	CS 1	0.66 ^{aB}	0.67 ^{bA}	0.67 ^{cA}	0.67 ^{aA}	0.59 ^{dC}	0.55 ^{dD}	**
	CS 2	0.64 ^{cC}	0.67 ^{bB}	0.68 ^{bA}	0.67 ^{aB}	0.63 ^{aD}	0.64 ^{aC}	**
	CS 3	0.60 ^{dC}	0.66 ^{bB}	0.69 ^{aA}	0.66 ^{bB}	0.60 ^{cC}	0.60 ^{bC}	**
Sig.		**	**	**	**	**	**	
TSS (° -Bx)	CTRL	49.91 ^{cF}	56.15 ^{cD}	56.91 ^{bC}	53.33 ^{dE}	59.72 ^{cB}	60.61 ^{dA}	**
	CS 1	50.36 ^{bD}	58.72 ^{bB}	55.00 ^{dC}	58.20 ^{cB}	55.10 ^{dC}	65.10 ^{aD}	**
	CS 2	53.56 ^{aD}	61.35 ^{aB}	57.20 ^{bC}	63.32 ^{aA}	60.91 ^{bB}	61.00 ^{cA}	**
	CS 3	50.42 ^{bF}	63.61 ^{aB}	61.51 ^{aC}	60.01 ^{bE}	65.14 ^{aA}	61.00 ^{bD}	**
Sig.		**	**	**	**	**	**	
pH	CTRL	3.86 ^{bC}	3.84 ^{cC}	3.86 ^{dC}	3.91 ^{cB}	3.96 ^{dA}	3.90 ^{cB}	**
	CS 1	3.85 ^{bD}	3.89 ^{bC}	3.91 ^{cC}	3.95 ^{bB}	3.99 ^{cA}	3.90 ^{cB}	**
	CS 2	3.93 ^{aD}	3.91 ^{aE}	3.96 ^{bC}	3.97 ^{bC}	4.05 ^{bA}	4.00 ^{bB}	**
	CS 3	3.94 ^{aD}	3.92 ^{aE}	4.00 ^{aBC}	4.01 ^{aB}	4.08 ^{aA}	3.99 ^{aC}	**
Sig.		**	**	**	**	**	**	
Molds (Log UFC/g)	CTRL	n.d.	-	-	-	-	n.d.	
	CS 1	n.d.	-	-	-	-	n.d.	
	CS 2	n.d.	-	-	-	-	n.d.	
	CS 3	n.d.	-	-	-	-	n.d.	

Small letters within a column and capital letters within a row show significant differences as assessed by Tukey's *post hoc* test. Abbreviations: **, significance at $p < 0.01$; *, significance at $p < 0.05$; n.s., not significant.

4.3.2.3 Total phenolic content and antioxidant activity

The addition of CSE influenced significantly ($p < 0.05$) also the total phenolic content in gummy candies, closely linked to the extract's concentration used in the formulation (Table 6).

Immediately after preparation, the phenolic compounds in all CS gummy candies were significantly higher than CTR (265.07µg GAE g^{-1}, 272.70 µg GAE g^{-1}, 282.31 µg GAE g^{-1}, 317.09 µg GAE g^{-1} for CTR, CS1, CS2 and CS3, respectively) and this trend was maintained throughout their storage. However, all candies, enriched or not, showed a significant ($p \leq 0.05$) decrease in phenolic compounds during storage. This trend may has been caused by storage conditions (packaging and temperature), both the presence of oxygen and the temperature (25 °C) could have advantaged the natural degradation and the trigger of chemical reactions, causing of decrease in these precious compounds. Moreover, the change of moisture observed during storage has facilitated the mobilization of phenolic compounds present in the matrix and increased their susceptibility to degradation (Ergun et al., 2010). Nevertheless, the values of phenolic compounds found in candies enriched with CSE in this study were significantly higher than those of other potentially functional candies formulated with banana and Malaysian sting-less bee honey (183 µg GAE g^{-1}) [35] and comparable with those obtained in previous studies using phenolic extract of liquid Rosemary Extract (190-273 µg GAE g^{-1},) (Cedeño-Pinos et al., 2020), peppermint (160-380 µg GAE g^{-1}) (Sarabandi, et al., 2022) ,and Gummy Candies Made with sugars/fructans and green Propolis (153- 271 µg GAE g^{-1}) (Cedeño-Pinos et al., 2021).

Regarding the antioxidant activity, the values show significant differences ($p < 0.05$) among all gummy candies tested (Table 6). The antioxidant activity was dose-dependent since a significant increase was encountered after the addition of crescent percentages of CSE. The distinction between enriched and not enriched candies was evident immediately after the formulation and this tendency was maintained for the whole conservation, with ranges from 24.44 to 15.06 μmol TE g^{-1} for CTR, 38.65 – 30.25 μmol TE g^{-1} for CS1, 42.81 – 31.50 μmol TE g^{-1} for CS2 and 45.75 – 28.20 μmol TE g^{-1} for CS3.

Table 6 Total phenolic content (TPC) and antioxidant activity (ABTS assay) of gummy candies during storage period.

Parameter	Sample	Storage time (days)						
		0	**15**	**30**	**60**	**90**	**120**	**Sig.**
TPC (μg GAE g^{-1})	CTRL	265.11 ^{cA}	235.40 ^{cB}	240.01 ^{bB}	188.86 ^{bC}	179.91 ^{bC}	131.40 ^{bcD}	**
	CS 1	272.71 ^{bcA}	262.31 ^{bAB}	246.80 ^{bB}	205.82 ^{bC}	207.65 ^{bC}	161.12 ^{aD}	**
	CS 2	282.38 ^{bA}	256.89 ^{bB}	241. 60 ^{bC}	191.24 ^{bD}	189.52 ^{aD}	128.00 ^{cE}	**
	CS 3	317.15 ^{aA}	295.10 ^{aAB}	278.91 ^{aB}	240.96 ^{aC}	224.06 ^{aC}	147.90 ^{abD}	**
Sign		**	**	**	**	**	**	
ABTS (μmol TE g^{-1})	CTRL	24.43 ^{cA}	18.41 ^{cBC}	17.07 ^{cBC}	14.63 ^{bCD}	19.68 ^{bB}	15.06 ^{bD}	**
	CS 1	38.65 ^{bA}	36.61 ^{bAB}	33.73 ^{bCD}	29.59 ^{aC}	33.99 ^{aAB}	30.25 ^{aC}	**
	CS 2	42.81 ^{aA}	37.61 ^{abB}	37.80 ^{aB}	29.83 ^{aC}	35.78 ^{aB}	31.49 ^{aC}	**
	CS 3	45.75 ^{aA}	41.20 ^{aB}	38.98 ^{aB}	31.86 ^{aCD}	34.13 ^{aC}	28.20 ^{aD}	**
Sig.		**	**	**	**	**	**	

Small letters within a column and capital letters within a row show significant differences as assessed by Tukey's post hoc test. Abbreviations: **, significance at $p < 0.01$; *, significance at $p < 0.05$; n.s., not significant.

Comparing the results between TPC and antioxidant activity, it was evident the radical scavenging activity was influenced not only by phenolic compounds but also by other compounds present in the CSE. While showing an important decrease in phenolic compounds, the antioxidant activity of enriched candies remained high until the end of storage. Probably, this can be explained by the simultaneous action of various compounds among which the most important were chlorogenic acid and

melanoidins (Cossu et al., 2021; Murthy et al., 2012). The presence of these compounds explains the difference between the values of total phenolic compounds and the antioxidant activity of CS1, CS2, and CS3 from the beginning to the end shelf-life of gummy candies. The results of antioxidants quantified in CS1, CS2, and CS3 were similar and greater to those observed in candies formulated with other ingredients (Samakradhamrongthai, et al., 2021;Marsi et al., 2023; Rivero et al., 2020; Rivero et al., 2021).

4.3.2.3 Textural properties of gummy candies

The textural changes observed during storage of the gummy candies are shown in Table 7. The obtained results indicated that the addition of CSE significantly influenced the hardness, chewiness, stickiness, of all treated samples. Nevertheless, it was equally evident that the factor that most affected the maintenance of structural characteristics during the shelf-life of gummy candies was the time and condition of storage.

As expected, all chemical variations had a significant effect on all the analyzed textural variables. Certainly, a fundamental role in hardness, cohesiveness, chewiness, and stickiness was played by the loss of moisture. Previously, it was reported that the change of moisture over time caused a rapid increase in the viscosity of the surface area (higher stickiness) and an increase in the glass transition temperature of the candies (Handayani et al., 2021). This last phenomenon determines a greater mobilization of the compounds and, subsequently, the crystallization of the sugars present in the gummy matrix and an excessive hardening of the candy (Dzah et al., 2020). The dynamics described above occurred after the 60th day of storage and resulted in significant structural

change in all samples. However, the enriched candies maintained better structural characteristics over time in terms of stickiness, springiness, and hardness than no enriched samples. This was probably due to the nature of the binding of water to the components present in the extract, the presence of fibers could be the cause of the maintenance of consistency (Miranda et al., 2020; Cedeño-Pinos at al., 2020).

Table 7 Textural proprieties of gummy candies during the storage period.

Parameter	Sample	Storage time (days)						
		0	**15**	**30**	**60**	**90**	**120**	**Sig.**
Hardness (N)	CTRL	1.75^{abE}	3.01 ^{aD}	3.02 ^{abD}	6.17 ^{aC}	19.22 ^{aA}	13.71 ^{abB}	**
	CS 1	1.47 ^{bE}	2.45 ^{bDE}	2.66 ^{bD}	5.59 ^{aC}	13.74 ^{cA}	11.01 ^{bB}	**
	CS 2	1.81 ^{aC}	2.82 ^{abC}	2.97 ^{abC}	6.06 ^{aB}	17.46 ^{bA}	18.10 ^{aA}	**
	CS 3	1.60 ^{abE}	2.89 ^{abDE}	3.16 ^{aD}	6.66 ^{aC}	17.59 ^{bA}	14.06 ^{abB}	**
Sign		*	*	*	ns	**	*	
Springiness (mm)	CTRL	0.95 ^{bA}	0.92 ^{aA}	0.93 ^{aA}	0.98 ^{aAB}	0.84 ^{aB}	0.88 ^{aAB}	**
	CS 1	0.93 ^{abA}	0.94 ^{aA}	0.92 ^{aA}	0.91 ^{aAB}	0.84 ^{aB}	0.86 ^{aAB}	*
	CS 2	0.97 ^{aA}	0.95 ^{aA}	0.94 ^{aAB}	0.89 ^{aB}	0.89 ^{aB}	0.9 ^{aBC}	**
	CS 3	0.97 ^{aA}	0.93 ^{aABC}	0.93 ^{aAB}	0.92 ^{aABC}	0.89 ^{aBC}	0.87 ^{aC}	*
Sig.		*	ns	ns	ns	ns	ns	
Cohesiveness (No unit)	CTRL	0.95 ^{aA}	0.95 ^{aA}	0.94 ^{aAB}	0.91 ^{aBC}	0.89 ^{aC}	0.88 ^{bC}	**
	CS 1	0.96 ^{aA}	0.95 ^{aA}	0.95 ^{aAB}	0.91 ^{aBC}	0.90 ^{aC}	0.89 ^{aC}	**
	CS 2	0.96 ^{aA}	0.95 ^{aA}	0.95 ^{aA}	0.91 ^{aB}	0.91 ^{aB}	0.91 ^{abB}	**
	CS 3	0.96 ^{aA}	0.95 ^{aA}	0.94 ^{aA}	0.91 ^{aB}	0.87 ^{aC}	0.88 ^{bC}	**
Sig.		ns	ns	ns	ns	ns	*	
Chewiness (N x mm)	CTRL	9.02 ^{cC}	17.56 ^{bC}	16.36 ^{bC}	33.22 ^{bB}	58.59 ^{aA}	54.11 ^{aA}	**
	CS 1	8.99 ^{cD}	23.27^{aCD}	17.13 ^{bD}	33.65 ^{bC}	74.40 ^{aA}	54.65 ^{aB}	**
	CS 2	12.65 ^{aD}	23.27 ^{aD}	21.34 ^{abD}	35.25 ^{bC}	73.12 ^{aA}	61.40 ^{aB}	**
	CS 3	10.80 ^{bD}	24.67 ^{aC}	24.57 ^{aC}	47.35 ^{aB}	59.23 ^{aA}	66.24 ^{aA}	**
Sig.		**	**	*	**	ns	ns	
Stickiness (N)	CTRL	-0.05 ^{aA}	-0.21 ^{aA}	-0.41 ^{aAB}	-0.82 ^{abABC}	-1.60 ^{aD}	-1.05 ^{abCD}	**
	CS 1	-0.15 ^{bA}	-0.41 ^{aAB}	-0.69^{aABC}	-1.06 ^{bCDE}	-1.40 ^{aDE}	-1.53 ^{bE}	**
	CS 2	-0.10 ^{abA}	-0.43 ^{aAB}	-0.76 ^{aAB}	-1.03 ^{abB}	-1.04 ^{aB}	-0.38 ^{aAB}	*
	CS 3	-0.05 ^{aA}	-0.19 ^{aA}	-0.41 ^{aA}	-0.54 ^{aAB}	-1.47 ^{aB}	-0.55 ^{abAB}	**
Sig.		*	ns	*	*	ns	*	

Small letters within a column and capital letters within a row show significant differences as assessed by Tukey's *post hoc* test. Abbreviations: **, significance at $p < 0.01$; *, significance at $p < 0.05$; n.s., not significant.

4.3.2.4 Quantitative Descriptive Sensory Analysis of gummy candies

The results of the quantitative descriptive sensory analysis of gummy candies is illustrated in Figure 3-5.

The visual appearance (brillance, intensity of yellow, orange and brown) shows significant variation of yellow and brown intensity related to the different formulations and the storage times: it was probably due to the presence of melanoidins in CSE (Echavarría et al., 2012) and, consequently, its concentration in enriched gummy candies. Specifically, at the start of storage (Figure 3a), the increase in the concentration of CSE in the formulation has resulted in a significant ($p < 0.01$) variation of the intensity of yellow (decrease) and brown (increase) while for the orange parameter, the candy attribute scores suggested that a 1% concentration of CSE (CS1 sample) significantly improved its intensity ($p<0.05$). At the end of storage period, Figure 3b suggests that the judged visual parameters were better for the CSE2 sample with statistically significant differences ($p >0.05$) when compared to other tested samples.

The scores obtained for the descriptors of structure, at the beginning of conservation, showed that the addition of CSE to the formulation does not lead to a variation. (Figure 3a).At the end of storage (Figure 3b) a better maintenance of the structure was recorded for samples CS2 and CS4 which showed score significantly higher ($p >0.05$) than CS1 and CTR, especially for descriptors related to consistency and adhesion.

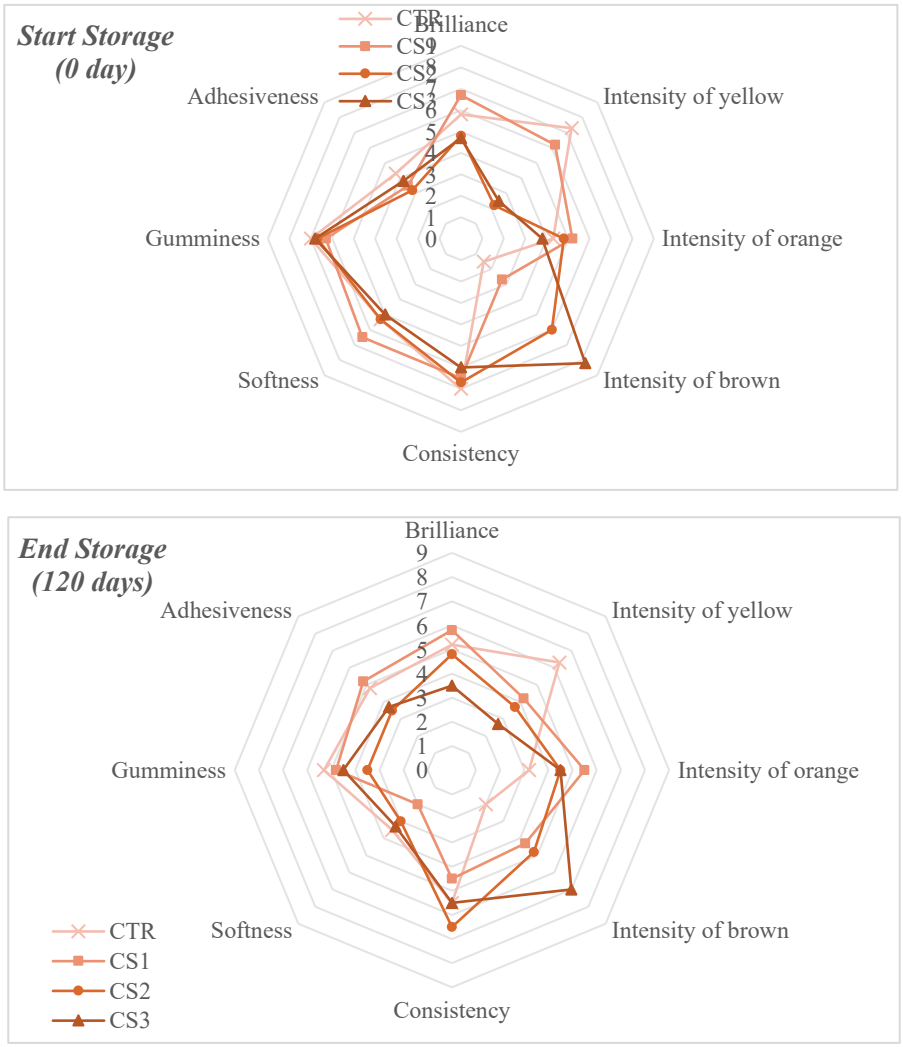


Figure 3 Spider plot of score means for visual (brilliance, intensity of yellow, orange and brown) and texture (consistency, softness, gumminess, adhesiveness) descriptors at start (a) and end (b) storage period

Regarding the olfactory and teste descriptors, no statistically significant differences ($p < 0.05$) were recorded between samples at the start of the storage (Figure 4a). These results were to be considered positive, since suggested that the addition of CSE in the gummy candy formulation does not alter the olfactory (intensity, fruity and citrus) and taste characteristics (sweet, fruity, spicy, citrus, astringent and aftertaste). However, during storage taste sensations tended generally to increase in all the candy samples, probably due to the change in a_w values, as reported by Ergun et al. (De Moura et al., 2019) In particular, regarding the taste

sensations, CS2 candies stood out at the end of preservation for the highest scores ($p > 0.05$) for sweet and fruity tastes (Figure 4b). Aftertaste tended to increase in all candies during storage with fewer variations in CS-enriched ones than in CTR (Figure 4b). As regards the olfactory sensations, at the end of storage no statistically significant difference was found between the analysed samples (Figure 4 b).

Figure 4 Spider plot of score means for olfactory (intensity, fruity and citrus) and teste (sweet, fruity, spicy, citrus, astringent and aftertaste) descriptors at start (a) and end (b) storage period.

The results obtained for the total acceptability of the gummy sweets tested in this study (Figure 5) showed that, at the start of storage, although all the gummy candies recorded scores above the total acceptability limit set at 5, CSE-enriched candies scored higher than CTR. This trend was maintained until the end of the storage period (120 days), when a significantly higher score ($p > 0.05$) was recorded for candy CS2 (7.33) compared to CS1 (6.3), CSE3 (6.0) and CTR (6.5).

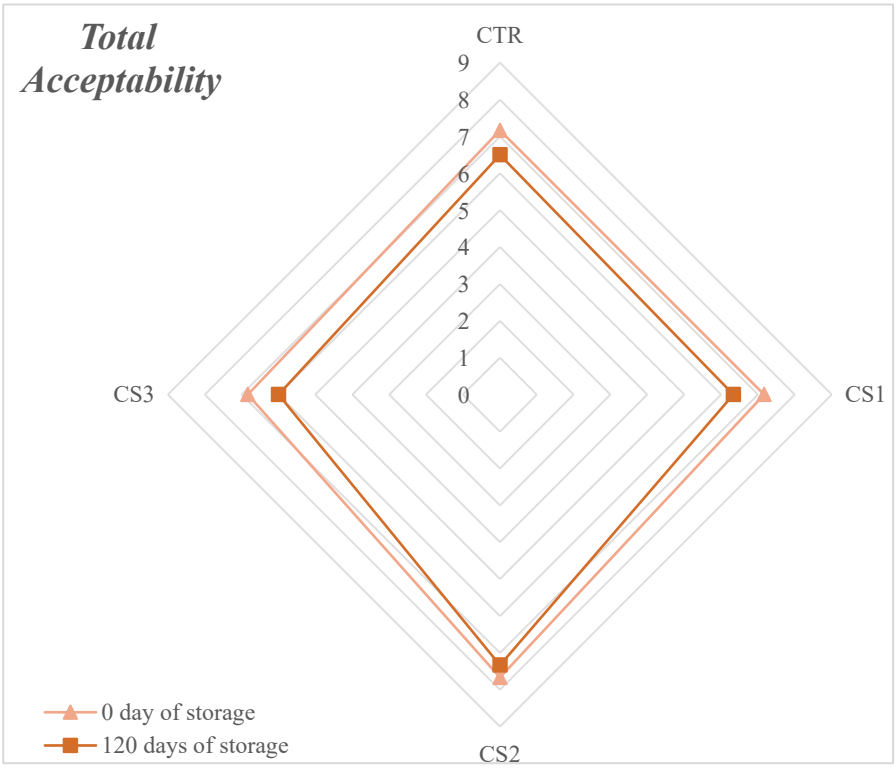


Figure 5 Total acceptability of the gummy candies at the start (0 day) and the end (120 days) storage period.

4.4 Conclusions

The addition of coffee silverskin has contributed positively to the chemical, physical, microbiological, and textural characteristics of candies. After 120 days of storage, the CS2 and CS3 candies showed the best results for antioxidant activity, and textural, and sensorial characteristics in comparison with CTR.

The results illustrated in this paper show that CSE could be a valid ingredient for the antioxidant enrichment of food products. The formulation of confectionery products with the aid of agri-food waste, as Coffee Silverskin, could have multiple advantages such as offering a vehicle of bioactive compounds for a large group of consumers, from children to adults, encouraging the transition of agri-food industries to a circular economy, enhance the production waste of the food sector and reduce environmental pollution resulting from the incorrect disposal of agri-food waste.

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Chapter 5 Lemon by-products to improve the storage quality of Clementine (Citrus x Clementina) Fruits

5.1 Quality Evaluation of Ready-to-Eat Coated Clementine (Citrus x Clementina) Fruits

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The personal contribution of the author was: conceptualization; methodology, formal analysis, software, data curation, writing—original draft preparation,

Abstract

Conventional and innovative preservation treatments were compared to extend the shelf life of ready-to-eat Clementine (Citrus x Clementina) segments. This research aimed to find an environmentally friendly packaging typology for this fruit while preserving quality and meeting the needs of the consumer in terms of practicality of use and food safety. The experimental plan envisaged both the use of conventional storage techniques, such as modified atmosphere packaging (O_2 5%, CO_2 5%, and N_2 90%), and the use of innovative storage techniques, such as an alginate-based (1.5%) edible coating. Quality changes were monitored by evaluating several indexes, such as color, texture, weight loss, respiration rate, pH, solid soluble content, bioactive compounds, antioxidant activity, organic acids, and microbiological contamination for 21 days at 4 °C. Moreover, a panel of judges

assessed the sensory characteristics. Ready-to-eat Clementine segments, produced with edible coatings, possessed better sensory and textural properties and similar physic-chemical characteristics than those packaged in a modified atmosphere. The coating favored the creation of a controlled environment with low oxygen stress, which resulted in a reduction in enzymatic activity and oxidation for 20 days of storage at 4 °C. The results suggest that an edible coating could be a sustainable alternative to a modified atmosphere for the shelf-life extension of ready-to-eat Clementine segments.

Keywords: Citrus x Clementina; edible coating; ready-to-eat fruits.

5.1.1 Introduction

In recent years, the increased focus on the health properties of foods and the fast-paced lifestyle of the modern consumer have led to an exponential increase in demand for ready-to-eat fruits.

The Clementine (Citrus x Clementina) is a citrus fruit. It is a hybrid between the Mediterranean mandarin and the sweet orange, whose maturation typically takes place in autumn; they have found their natural habitat in Calabria, a region in Southern Italy. Often, the Clementine is mistakenly confused with the mandarin, from which it is distinguished by its sweet taste, the absence of seeds (with rare exceptions), the ease of being peeled, a more intense orange color in the peel, and the absence of the characteristic scent of the mandarin. Very fragrant and sweet, Clementine fruits are eaten fresh or used for the preparation of syrups, juices, jams, and in many pastry recipes for the preparation of cakes and pies, or to obtain ice creams, sorbets, and jellies. This citrus fruit has been recognized by many authors as source of countless bioactive compounds with health-promoting properties for

humans, such as vitamins (in particular, C), carotenoids, flavonoids, and phenolic acids (Turner et al., 2013; Del Caro et al., 2004). However, post-harvest operations, such as peeling and cutting, can significantly affect the shelf life, as they favor metabolic processes that cause a sudden qualitative decay (Olivas et al., 2005). This type of vegetable processing in fact involves a faster decay of minimally processed fruits because it triggers a series of chained reactions that determine enzymatic browning, softening, microbial contamination in vegetables tissues, and the final production of volatile substances (Zappia et al., 2018; Rojas-Graru et al., 2007). These reactions drastically reduce the chemical, physical, and sensorial characteristics, as well as food safety. For these reasons, maintaining the qualitative parameters and delaying the growth of pathogen and spoilage microorganisms during storage are real challenges for the fresh fruit industry (Olivas et al., 2005).

Nowadays, fruits are stored using low temperatures (equal to or less than 4 °C), often in combination with modified atmosphere packaging (MAP), which represent packages with unbalanced gaseous composition with respect to the normal atmospheric gas composition (low concentration of oxygen and high concentration of carbon dioxide) to counteract reactions that, assisted by the presence of high oxygen rates, lead to chemical and microbiological degradation of the fruit. Nevertheless, this method has some limitations due to the loss of its effect after opening or possible mechanical damage during transport/sale (holes, cuts, etc.) (Celine et al., 2020). Edible coatings are a valid and environmentally sustainable technology to modify the atmosphere to extend the shelf life of ready-to-eat fruits (Ungnreanu et al., 2023; Embuscado et al., 2009). Sodium alginate and a calcium chloride solution can be used to formulate the coating on the surface of vegetables

(Chen et al., 2009). In the presence of calcium bivalent ions (Ca^{++}), there is a phenomenon of molecular cross-linking that determines the strengthening of chemical bonds between the components of sodium alginate and promotes the barrier effect of migration of the coating water (Embuscado et al., 2009). Several studies show that edible alginate-based coatings have the potential to supply a selective barrier to moisture, carbon dioxide, and oxygen, improve mechanical/textural properties, and prevent flavor loss (Embuscado et al., 2009; Chen et al., 2021). In addition, it has been reported that edible coatings allow control of the processes of transpiration and respiration (which cause fast weight loss and fruit dissection), slow down enzymatic activity, and help to preserve the healthy characteristics of the fruit (Chen et al., 2021;Castro-Yobal et al., 2021;Thivya et al., 2021) .

The replacement of the use of a modified atmosphere with edible coatings to extend the shelf life of perishable products could represent an eco-friendly choice as the materials used for the realization of the coatings are obtained from renewable sources (Castro-Yobal et al., 2021;Thivya et al., 2021; Zappia et al., 2023; Pelissari et al., 2018).

The aim of this work was to test the effect of quite recent preservation methods (MAP) and innovative ones, such as an edible alginate-based coating, on keeping the chemical, physical, microbiological, and sensory characteristics of ready-to-eat Clementine segments. The quality change was monitored by evaluating several microbiological, sensory, and physic-chemical indexes during storage for 21 days at 4 °C.

Previous studies have evaluated the influence of edible coatings on whole citrus fruits. Alvarez et al. (Alvarez et al., 2023) saw that pectin-based coatings enriched with eugenol preserved the chemical-physical characteristics of 'Valencia' oranges and reduced the incidence of sour rot caused by P. Italicum. Jurić et al. (2023) noted that layer-by-layer hydroxypropyl methylcellulose/chitosan or single chitosan coating preserved the overall quality of mandarin fruit both at room and cold temperatures for 10 and 28 days, respectively. Rasouli et al. (2019) evidenced that an edible coating based on Aloe vera gel and salicylic acid reduced electrolyte leakage, chilling injury, and malondialdehyde accumulation and preserved sensorial, textural, and microbiological characteristics of the orange 'Thomson Navel.' While edible coatings on whole citrus fruits have already been tested (Alvarez et al., 2023; Jurić, et al., 2023; Rosuli et al., 2019), no coating has yet been tested on segments of Clementine to produce ready-to-eat fruits. In addition, there are no studies concerning the packaging and storage of Clementine segments, so this research could be useful for disseminating new knowledge on equally new possibilities for technological proposals and the marketing of quality fruit.

5.1.2 Materials and Methods

5.1.2.1 Reception and Pretreatment of Raw Material

The Clementine fruits (*Citrus x Clementina*) were bought at a local market and transported to the FoodTec laboratory of the University Mediterranean of Reggio Calabria, and those with defects (presence of mold, physical damage, parasitic attacks, etc.) were removed.

Citrus fruits with weights between 80–90 g, heights ≥ 50 mm, widths ≥ 60 mm, and external peel 'albedo' of a completely orange color were selected. Subsequently, to decrease the microbial contamination on the 'albedo,' the whole fruits were dipped in a solution of sodium hypochlorite (200 ppm) for 2 min, washed with distilled water, and dried on stainless steel grids in a vertical laminar flow hood (UV lamp 30 W, mod. ASALAIR 1200 FLV, Asal Srl, Milan, Italy) for 30 min at room temperature in forced air (Rosuli et al., 2019).

The preparation of the raw material and the coating of the Clementine segments were performed in a vertical laminar flow hood (UV lamp 30 W, mod. ASALAIR 1200 FLV, Asal Srl, Milan, Italy).

All tools used during preliminary and coating operations were sanitized before use with a solution of sodium hypochlorite (50 ppm).

5.1.2.2 Preparation Coating Solution

Food grade sodium alginate (Sigma-Aldrich, Merk Life Science s.r.l., Milano, Italy), glycerol (Carlo Erba reagents, Cornaredo, Italy), and calcium chloride (Labochimica s.r.l., Campodarsego, Italy) were used to prepare the solutions to create the edible coating on the surface of the Clementine segments.

The sodium alginate solution (1.5% *w*/*v*) was prepared by dissolving the sodium alginate powder in distilled water at 70 °C with magnetic stirring for 60 min. Then, the solution was cooled at room temperature (up to 30 °C), and glycerol (1.5% *w*/*v*) was added as a plasticizer to increase the coating flexibility. Calcium chloride solution (2% *w*/*v*) was prepared by dissolving calcium chloride in distilled water under magnetic stirring at room temperature for 30 min (Glicerina et al., 2022).

2.1.2. Treatment and Storage of Clementine Slices

Clementine fruits, after the treatment described in Section 2.1, were peeled, and the segments were separated manually. Subsequently, a part of these segments was coated with alginate solution (AL), and the remaining segments were directly packaged in a modified atmosphere (MAP) with O_2 5%, CO_2 5%, and N_2 90% gas composition; the control sample (CTR) was packaged in a normal atmosphere.

Regarding the application of the edible coating on the surface of ready-to-eat Clementine fruits, the segments were dipped for 2 min in sodium alginate solution (1.5% *w*/*v*) and recovered, and the excess of the solution was air dried at room temperature for 1 min. Later, they were again immersed in calcium chloride solution (2% *w*/*v*) for 2 min to induce the cross-linked reaction, and they were air dried at room temperature up to complete drying (Glicerina et al., 2022).

Clementine segments (about 100 g) were packaged in a PP tray that was heat sealed with PP/PE film using a packaging machine (Orved, VGP 25N, Musile di Piave, Italy). The samples were stored at 4 °C for 21 days under constant lighting to simulate the real conditions of sale.

Physical, chemical, and microbiological analyses were carried out at 0, 3, 7, 14, and 21 days of storage. Sensory analyses were conducted at the beginning and end of storage. Figure 1 shows a schematic representation of this study on ready-to-eat Clementine segments.

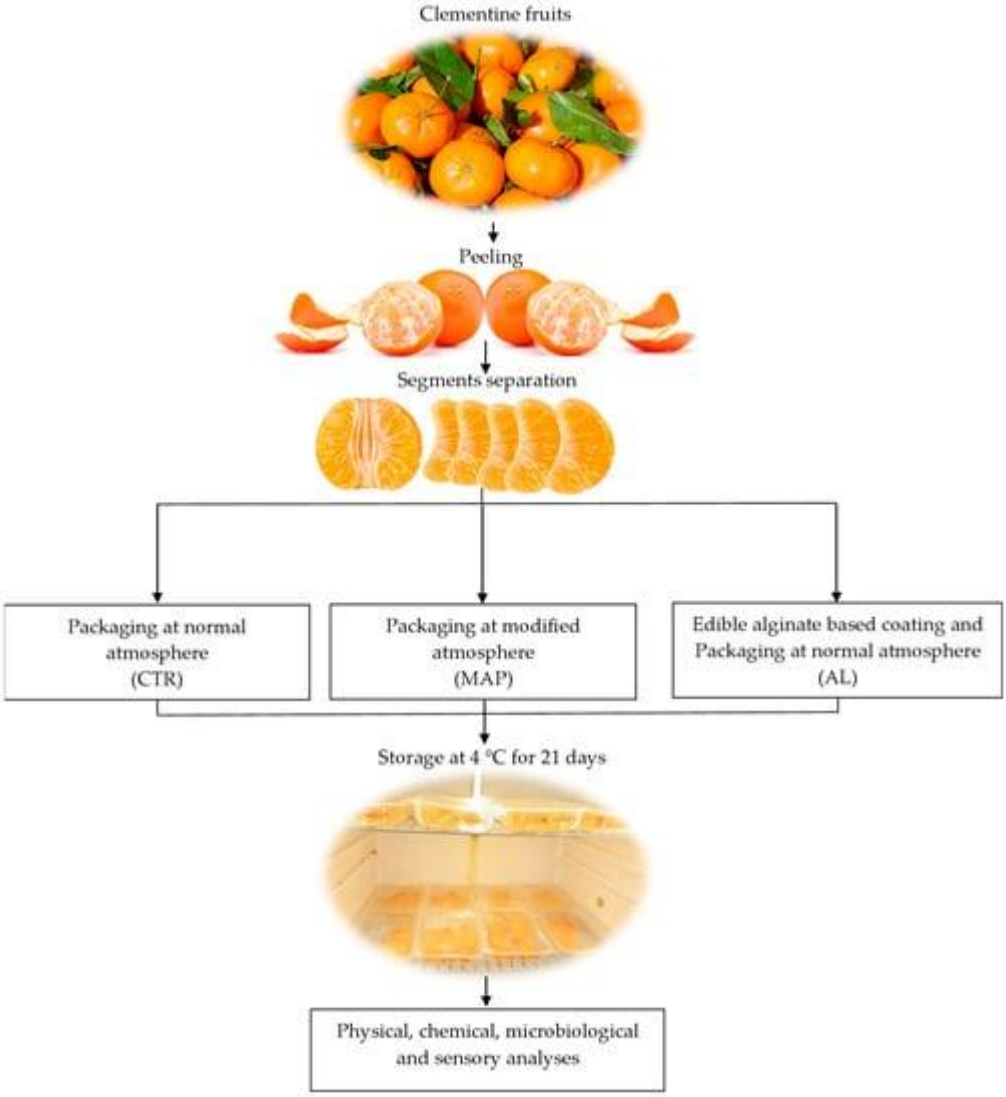

Figure 1. Schematic representation of this ready-to-eat Clementine segments study.

5.1.2.3 Color Measurement

The Clementine surface color parameters were determined according to the CIE L*a*b* color system by colorimeter (Minolta CM-700d Spectrophotometer, Konica Minolta, Inc., Sakai, Osaka, Japan) using a D65 illuminant. The L* parameter represents the lightness of the sample on the 0–100 scale, where 0 is the black and the 100 is the white. The positive a* represents the red content and the negative a* represents the green content of the sample on the red/green axis. The

positive b* represents the yellow content and the negative b* represents the blue content of the sample on the yellow/blue axis. Color variables were recorded for each sample per treatment (12 segments × 2 replicates).

5.1.2.4 Weight Loss, Moisture, and Headspace Gas Composition

Weight loss was determined according with the AOAC standard method (Association of Official Analytical Chemists, 1994), expressed in percentage and calculated by the following Equation (1):

$$\text{Weight loss } (\%) = \frac{W_b - W_m}{W_b} \times 100 \quad (1)$$

where W_b is the weight at the beginning of storage and W_m is the weight monitored after 3, 7, 14, and 21 days of storage.

The moisture content was assessed gravimetrically by the AOAC method (Association of Official Analytical Chemists, 2005) as the difference in weight pre- and post-drying in the oven at 105 °C until a constant weight. The moisture content was expressed in percentage and calculated as follows (Equation (2)):

$$\text{Moisture content } (\%) = \frac{W_i - W_f}{W_i} \times 100 \quad (2)$$

where W_i is the weight of the fresh samples and W_f is the weight of the samples after drying.

The headspace composition was recorded using a gas analyzer (PBI, DANSENSOR, Ringsted, Denmark, CP O_2/CO_2) equipped with a needle to withdraw and analyze the gaseous composition of the tray's headspace. The needle was inserted into the tray using a neoprene plastic pad to prevent gases from the surrounding atmosphere from entering during measurement.

5.1.2.5 Textural Analysis

The textural analysis was conducted with a penetration test to determine the hardness of the Clementine segments according to the method proposed by Glicerina et al. (2022) using a TA-XT Plus Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK) equipped with a 5 mm diameter stainless steel probe (P/5). Data acquisition and curve integration were carried out using Exponent software 6.1.4.0 (Stable Micro Systems Ltd., Godalming, UK). The parameters used for this test were: penetration distance of 3 mm, test speed of 1.0 mm/s, and post-test speed of 3.0 mm/s.

The hardness was expressed in grams (g) and estimated as the maximum peak of the curve recorded during the penetration test (the highest force necessary to penetrate the entire segment). Twenty replicates were used for each sample.

5.1.2.6 Sensory Analysis

A sensory quantitative descriptive analysis (QDA) was performed to assess the sensorial attributes of ready-to-eat Clementine segments.

Ten participants (aged 21–42) with earlier sensory analysis experiences were recruited to evaluate the visual, gustatory, olfactory, and structural attributes of the fruits. The sensory analysis was based on a 0-to-9-point hedonic scale where 0 showed the absence of the attribute and 9 showed an extremely high attribute value. The acceptability limit was considered to be 4.5. The visual appearance (intensity of the color, form, glossiness, uniformity of the surface), aroma (intensity, fruity, citrus, spicy), taste (sweet, salt, acid, bitter, citrus, fruity, astringent, aftertaste), texture descriptors (consistency, chewiness, moisture, crunchiness, turgidity), and

total acceptability were evaluated. The results were expressed as an average of the judgements obtained during the tasting.

5.1.2.7 Chemical Analysis

About 70 g of Clementine segments was homogenized using an Ultra-Turrax (T 25 digital, IKA, Staufen, Germany) and then transferred in a falcon tube and centrifuged (NF 1200R, Nüve, Ankara, Turkey) for 10 min at 10,000 rpm at 4 °C. The supernatant was recovered, filtered through a Buchner apparatus with a 0.45 mm filter paper, and filtered again with a PTFE 0.45 μm (diameter of 15 mm) syringe filter. The obtained juice was used to determine the pH, total soluble solids (TSS), titratable acidity (TA), total phenolic content (TPC), total flavonoid content (TFC), and total antioxidant activity (TAA).

5.1.2.7.1 Total Soluble Solids (TSS) and Titratable Acidity (TA)

The total soluble solids were estimated by placing a few drops of juice on the prism of a digital hand refractometer (DBR 047 SALT, Giorgio Bormac s.r.l, Carpi (MO), Italy) and expressed in degrees Brix (°Bx) at 25 °C.

As regards the titratable acidity, 5 mL of juice was diluted with 50 mL of deionized water and titrated with 0.1 M NaOH until pH 8.1 using a digital pH meter (Crison Basic 20, Crison instruments, Alella, Spain). The results were expressed as g citric acid/100 g, as the most abundant acid in citrus (Legua et al., 2014).

5.1.2.7.2 Total Phenolic Content (TPC)

The TPC was determined with a colorimetric method described by Jurić S. et al. (2023), with some modifications. Briefly, 0.1 mL of juice, 5 mL of distilled water, and 0.5 mL of Folin-Ciocalteau reagent (diluted in 1:2 ratios with distilled water) were mixed in a 10 mL volumetric flask. After 2 min, 1.5 mL of 20% sodium

carbonate solution (*v*/*v*) was added. The reaction mixture was made up to volume with distilled water and kept in the darkroom for 2 h at room temperature. The solution used as a blank was prepared by replacing the sample with water in the reaction mixture. The absorbance was recorded at 765 nm against a blank using a spectrophotometer (Perkin-Elmer UV-Vis k2, PerkinElmer Inc., Waltham, MA, USA) and by comparing with a gallic acid calibration curve (1–10 mg L^{-1}). The results were expressed as mg of gallic acid equivalents/kg of fresh weight.

5.1.2.7.3 Total Flavonoid Content (TFC)

The TFC was carried out with a modified method proposed by Jurić S. et al. (2023). In a 5 mL volumetric flask was mix 0.2 mL of juice, 2 mL of distilled water, and 0.15 mL of 5% of sodium nitrite (*v*/*v*). After 5 min, 0.15 mL of 10% aluminum chloride (*v*/*v*) was added to the reaction mixture, which was then incubated at room temperature for 6 min. Subsequently, 1 mL of 1 M sodium hydroxide was added, made up to volume with distilled water, and kept at room temperature for 10 min. A blank solution was prepared by replacing the sample with water in the reaction mixture. The absorbance was recorded at 360 nm against a blank using a spectrophotometer (Perkin-Elmer UV-Vis k2, PerkinElmer Inc., Waltham, MA, USA) and by comparing with a quercetin calibration curve (1–20 mg L^{-1}). The results were expressed as mg of quercetin equivalents/g of fresh weight.

5.1.2.7.4 Determination of Organic Acids

For the determination of organic acids (oxalic, malic, ascorbic, and citric acids) in Clementine samples, HPLC (High-performance liquid chromatography) analysis was used according to the literature (Jurić et al., 2023). Clementine juices

were diluted when necessary and filtered by a PTFE 0.45 μm (diameter of 15 mm) syringe filter before analysis.

A Knauer HPLC Smartline Pump 1000, equipped with a Knauer Smartline UV Detector 2600 and a thermostat, was used. The separation of organic acids was performed on SYNERGI HYDRO-RP (250 mm × 4.6 mm i.d., 4 μm) at 22 °C. The injected sample volume was 20 μL. The analysis was carried out in isocratic elution at a flow rate of 0.7 mL min with potassium phosphate 20 mM at pH 2.9 as the mobile phase. The detection wavelengths were 254 nm for ascorbic acid and 210 nm for malic, oxalic, and citric acid. The results are expressed as mg of acid/L of juice.

5.1.2.7.5 Total Antioxidant Activity (TAA)

The antioxidant activity of Clementine segments was measured using the ABTS and DPPH assays following the procedures proposed by De Bruno et al. (De Bruno et al., 2023), appropriately modified.

Concerning the DPPH assay, a 6×10^{-5} methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was prepared. The analysis was carried out by reacting in a cuvette 20 μL of Clementine juice at 2980 μL of methanolic DPPH radical solution. After 30 min of incubation in the dark at room temperature, the decrease in absorbance to 515 nm (proportional to the radical scavenging activity of the sample) was recorded using a spectrophotometer (Perkin-Elmer UV-Vis k2, PerkinElmer Inc., Waltham, MA, USA). Methanol was used as a blank.

Regarding the ABTS assay, the preparation of ABTS solution involved the reaction of 7 mM of ABTS (2,2-Azino-bis 3-ethylbenzothiazoline-6-sulfonic acid) solution and 2.45 of mM potassium persulfate. The mixture was kept in the dark for

12–16 h at room temperature. Then, the ABTS radical solution was diluted with ethanol up to 0.7 of absorbance at 734 nm, determined spectrophotometrically (Perkin-Elmer UV-Vis k2, PerkinElmer Inc., Waltham, MA, USA). The analysis of the samples was performed by mixing 20 µL of Clementine juice at 2980 µL of methanolic ABTS solution. The decrease in absorbance was read after 6 min of dark incubation at room temperature. Ethanol was used as a blank.

The results of both the DPPH and ABTS assays were expressed as mM Trolox equivalent kg^{-1} of fresh Clementine fruits plotted against the Trolox concentration (from 1 to 24 µM).

5.1.2.3 *Microbiological Analysis*

The total bacterial count (TBC) and yeasts and molds (Y&M) were detected to evaluate the microbiological contamination of Clementine segments following Glicerina et al. (2022), with some modifications. For the microbial analysis, 5 g of each sample was placed in a sterile bag with a Ringer solution and homogenate using Stomacher (BagMixer® 400 P, Interscience, Saint-Nom-la-Bretèche, France) for 3 min. The obtained samples were serially diluted, and 1 mL of each dilution was used for microbiological analysis. Dichloran Rose Bengal Chloramphenicol (DRBC) was used for the Y&M and the TBC. The plates, after solidification, were incubated at 25 °C, and colonies enumeration was made after 5 days and after 2 days for the Y&M and TBC counts, respectively. The results were expressed as log10 CFU/g of sample.

5.1.2.4 Statistical Analysis

The analytical data were reported as the mean value ± standard deviation. The analysis of variance (one-way ANOVA) was conducted by SPSS Software (Version 15.0, SPSS Inc., Chicago, IL, USA) by applying the Tukey post hoc test at $p < 0.05$

5.1.3. Results

5.1.3.1 Color Measurements

The color of food is a very important index of quality for the consumer. The results reported in Table 1 evidence no significant differences for L* and b* parameters among the samples and during storage times, whereas some light variations of the red color only appeared after 3 days. At the end of storage, all of the color parameters were similar among the Clementine samples.

Table 1. Colorimetric coordinates of Clementine samples during storage.

Parameter	Sample	Time					
		0	3	7	14	21	Sig.
L*	CTRL	50.19±1.69	50.72±1.42	53.25±1.22	52.86±2.65	51.71±1.67	n.s.
	MAP	48.97±1.84	50.94±1.54	52.31±1.98	50.68±2.03	52.51±1.88	n.s.
	AL	54.80±1.33	52.62±1.69	53.84±1.16	52.57±1.54	53.92±2.10	n.s.
Sign.		n.s.	n.s.	n.s.	n.s.	n.s.	
a*	CTRL	8.30±1.21^{b}	8.41±1.27 ^{b}	8.74±1.01	8.85±2.14	8.67±1.32	n.s.
	MAP	9.73±1.00 ^{a}	8.35±1.58 ^{b}	8.42±1.18	8.43±1.14	9.25±1.48	n.s.
	AL	8.18±1.3 ^{b}	9.27+1.01 ^{a}	8.97±1.53	8.60±1.96	9.46±1.56	n.s.
Sign.		*	*	n.s.	n.s.	n.s.	
b*	CTRL	19.48±1.07	20.90±1.54	19.78±1.43	21.71±97	20.21±1.82	n.s.
	MAP	19.66±1.21	19.92±1.36	21.23±1.61	21.90±2.15	21.83±1.59	n.s.
	AL	20.27±1.5	20.48±1.41	21.63±1.53	20.60±2.63	21.39±1.58	n.s.
Sign.		n.s.	n.s.	n.s.	n.s.	n.s.	

Small letters within a column and capital letters within a row show significant differences as assessed by Tukey's post hoc test. **, significance at $p < 0.01$; *, significance at $p < 0.05$; n.s., not significant

5.1.3.2. Moisture, Weight Loss, and Headspace Gas Composition

The trends of moisture, weight loss, and headspace gas composition are shown in Table 2. All samples showed very lightweight loss with values equal to 0.1% after 21 days of storage. Similar moisture contents (85%–86%) were observed in all samples.

Table 2. Moisture, weight loss, and headspace gas composition of Clementine segments during storage.

Parameter	Sample	Time					
		0	3	7	14	21	Sig.
Moisture (g/100 g)	CTRL	85.02±0.55 ^{B}	88.46±2.00 ^{B}	87.60±1.90 ^{AB}	83.2±2.16 ^{A}	85.59±1.85 ^{AB}	*
	MAP	85.65±1.50	86.10±1.50	82.93±1.16	83.3±1.66	86.69±0.98	n.s.
	AL	85.38±2.00	85.56±0.30	86.74±1.97	85.8±1.35	86.77±1.23	n.s.
Sign.		n.s.	n.s.	n.s.	n.s.	n.s.	
Weight loss (g/100 g)	CTRL	-	0.03±0.01 ^{A}	0.02±0.01 ^{A}	0.07±0.02 ^{B}	0.10±0.03 ^{B}	**
	MAP	-	0.03±0.01 ^{A}	0.02±0.01 ^{A}	0.08±0.02 ^{B}	0.10±0.03 ^{B}	**
	AL	-	0.05±0.02 ^{A}	0.05±0.01 ^{A}	0.07±0.01 ^{AB}	0.10±0.04 ^{B}	**
Sign.			n.s.	n.s.	n.s.	n.s.	
O_2 (%)	CTRL	21.00±0.00^{aA}	14.5±1.11^{aBC}	14.3±1.90^{aBC}	7.1±0.99^{bB}	5.6±0.71^{bC}	**
	MAP	5.00±0.00^{bA}	3.4±0.98^{bAB}	1.8±0.40^{bA}	1.2±0.43^{cA}	1.1±0.05^{cA}	**
	AL	21.00±0.00^{aA}	16.4±0.43^{aB}	13.3±0.63^{aBC}	8.25±0.57^{aC}	8.3±0.68 ^{aC}	**
Sign.		**	**	**	**	**	
CO_2 (%)	CTRL	0.02 ^{bC}	9.4±1.37^{B}	10.0±2.30^{bB}	13.0±1.13^{bB}	19.9±0.14 ^{aA}	**
	MAP	5.00 ^{aC}	9.9±0.76^{B}	13.5±1.56 ^{aBC}	17.2±2.3 ^{aA}	15.8±1.79 ^{bBC}	**
	AL	0.02 ^{bC}	9.2±0.43 ^{B}	9.8±0.81 ^{bB}	14.9±0.10^{abA}	14.3±0.49 ^{bA}	**
Sign.		**	n.s.	*	**	*	

Small letters within a column and capital letters within a row show significant differences as assessed by Tukey's post hoc test. **, significance at $p < 0.01$; *, significance at $p < 0.05$; n.s., not significant

The results of gas composition denoted the effect of fruit metabolism during storage in a different trend, depending on the applied technology. The oxygen decreased during monitoring times from 21% to 5.6% (CTR) or from 8.3% (AL) and 5% to 1.1% (MAP), with a consequent increase in the carbon dioxide percentage (14.3%–19.9%).

5.1.3.3 Textural and Sensory Analysis

As illustrated in Figure 2, a general increase in hardening was observed in all samples during the storage time, whereas the samples showed significant differences. The coated segments (AL) possessed a higher firmness (279.3 g) than the MAP (164.9 g) and CTR (206.2 g). This trend was probably due to reactions that cause tissue breakdown or thickening.

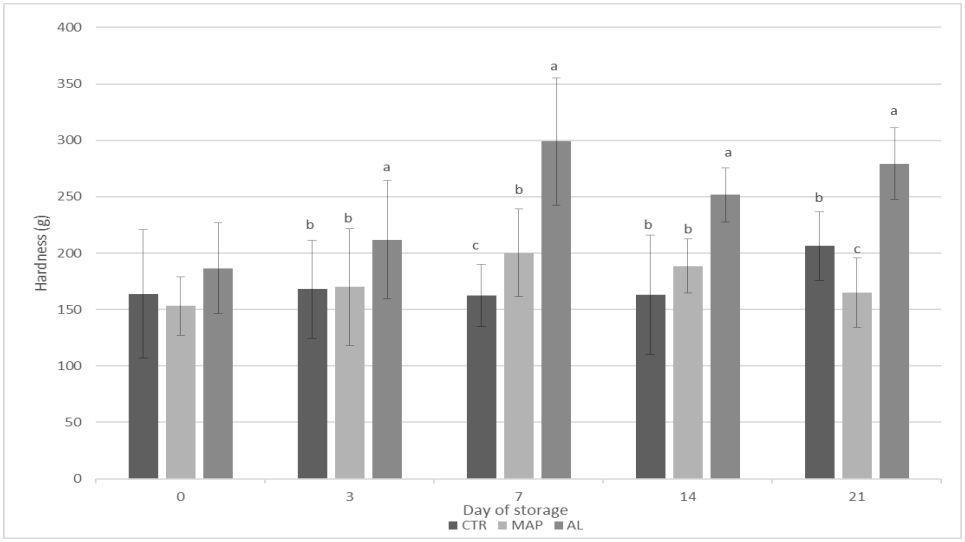

Figure 2. Textural proprieties in Clementine segments during the storage. Different letters show significant differences between each group ($p < 0.05$). Abbreviations: CTR segments uncoated and packaged in normal atmosphere; MAP segments packaged in modified atmosphere; AL segments with edible alginate-based coating.

The sensory analysis of samples (Table 3) showed a lower score for sweet (5.5), fruity (5), and citrusy (5.3), while a higher score was given for bitter (6.2) in AL than CTR and MAP (around 6.7 for positive gustatory hints and, respectively, 5.7 and 4.5 for negative ones), among the gustatory hints. However, the judgments about the visual and structural characteristics indicated that AL were the most appreciated Clementine samples by panelists at up to 20 days of storage (color score: 8; firmness score: 7). Finally, regarding the total acceptability, the coated

Clementine segments obtained a higher score than MAP and CTR after 21 days of storage at 4 °C (6.2).

Table 3. Sensory parameters of Clementine segments during storage.

Parameter	Sample	Time		
		0	21	Sig.
Sweet	CTRL	6.80±1.30^{aA}	4.00±0.08^{bB}	**
	MAP	6.70±1.20^{aA}	5.00±0.60^{aB}	**
	AL	5.50±1.00 ^{b}	5.00±0.80 ^{a}	n.s.
Sign.		*	*	
Bitter	CTRL	5.70±0.90^{ab}	6.00±1.20^{ab}	n.s.
	MAP	4.50±1.00^{b}	4.00±1.30^{a}	n.s.
	AL	6.20±0.70^{a}	7.00±1.20^{b}	n.s.
Sign.		*	**	
Fruity	CTRL	6.70±0.70^{a}	6.00±1.00	n.s.
	MAP	6.70±0.70^{a}	6.00±1.50	n.s.
	AL	5.00±0.80^{bB}	6.00±0.80 ^{A}	*
Sign.		*	n.s	
Citrusy	CTRL	6.70±0.70^{a}	6.00±1.20^{ab}	n.s.
	MAP	6.5±0.50^{aA}	5.00±0.80^{aB}	**
	AL	5.30±1.20^{bB}	7.00±0.60^{bA}	*
Sign.		*	**	
Colour	CTRL	7.00±0.06^{b}	6.50±1.30^{b}	n.s.
	MAP	6.50±0.05^{b}	6.00±0.60^{b}	n.s.
	AL	9.00 ±0.05^{a}	8.00±0.60^{a}	n.s.
Sign.		**	*	
Firmness	CTRL	6.8±1.1^{a}	6.2±0.9 ^{a}	n.s.
	MAP	7.2±1.1^{ab}	6±1.00 ^{a}	n.s.
	AL	8.0±0.6 ^{b}	7±1.2 ^{b}	n.s.
		*	*	
Overall acceptability	CTRL	8.00±0.50 ^{aA}	5.00±0.50 ^{bB}	**
	MAP	8.00±1.00 ^{aA}	6.00±0.50 ^{aB}	**
	AL	7.2±0.50 ^{bA}	6.2±0.50 ^{aB}	**
Sign.		*	*	

Small letters within a column and capital letters within a row show significant differences as assessed by Tukey's post hoc test. **, significance at $p < 0.01$; *, significance at $p < 0.05$; n.s., not significant

5.1.3.4 Total Soluble Solids and Titratable Acidity

The total soluble solids (TSS) and titratable acidity (TA) are important quality indices that show the freshness of Clementine fruits and that are closely related to

the sweet taste that makes them particularly appreciated by the final consumer. In this study, significant differences among the tested samples were noted after 7 days of storage. The results reported in Table 4 show the trend in all samples during storage.

Table 4. Total soluble solids and titratable acidity of Clementine segments during storage.

Parameter	Sample	Time					
		0	**3**	**7**	**14**	**21**	**Sig.**
SSC (Brix °)	CTRL	12.1±0.05^{AB}	12.2±0.17^{aA}	10.0±0.72^{bC}	11.6±0.1^{aAB}	11.5±0.38^{B}	**
	MAP	11.9±0.4^{A}	12.8±0.1^{aA}	11.5±0.71^{aA}	11.8±0.07^{bA}	12.3±0.98 ^{A}	n.s
	AL	11.9 ±0.13^{A}	12.2±0.23^{aA}	12.6±0.78^{aA}	12.2±0.07^{cA}	11.9±0.06 ^{A}	n.s
Sig.		n.s.	n.s.	*	**	n.s.	
Titratable acidity (%)	CTRL	0.59±0.03^{aA}	0.54±0.02^{aA}	0.53±0.04^{aA}	0.50±0.05^{aB}	0.51 ±0.06^{aAB}	*
	MAP	0.57±0.1^{aA}	0.57±0.08^{abA}	0.54±0.08^{aA}	0.52±0.01^{aA}	0.52±0.05^{aA}	n.s.
	AL	0.54±0.03^{aA}	0.54±0.01^{bA}	0.56±0.05^{aA}	0.54 ±0.03^{aA}	0.54 ±0.01^{aA}	n.s.
Sign.		n.s	*	n.s.	n.s.	n.s.	

Small letters within a column and capital letters within a row show significant differences as assessed by Tukey's post hoc test. **, significance at $p < 0.01$; *, significance at $p < 0.05$; n.s., not significant

As regards the Clementine segments coated with alginate, a constant trend was recorded until the end of storage (11.9 °Bx and 0.54% for TSS and TA, respectively). Similar results were observed for MAP samples with parameters from 11.9 to 12.3 °Bx and from 0.57% to 0.52%, from the beginning until 20 days of storage. In contrast, the CTR samples showed a statistically significant variation in these parameters due to the physiological mechanisms of the fruits following the deprivation of the albedo.

5.1.3.5 Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Organic Acid, and Total Antioxidant Activity (TAA)

The healthy properties of Clementine fruits are due to a series of valuable compounds including polyphenols, flavonoids, and organic acids, whose variations observed in this study are reported in Figure 3 and Table 5. During the first days of storage, there was an increase in TPC and TFC for all of the analyzed samples. This phenomenon is due to enzymatic activities that occur immediately after the peeling of the fruits. A significant increase was found after 7 and 14 days of storage. However, on the 21st day of shelf life, the AL segments showed, significantly, the highest antioxidant contents compared to MAP and CTR (571.80 mg GAE kg^{-1} and 432.85 mg QE kg^{-1}).

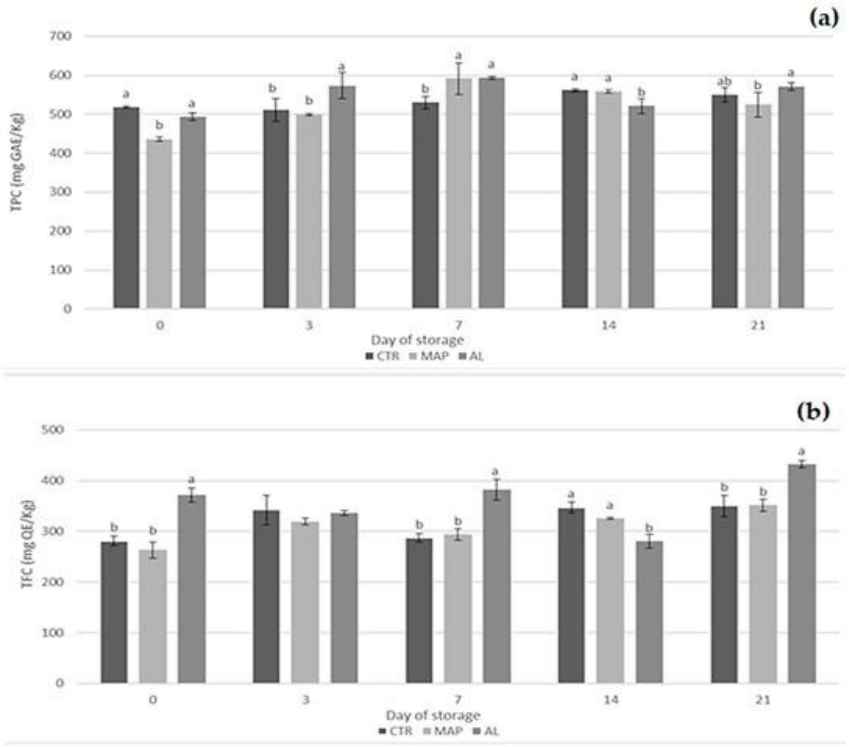

Figure 3. Total phenolic content (TPC) (**a**) and total flavonoid content (TFC) (**b**) in Clementine segments during storage. Different letters (**a**,**b**) show significant differences between the means of each group as assessed by Tukey's post hoc test ($p < 0.05$).

As regards the organic acid content (Table 5), both MAP and AL showed beneficial effects on the maintenance of citric, ascorbic, malic, and oxalic acids during the product's shelf life. Instead, in CTR samples, a drastic decrease was

observed, especially in terms of ascorbic acid after three days of storage (from 153.64 to 101.23 mg L−1).

Table 5. Organic acid trends of Clementine segments during storage.

Parameter (mg L $^{-1}$)	Sample	Time					
		0	3	7	14	21	Sig.
Oxalic acid	CTRL	190.24±5.70^{A}	150.59±2.70^{B}	161.88±2.70^{B}	152.86±1.60^{B}	204.30±2.80^{A}	**
	MAP	227.37±8.20^{A}	152.11±9.40^{B}	247.45±7.20^{A}	118.81±1.80^{B}	208.89±2.80^{AB}	**
	AL	150.58±6.30	147.59±5.30	186.93±5.80	186.24±90	211.57±12.40	n.s.
Sign.		n.s.	n.s	n.s.	n.s.	n.s.	
Citric acid	CTRL	4818.34±83.40	4478.34±28.70	4925.01±96.70	4430.29±43.40	4489.26±41.20	n.s.
	MAP	5502.17±39.70	5061.70±47.00	4367.87±48.90	4996.61±72.80	4444.42±76.30	n.s.
	AL	5005.19±20.70	4235.22±59.40	4997.44±43.40	4632.13±72.00	4860.58±64.50	n.s.
Sign.		n.s	n.s.	n.s.	n.s.	n.s.	
Malic acid	CTRL	861.98±42.80^{A}	825.72±62.80^{A}	662.36±54.00^{abAB}	538.78±42.90^{B}	681.32±44.70^{AB}	**
	MAP	615.39±38.10	625.48±58.00	504.96±46.10^{b}	481.06±51.90	498.81±58.70	n.s.
	AL	624.55±54.30^{AB}	713.00±49.00	716.35±68.70^{aA}	483.06±61.40^{B}	625.54±67.10^{AB}	*
Sign.		n.s	n.s	*	n.s.	n.s.	
Ascorbic acid	CTRL	153.64±19.25^{A}	101.23±25.30^{bAB}	89.04±5.88^{cB}	97.19±1.65^{bAB}	90.46±1.00^{bB}	**
	MAP	118.74±6.10	110.71±4.30^{ab}	133.20±0.50^{b}	121.99±22.30^{ab}	127.93±23.60^{ab}	n.s.
	AL	124.49±18.80^{B}	147.18±35.50^{aAB}	169.82±14.10^{aA}	168.56±4.70^{aA}	167.85±8.40^{aA}	**
Sign.		n.s.	*	**	*	*	

Small letters within a column and capital letters within a row show significant differences as assessed by Tukey's post hoc test. **, significance at $p < 0.01$; *, significance at $p < 0.05$; n.s., not significant

The antioxidant activity (Table 6) recognized in citrus is due not only to the presence of phenolic compounds but also to the presence of organic acids. In this study, the synergy between the compounds mentioned above in counteracting the activity of free radicals was particularly evident from the results obtained from the ABTS and DPPH assays. In particular, the AL segments showed a gradual increase in radical scavenging activity from 1.26 to 1.76 and from 0.43 to 0.50 mM Trolox kg−1, respectively. This was probably related to the previously described

maintenance of the concentration of organic acids and the increase in TPC and TFC over time.

Table 6 Total antioxidant activity (TAA) of Clementine segments during storage.

Parameter (mM TE kg $^{-1}$)	Sample	Time					
		0	3	7	14	21	Sig.
ABTS	CTRL	1.33 ±0.20^{aB}	1.80 ±0.05^{aA}	1.31±0.23^{aB}	1.24±0.05^{aB}	1.42±0.06^{bB}	**
	MAP	1.05 ±0.08^{bA}	1.21 ±0.25^{bA}	1.46±0.35^{aA}	1.30±0.04 ^{aA}	1.39 ±0.12^{bA}	n.s.
	AL	1.26 ±0.12^{abB}	1.97±0.15^{aA}	1.27±0.17^{aB}	1.32±0.08 ^{aB}	1.76 ±0.17^{aA}	**
Sign.		*	**	n.s	n.s.	**	
DPPH	CTRL	0.43±0.01^{aA}	0.39±0.02^{cAB}	0.39±0.02^{abAB}	0.32 ±0.01^{cB}	0.40±0.08^{aAB}	*
	MAP	0.44±0.05^{aAB}	0.47 ±0.01^{aA}	0.31 ±0.07^{bB}	0.36 ±0.02^{bAB}	0.43 ±0.1^{aAB}	*
	AL	0.43±0.04^{aB}	0.44 ±0.01^{bB}	0.41 ±0.01^{aB}	0.41 ±0.01^{aB}	0.50 ±0.03^{aA}	**
Sign.		n.s.	**	*	**	n.s.	

Small letters within a column and capital letters within a row show significant differences as assessed by Tukey's post hoc test. **, significance at $p < 0.01$; *, significance at $p < 0.05$; n.s., not significant

5.1.3.6 Microbiological Analyses

Microbiological analysis did not detect the presence of total microbial charge, yeast, or molds.

5.1.4 Discussion

Maintaining the quality of ready-to-eat fruits over time is a real challenge for the fresh fruit industry. Mechanical operations, such as cutting or peeling, cause stress on the fruit, which can increase metabolic activity, exposure to microbiological contamination, and activation of enzymatic pools responsible for adverse effects (e.g., discoloration, loss of turgidity, and loss of nutritional proprieties). In this study, the quality of Clementine segments was studied in

relation to two typologies of packaging, monitoring chemicals, and physical, microbiological, and sensory parameters during 21 days of storage at 4 °C.

The observed trend for moisture and weight loss was probably due to the specific characteristics of the packaging conditions and the metabolic activity of segments after peeling. In fact, the high barrier properties of the packaging prevented the diffusion of the aeriform from the inside to the outside of the tray. However, it should be noted that in the trays containing the CTR samples, an excess of fog was found during storage, indicating an intense metabolic activity due to transpiration and respiration processes accelerated by the deprivation of the peel from the fruit (Figure 4). This was also confirmed by the concentration of oxygen and carbon dioxide in the headspace of the trays during storage.

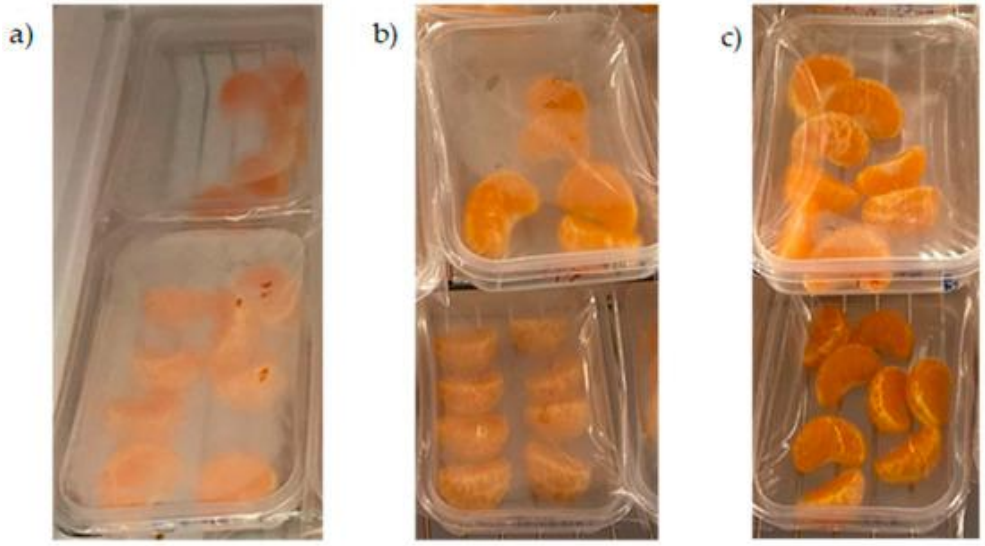

Figure 4. Ready-to-eat Clementine segments packaged at normal atmosphere (CTR) (**a**), at modified atmosphere (MAP) (**b**), and edible-alginate coated (**c**).

Comparing the oxygen and carbon dioxide levels of AL and CTR, it can be inferred that the coating applied on the segments slowed down their metabolic process.

The sensory characteristics, as well as the color and texture, play a key role in the choice of purchase by consumers. However, they have still been poorly studied. Previously, it was reported that the typical sweet taste of Clementine fruits can be strongly influenced by the constituent variables of the fruit (ratio of organic acid and solid soluble content), and also by the external stresses to which they are subjected pre/at harvest (cultivar, environmental condition, maturity/harvest period, physical damage, parasitic attacks, mold, etc.) and post-harvest (temperature, ratio of O_2/CO_2, permeability of packaging, microbiological attack, enzymatic reactions of degradation) (Choen et al., 1990; Wigati et al., 2023). Moreover, various volatile compounds, such as limonene, myrcene, α-pinene, and linalool, are decisive in giving the typical odor and taste to the fruits of Clementine and mandarin (Moshonas et al., 1997;Perez-Lopez et al., 2006;Barboni et al., 2009; Hagenmaie et al., 2002).

The sensory evaluations (Figure 5 and 6) revealed that the parameters related to the gustatory sensations were slightly influenced by the application of the coating at the end of storage. These results agree with earlier studies carried out on segments of oranges coated with alginate. It has been found that the calcium chloride used to promote the cross-linking of sodium alginate causes a greater feeling of bitterness in the fruit (Glicerina et a., 2022). However, the parameters related to structure and visual appearance were significantly improved in AL samples.

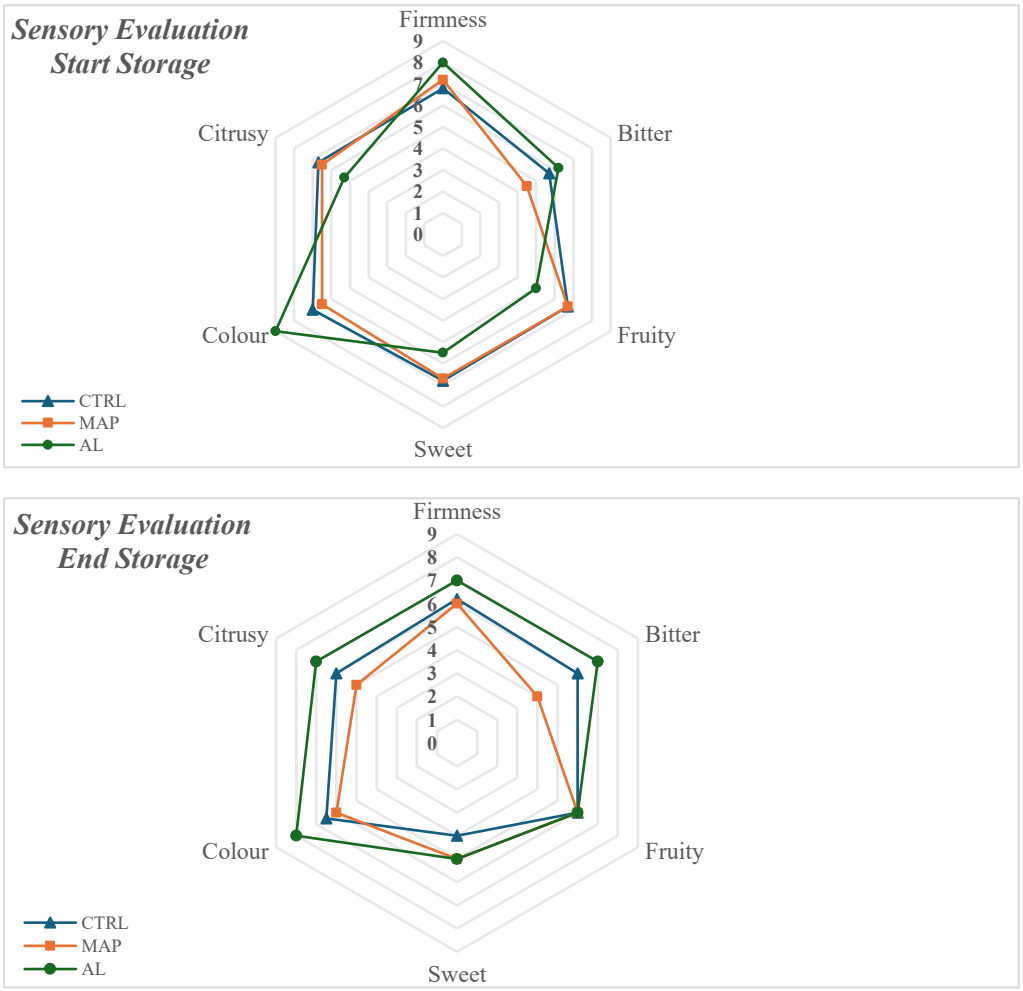

Figure 5 Spider plot of Sensory Evaluation at start (a) and end (b) storage of Clementine Segments

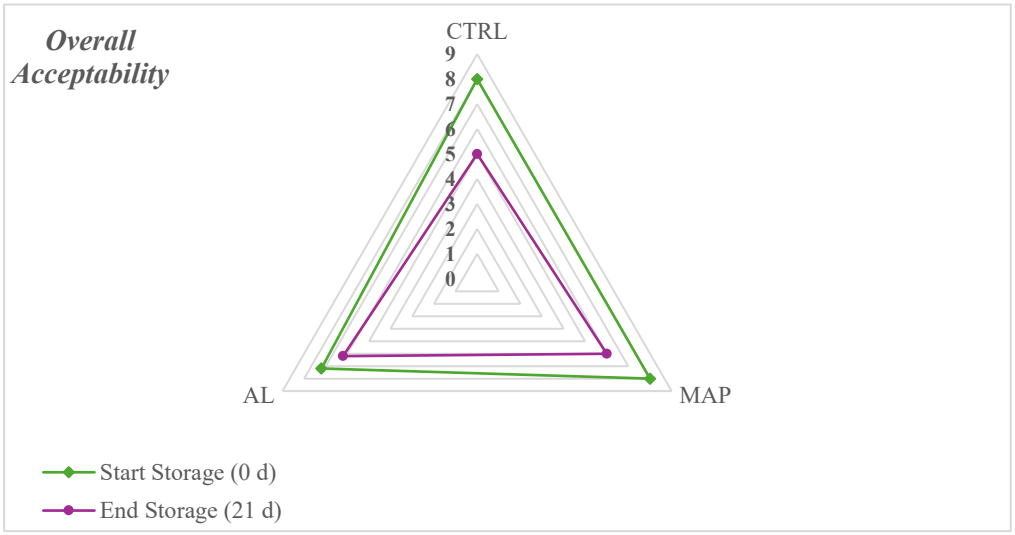

Figure 6 Spider plot of Overall Acceptability of Clementine Segmensts

The color and texture results confirmed the observations obtained through the panel test. Several studies show that edible alginate-based coatings can improve physical properties, such as mechanical properties, through cross-linking reactions between the structural components of the vegetable cell wall and Ca^{++} ions, and visual ones, through retaining the color of the fruits as unchanged and bright (Embuscado et al., 2009). Figure 7 shows the visual appearance of the Clementine segments after 21 days of storage.

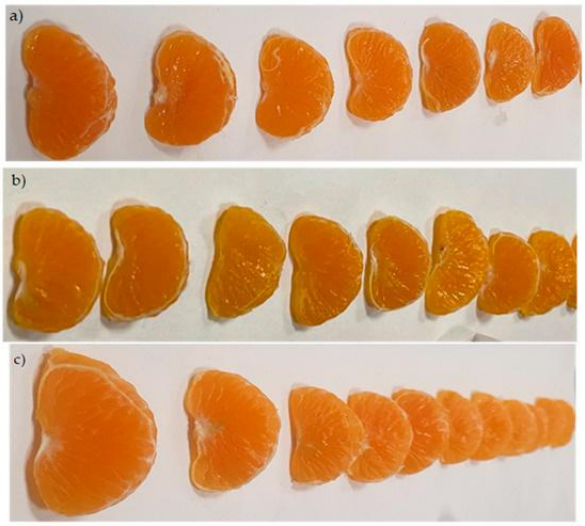

Figure 7 Visual appearance of Clementine segments after 21 days of storage. Segments at normal atmosphere (CTR) (**a**), packaged in modified atmosphere (MAP) (**b**), and edible-alginate coated (**c**).

As regards the chemical analyses, the TSS and TA levels in the juice bags are undoubtedly among the most important quality indices for Clementine fruits. During ripening, there is an increase in TSS and a decrease in TA that give citrus fruits the typical sweet taste (Hussain et al., 2017; Rapisarda et al., 2008). The optimal maturity values of TSS and TA should be between 10 and 13 °Bx and from 0.6 to 1.4%, respectively (Tietel et al., 2011). However, the values of these parameters can be significantly influenced by environmental and metabolic factors (Hijaz et al., 2020). Post-harvest, keeping these parameters within the limits

abovementioned ensures that the fruits keep the typical fresh and sweet taste over time. In this study, both the use of MAP and AL allowed for maintaining, unchanged, the basic characteristics after peeling during 21 days of storage, while CTR showed a marked qualitative decay after the 7th day of storage. These results suggest that both MAP and AL allow for slowing down the metabolic processes that underlie the qualitative decay of fruits. Also, the results obtained for the headspace confirm that CTR samples (without MAP and edible coating) produced a more intense metabolic activity due to respiration and transpiration processes that occurred after the peeling of the fruit (Banda et al., 2015; Kader et al., 2000). Currently, there are no studies on edible coatings on Clementine segments; however, similar results were obtained by the application of edible coatings on whole citrus fruits (Jurić et al., 2023) and Clementine fruits treated at pre-harvest with foliar application of Si-Ca and stored for 30 days (Ziogas 2022).

The health, nutritional, and antioxidant properties of Clementine fruits have been recognized by many authors (Saini et al., 2022; Sun et al., 2002; Harats et al., 1998; Ghanim et al, 2010; Codoñer-Franch et al, 2010; Tomas-Barberan et al., 1997) These properties are explained by the synergistic effect of phenolic compounds and organic acids (such as ascorbic, citric, and malic acid) present in fruits (Wigati et al., 2023). During storage, these compounds can be subjected to a decrease, depending on the storage conditions (packaging, temperature, lighting, and presence of oxygen and ethylene). In addition to physical and sensory characteristics, the purpose of this work was to investigate the effect of MAP and AL in preserving the bioactive compounds and antioxidant activity of ready-to-eat Clementine segments.

In our study, regarding TPC and TFC, significant differences were found among individual samples and during their shelf life. An initial increase in TPC and TFC was observed, followed by the maintenance of these constant values at the end of their shelf life. Several authors report that this phenomenon is mainly due to the activity of the enzyme PAL that, following the peeling operation, catalyzes the synthesis reactions of new phenolic compounds (Tomas-Barberan et al., 1997; Petriccione et al., 2015; Piscopo et al., 2019). However, this rise could be followed by rapid decay if strategies to contain the oxidation of newly formed compounds do not fit. In this case, the combined effect of packaging, temperature, and MAP/coating resulted in a good result in terms of the maintenance of the compounds throughout the shelf life. A similar trend of TPC and TFC was observed in previous studies of citrus segments and juices (Del Caro et al., 2004).

The organic acid composition of the fruits is crucial for both the sensory and nutritional characteristics. In Clementine fruit, the major organic acids are citric, malic, ascorbic, and oxalic, respectively, and storage conditions can significantly affect their concentration (Jurić et al., 2023;Wigati et al., 2023;Alos et al., 2004). The obtained results denoted that MAP and AL maintained the levels of major organic acids present in the Clementine fruits over time. In particular, the level of ascorbic acid remained constant for all times of storage. In contrast, a drastic reduction of ascorbic acid was shown in the CTR sample. This is probably because only the packaging with barricaded material did not allow for slowing down the metabolic reactions that lead to its reduction. A previous study demonstrated that edible coatings and low temperature slow the activity of the enzyme aconitase and NADP-malic and gene expression levels, but they increase higher levels of citrate

synthase and NAD-diseased dehydrogenase, resulting in reduced degradation of major acid present in the citrus fruit (Zhou et al., 2019).

In summary, edible alginate-based coating preserves and improves the antioxidant capacity of the food because, during storage, there was an increase in TPC and TFC and maintenance of the initial level of organic acids. These results are significantly better than those obtained in CTR and MAP. In fact, at the end of shelf life, all the results related to antioxidant activity and nutritional properties (TPC, TFC, and organic acid) were significantly better in AL than MAP and CTR.

Finally, the microbiological analysis revealed that all the samples showed an absence of total bacteria, yeasts, and molds. This phenomenon is certainly the result of the processing conditions of the raw material (environment carefully sanitized), storage (low temperature, sealed tray, MAP, edible coating), and the constituent characteristics of the fruit, such as phenolic compounds and acidity that counteract/slow the proliferation of pathogenic and spoilage microorganisms.

5.1.5 Conclusions

An alginate-based edible coating favored the creation of a controlled environment with low oxygen stress, which resulted in a reduction in enzymatic activity and oxidation. In fact, coated samples (AL) showed better chemical and texture characteristics than CTR and MAP in terms of the highest content of total polyphenols and flavonoids, together with oxalic, citric, and ascorbic acids after 21 days of storage at 4 °C. In addition, the total acceptability scores suggest that coated samples retain their characteristics to a greater extent, even after 21 days of storage.

The use of an alginate-based edible coating may then be a better choice to preserve the quality of Clementine segments than packaging in a modified atmosphere. The use of natural and renewable resources to extend the shelf life of ready-to-eat fruits could represent a real opportunity for the fruit industries, for the consumer, and for the environment.

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5.2 Improvement of storage quality of ready-to-eat Clementine fruits by Lemon by-products

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The research in the present chapter is reported as an article published in the "*Agricolture*" (2024; 14(9):1488. https://doi.org/10.3390/agriculture14091488). The personal contribution of the author was: conceptualization; methodology, formal analysis, investigation, software, data curation, writing—original draft preparation,

Abstract

In this study, the effect of the antioxidant extract from lemon by-products (*Citrus Limon L.*) integrated into edible alginate-based coating was evaluated to preserve the storage quality of ready-to-eat Clementine (*Citrus x Clementina*) fruits. The effect of different coatings (1.5% of alginate, 1.5% of alginate + 2-4% of lemon by-product extract) was assessed by physical, chemical, microbiological, sensorial, and structural analysis of ready-to-eat Clementine fruits stored for 21 days at 4 ºC. Ready-to-eat Clementine fruits coated with alginate and extract from lemon by-product showed greater levels of polyphenols, flavonoids, antioxidant activity, and organic acids. Microbiological analysis reveals the dose-dependent effect of extract to contrast the growth of mesophilic bacteria, yeast and moulds during the storage. Sensory analysis confirmed that enriched coating improved visual, structural, and olfactory parameters until the end of storage. The evidence in this study proves that

antioxidant extract from lemon by-products was a great sustainable treatment to preserve ready-to-eat fruits.

Keywords: Antioxidant extract; *Citrus x Clementina*; Edible coating; Lemon by-product; Ready-to-eat fruits.

5.2.1 Introduction

Seasonal fruits have always been considered an ally for human health. Many studies confirm that the different mechanisms of action of bioactive compounds present in fruits (polyphenols, flavonoids, vitamins, fibres etc.) promote human health and counteract the onset of various human diseases (Mazzoni et al., 2021; Fabroni et al., 2016).

Clementine (*Citrus x Clementina*) is a typical autumn citrus cultivated in Italian temperate areas, especially in the Calabria region (South of Italy), where it finds the appropriate conditions for optimal ripeness. Very appreciated for their sweetness and the absence of seeds, Clementine fruits have also been recognized as a source of innumerable healthy compounds, such as flavonoids and ascorbic acid (Vitamin C). Still, the habits of the modern consumer often hinder their consumption because the washing, peeling, and cutting operations require time and tools not always available, especially in the case of meals consumed outside the home and in a short time.

Currently, the main challenge for the fruit industries is to meet the needs of consumers, both in terms of nutritional quality and high-service content regarding user-friendliness. For these reasons in recent decades, the demand and the sale of ready-to-eat fruits has increased (Temgire et al., 2021). The main problem of ready-

to-eat fruits is related to the operations they undergo before packaging (peeling, cutting) that predispose them to a rapid physical, chemical, sensory and microbiological decay (loss of colour, smell, taste, texture, reduced health compounds, faster grown of pathogenic and spoilage microorganisms) compared to their entire fruit Zahr et al., 2023;Adetunji et al., 2023; Díaz-Mula et al., 2023). Nowadays, to slow these phenomena, "obstacle strategies" are used, which consist of the simultaneous use of synthetic phenolic compounds, such as Butylate hydroxyanisole (BHA), (Makahleh et al., 2015; Ghatk et al., 2016), modified atmosphere packaging (MAP) and low temperatures that lead to a substantial increase in the unit cost of food. Modern consumers perceive the conventional conservation methods negatively: prolonged intake of synthetic preservatives leads to collateral effects on human health (Liu et al., 2020; Wang et al., 2021) while conventional packaging strategies can be polluting and unsafe for improper sales/distribution conditions (mechanical damage to trays, high temperatures etc.) causing a faster quality decrease and excessive food loss (Thompson et al., 2018; Rao et al., 2015). In recent times, polymer-based edible coating, such as sodium alginate added with natural preservative compounds (Giacondino et al., 2023; Aziz et al., 2018; Hasan et al., 2022; Ramakrishnan et al., 2023; Hao et al., 2017; Shankar et al., 2019) has been evaluated as an eco-friendly winning treatment to find a solution to meet the actual consumer, environmental and industry needs (Han et al., 2005).

Extracts of citrus by-products have been recognized as rich in bioactive compounds (polyphenol, flavonoid, ascorbic acids) with high antioxidant and antimicrobial activity (Andrade et al., 2022; Singh et al., 2020; Nardella et al., 2022;

Gattuso et al., 2023) suitable to improve the shelf life and the presence of health-beneficial compounds in ready-to-eat fruits (Al-Dalai 2019).

Sodium alginate is a natural, edible, and biodegradable anionic polysaccharide derived from brown algae that has already been used to improve chemical and physical characteristics and to prolong the storage of various fruits such as apple, sweet cherry, mango, avocado, and peach (Chen et al., 2021; Díaz-Mula et a., 2012; Rastegar et al., 2019; Iñiguez-Moreno et al., 2021; Li et al., 2019). The induction to cross-linking sodium alginate in the presence of bivalent ions, such as Ca^{++}, determines the modification of its chemical structure and the formation of an impermeable barrier around fruits that improves its structural characteristics and slows down physiological, chemical, physical and microbial processes that are the basis of the qualitative decay of ready-to-eat fruits (Ungureanu et al., 2023; Embuscaldo et al., 2009; Castro-Yobal et al., 2021).

Our previous studies found that alginate-based coating is an excellent alternative to modified atmosphere packaging in ready-to-eat Clementine fruits (boninsegna et al., 2023). However, there are no scientific studies on the application of edible coatings on Clementine segments enriched with antioxidant by-product extract. This study aimed to evaluate the efficiency of different edible alginate-based coatings to maintain the chemical, physical, sensory, and microbiological quality of ready-to-eat Clementine fruits.

Therefore, the purpose of the study is to i) Test the influence of lemon by-product extract on improving the characteristics of edible coating alginate-based, ii) test the effectiveness of lemon by-product extract incorporated in edible alginate-based coatings to preserve the quality and safety of ready-to-eat Clementine fruits

during storage and iii) offer a natural and sustainable alternative to preserve of ready-to-eat fruits.

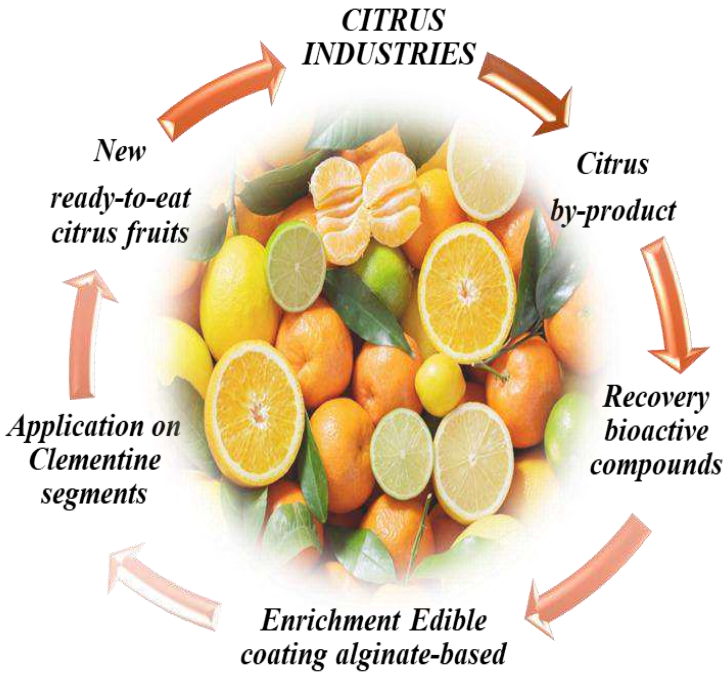

Figure 1 Graphical representation of study objectives

5.2.2 Materials and Methods

5.2.2.1. Extraction of bioactive compounds from Lemon pomace (LP)

Lemon pomace, the by-product of the industrial processing of lemons (peel, pulp, and seeds) was supplied by a local citrus cooperative (Citrus Juices SRL, Reggio Calabria, Italy). Immediately after receipt at the FoodTec laboratory of the University Mediterranean of Reggio Calabria, lemon pomace was dried for 3 hours at 50 °C ± 5 °C, until reaching a moisture content of 10%, ground and homogenized. The obtained sample (LP) was then used to prepare extracts. The solid-liquid extraction was performed by mixing 100 g of LP with 400 mL of hydroalcoholic solvent (EtOH 50%), on a stirring plate at 60 °C for 60 minutes. The extract obtained (LPE) was centrifuged (6000 rpm) for 10 minutes at 4 °C (NF 1200R,

Nüve, Ankara, Turkey). Subsequently, the supernatant was recovered, filtered through a Buchner funnel with 0.45 mm filter paper, then refiltered with 0.45 mm PTFE filter and stored at −21 °C until further analyses.

5.2.2.2 Clementine fruit samples' preparation

The Clementine fruits (*Citrus x Clementina*) were bought at the local market sited in Reggio di Calabria (Italy) in February 2023, transported to the FoodTec laboratory of the University Mediterranean of Reggio Calabria, and picked for similar size (weight: 80-90 g, height: > 50mm, width:> 60mm) and colour (completely orange 'flavedo') (Boninsegna et al., 2023). The whole fruits were then sanitized by immersing in a sodium hypochlorite solution (200 ppm) for 2 minutes, rinsed with distilled water, and dried on stainless steel grids in a vertical laminar flow hood (UV lamp 30 W, mod. ASALAIR 1200 FLV, Asal Srl, Milan, Italy) at room temperature and forced air (Boninsegna et al., 2023). Clementine fruits were peeled, reduced manually into segments, and divided into 4 groups to apply the treatments provided in the experimental plan.

To prepare the coating solution, 1.5 % (w/v) of sodium alginate powder (Sigma-Aldrich, Merk Life Science S.r.l., Italy) was dissolved in distilled water under stirring (70 °C, 60 min). Then, the temperature was reduced by 30 °C, and 1.5 % (w/v) of glycerol (Carlo Erba reagents, Italy) was added under continuous stirring (30 °C, 30 min) (Boninsegna et al., 2023). The obtained solution was named AL. For the other coating solutions, 2% and 4% (v/v) of LPE were added to the formulation of AL (AL-LPE 2% and AL-LPE 4%). Concomitantly, a $CaCl_2$ solution

(2% w/v) was prepared by dissolving calcium chloride (Labochimica s.r.l., Italy) in distilled water under stirring for 30 minutes (25 °C).

The coating solutions AL, AL-LPE 2%, and AL-LPE 4% thus prepared were used for the realization and edible coatings on ready-to-eat Clementine fruits.

The segments of Clementine fruits were dipped in AL, AL-LPE2%, and AL-LPE 4% solutions for 2 minutes, recovered and left for 1 minute at room temperature on stainless steel grids to remove excess solution. Then, segments were dipped in $CaCl_2$ solution for 2 minutes to induce a cross-linked reaction of sodium alginate, recovered, and put on stainless-steel grids at room temperature up to complete drying (about 3 hours) (Boninsegna et al., 2023). The operations of preparation, coating and drying of the Clementine fruits were conducted in a vertical laminar flow hood (UV lamp 30 W, mod. ASALAIR 1200 FLV, Asal Srl, Milan, Italy) to avoid microbiological contaminations. Uncoated samples were used as a control (CTR).

Clementine samples (about 100 g) were packaged in a PP tray heat-sealed with PP/PE film using a packaging machine (Orved, VGP 25N, Italy) and stored at 4 °C for 21 days under the light to recreate the real sale conditions. Clementine juice was obtained by homogenizing 70 g of sample (Ultra-Turrax, T 25 digital, IKA, Staufen, Germany) and centrifuging (NF 1200R, Nüve, Ankara, Turkey), for 10 minutes at 10,000 rpm and 4 °C. The supernatant was recovered, filtered by PTFE 0.45 μm (diameter 15 mm), and used for the chemical determinations.

Physical, chemical, and microbiological analyses were performed at 0, 3, 7, 14, and 21 days of storage. Sensory analyses were conducted at the beginning and the end of storage.

5.2.2.3 Chemical analyses of LPE and Clementine fruits

pH of Clementine juice and LPE was determined by using a digital pH meter (Crison Basic 20, Spain)

Clementine fruit moisture was quantified by the AOAC standard method (Official Methods: Gaithersburg, MD, USA, 2005) and expressed in percentage. Total soluble solids were quantified on Clementine juice by digital refractometer (DBR 047 SALT) and expressed in degrees Brix (°Bx) at 25 °C .

Diluted Clementine juice (1:10) was titrated with 0,1 M NaOH up to pH 8.1 (digital pH meter Crison Basic 20, Spain) to measure the titratable acidity (TA) and results were expressed as citric acid % (Legua et al., 2014).

The trend of organic acids (oxalic, malic, ascorbic, and citric acids) during the storage of Clementine fruits was identified with High-performance liquid chromatography (HPLC) following the procedure suggested by Jurić et al. (2023) with some modification. Briefly, 20 µL of juice was injected in Knauer HPLC Smartline Pump 1000, equipped with a Knauer Smartline UV Detector 2600 and SYNERGY HYDRO-RP (250 mm × 4.6 mm i.d., 4 µm). The thermostat was set at 22 °C and the separation was carried out in isocratic condition with potassium phosphate 20 mM at pH 2.9 at a flow rate of 0.7 mL min. Ascorbic acid was recorded at 254 nm and other organic acids at 210 nm, their concentration was reported as mg of acid 100 g^{-1}.

The total phenolic content (TPC) of LPE was carried out with the methods described by Imeneo et al. (2022), opportunely modified. 1 mL of diluted LPE (1:5), 1 mL of Folin- Ciocalteu reagent were mixed and, after a short incubation (8 minutes) at room temperature, 10 mL of Na_2CO_3 20%(w/v) were added to the

solution. The reaction mixture was made up to volume (25 mL) with deionised water and incubated at darkness and room temperature (25 °C) for 2 hours. The absorbance was recorded at 765 nm against a blank (reaction mixture without sample) by double-beam ultraviolet-visible spectrophotometer (Perkin-Elmer UV–Vis k2, Waltham, Massachusetts, U.S.A.). TPC of Clementine fruits was instead quantified following the method of Boninsegna et al. (2023). The results were compared with a gallic acid calibration curve and expressed as mg of gallic acid equivalent (GAE) g^{-1} of LPE dry weight (d.w.) and mg of gallic acid equivalents (GAE) Kg^{-1} of Clementine fruits.

To determine total flavonoid content (TFC), 1.2 mL of the diluted LPE (1:1) and 0.15 mL of 5 % (w/v) $NaNO_2$ were mixed and left at room temperature for 6 minutes, then 0.15 mL of 10 % $AlCl_3$ (w/v) were added. After 6 minutes, 2 mL of NaOH 1M was added and finally, distilled water was used up to a volume of 5 mL. The reaction mixture was left for 15 minutes in the darkness at room temperature (25 °C). Subsequently, the absorbance was registered at 515 nm versus a blank (reaction mixture without sample) by spectrophotometer (Perkin-Elmer UV–Vis k2, Waltham, Massachusetts, U.S.A.) (Imeneo et al., 2022). TFC of Clementine fruits were performed by the colourimetric methods described by Boninsegna et al. (2023). The results were expressed as mg of catechin equivalents (CE) g^{-1} of LPE d.w. and mg of catechin equivalents (CE) Kg^{-1} of Clementine fruits, using a catechin calibration curve.

The identification of individual flavonoids on LPE and Clementine fruits was performed according to Romeo et al. (2019). Briefly, 5 µL of the sample were injected in a UHPLC PLATIN blue system (Knauer, Berlin, Germany) equipped

with a binary pump, coupled with a PDA-1 (Photo Diode Array Detector) PLATINblue (Knauer, Germany), Knauer blue orchid C18 column (1.8 mm, 100 × 2 mm) and Clarity 6.2 software. Individual flavonoids were detected at 280 nm using water acidified with formic acid to pH 3.10 (A) and acetonitrile (B) as elution solvents. The elution program is reported in Table 1. External standards were used to quantify principal flavonoids (hesperidin, eriocitrin, narirutin, naringin, neoriocitrin). The obtained results were expressed as mg g^{-1} of LPE d.w. and mg Kg^{-1} of Clementine fruits.

Table 1 Elution program for principal flavonoid quantification in LPE.

Time (minutes)	***Eluent A (%)***	***Eluent B (%)***	***Flow (mL/min)***
Initial	95.00	5.00	0.40
3.00	95.00	5.00	0.40
17.00	60.00	40.00	0.40
17.50	0.00	100.00	0.40
20.00	95.00	5.00	0.40
21.00	95.00	5.00	0.40

The total antioxidant activity (TAA) of LPE and Clementine fruits was tested using the DPPH and ABTS assays. The radical solutions of DPPH and ABTS were formulated according to earlier reports Boninsegna et al., 2023; De Bruno et al., 2023), and then the analysis was conducted by mixing in a cuvette 20 µL of the sample with 2980 µL of methanolic DPPH or ethanolic ABTS radical solutions with a reaction time of 30 minutes and 6 minutes respectively. The decrease in the absorbance value due to the interaction between the antioxidants and the tested free radicals was recorded at 734 nm for ABTS assay and 515 nm for DPPH assay using a spectrophotometer (Perkin-Elmer UV- Vis k2, Waltham, Massachusetts, USA) versus a blank which was ethanol for ABTS assay and methanol for DPPH assay.

The results obtained were translated using a Trolox calibration curve and expressed as mM Trolox equivalent g^{-1} of LPE (d.w.) and mM Trolox equivalent kg^{-1} of Clementine fruits.

5.2.2.4 Physical analyses of Clementine fruits

The Clementine segments' colour was recorded with a tristimulus colourimeter (Minolta CM- 700d Spectrophotometer, Japan) and acquired on 12 segments for storage time and treatments. The determinations were made in triplicate according to the CIE L* a* b* parameters (L* for lightness, a* for red/green, and b* for yellow/blue tones) (Boninsegna et al., 2023).

Weight loss was estimated by the AOAC standard method (Association of Official Analytical Chemists, 1994) and expressed in percentage.

Changes in oxygen and carbon dioxide levels in the trays' headspace were checked using a gas analyser (PBI, DANSENSOR, CP O_2/CO_2) provided with a thin needle to take a representative gas sample from the headspace of packages. The determinations were made in triplicate, and the results were expressed as O_2 and CO_2 percentages.

The penetration test was carried out on ready-to-eat Clementine fruits to find the effect of alginate-based coatings on firmness (Ramakrishnan et al., 2023). TA-XT Plus Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK) equipped with Exponent software 6.1.4.0 (Stable Micro Systems Ltd., Godalming, UK) was used for the data acquisition and integration. The analysis was conducted by a 5 mm diameter stainless steel probe (P/5). The following conditions were used: penetration distance of 3 mm, test speed of 1.0 mm/s, and post-test speed of 3.0

mm/s [43]. The results of firmness (N) were expressed as a means of 20 replicates for each treatment for each storage time.

5.2.2.5 Microbiological analyses of Clementine fruits

Mesophilic bacteria (TBC), yeasts and moulds (Y&M) were verified following the protocols of Boninsegna et al. (2023) and Glicerina et al.(2022). Serial dilutions were prepared as described in earlier studies and plating on Dichloran Rose Bengal Chloramphenicol (DRBC) and Plate Count Agar (PCA) for enumeration of Y&M and TBC, respectively. The plates were placed at 25 °C and the enumeration was performed after incubation of 5 days for Y&M and 2 days for TBC. The results were reported as log10 CFU g^{-1}.

5.2.2.6 Sensory analyses of Clementine fruits

The sensory characteristics were evaluated through a quantitative descriptive sensory analysis (QDA) attended by 10 trained people aged between 21 and 42. Visual (colour intensity, shape, gloss, surface uniformity), olfactory (intensity, fruity, citrus, spicy), structural (consistency, chewiness, humidity, crunchiness, turgidity), and taste characteristics (sweet, salt, acid, bitter, citrus, fruity, astringent, aftertaste) were evaluated on a scale from 0 to 9 where 0 indicated no perception and 9 the maximum perception of the considered attribute. Concerning total acceptability, each participant has issued a judgment from 0 to 9 on the total acceptability of the ready-to-eat Clementine fruits, where the limit score was 4.5 (Boninsegna et al., 2023). The results were elaborated as a mean of the scores obtained for each sensorial attribute.

5.2.2.7 Statistical analysis

The analytical data were reported as mean value ± standard error. The analysis of variance (one-way ANOVA) was conducted by SPSS Software (Version 15.0, SPSS Inc., Chicago, IL, USA) applying the Tukey *post hoc* test at $p < 0.05$.

5.2.3 Results and discussion

5.2.3.1 Chemical Characterization of LPE

The chemical characteristics of the extracts play an important role in the formulation of edible coatings both in their structure and for the enrichment of bioactive compounds and the antioxidant and antimicrobial activity related to them. The retention of antioxidant extract within the polysaccharide matrix depends on multiple factors such as pH, ionic interactions, and the chemical composition of the polysaccharide and the extract (Dobson et al., 2019; Tietel et al., 2011; Ureña et al., 2024). Table 2 reports the chemical characterization of LPE.

The resulting pH value was 3.95 and could be considered optimal for the enrichment of edible alginate-based coating as indicated in previous studies (ranging from 3 to 5) on the physical-chemical properties of alginate (Duong et al, 2023). The extract pH has been considered as the major responsible for the thickness and consistency of the edible alginate-based coating since in acidic environments (pH < 3) ionic interactions determine a partial precipitation of alginate which results in a contraction of the coating and a decrease in its thickness (Malektaj et al., 2023; Andriamanantoanina et al., 2010).

LPE exhibited high content in TPC (12.67 mg GAE g^{-1}) and TFC (2.10 mg CE g^{-1}) with hesperidin and eriocitrin as the main abundant phenolic compounds, according to other studies on green extractions of bioactive compounds from lemon by-products (Imeneo et al., 2022). These results are also related to the measured antioxidant activity (22.97 μM TE g^{-1} for DPPH assay and 18.90 μM TE g^{-1} for ABTS assay). The retention of these compounds within the alginate-based coating could increase the functional characteristics of the fruit and provide an efficient natural barrier to counteract the proliferation of spoilage and pathogenic microorganisms, as already proven in experiments carried out on edible alginate-based coatings enriched with vegetable extracts of *Ficus hirta*, pomegranate peel, and grape seed extracts (Messaoud et al., 2016; Chen et al., 2016; Faba et al., 2018).

Table 2. Chemical Characterization of Lemon Pomace Extract (LPE)

Parameter	Results
pH	3.95±0.02
TPC (mg GAE g-1 d.w.)	12.67 ±0.17
TFC (mg CE g-1 d.w.)	2.10±0.11
Hesperidin (mg g-1 d.w.)	3.88±0.35
Eriocitrin (mg g-1 d.w.)	1.51±0.39
Narirutin (mg g-1 d.w.)	0.03±0.00
Naringin (mg g-1 d.w.)	0.01±0.05
Neoeriocitrin (mg g-1 d.w.)	0.01±0.04
DPPH (μM TE g-1 d.w.)	22.97±0.53
ABTS (μM TE g-1 d.w.)	18.90±0.29

5.2.3.2 Chemical, Physical, Microbiological, and Sensory Analyses Results of Clementine fruits

In citrus fruits, the variation in pH, TA, and TSS is a normal physiological process that determines the qualitative decay and can be assisted by the wrong practices of management, post-harvest operations, and the distribution/sale of fruits

(Tietel et al., 2011). The initial levels of TA e TSS in Clementine fruits were within the range of 0.51 - 0.58% and 11.75 - 12.13 °Brix respectively with significant differences ($p > 0.01$) of pH and TA values for AL-LPE 2% and AL-LPE 4% respect to CTR and AL, due to the different concentrations of extract used in the formulation of edible coatings as also confirmed by pH values (Table 3). This trend has been maintained throughout storage, with important variations from 7 days for CTR and to 14 and 21 days for AL e AL-LPE 4% respectively, while for AL-LPE 2% no difference was recorded up to 21 days at 4°C. CTR also showed important variations in TSS during storage unlike all samples treated with edible alginate-based coatings.

Total soluble solids and titratable acidity are considered among the most important indicators of citrus quality, their ratio is related to many factors including the cultivar, harvest time, and post-harvest treatments (Poiroux-Gonord et al., 2012; Hijaz et al., 2020; Lado et al., 2014). In Europe commercial maturity of mandarin and clementine fruits occurs when TA and TSS levels reach values above 0.3% and 8 °Brix, respectively (Lado et al., 2014). The reduction of these values indicates important sensory and chemical changes that affect the nutritional and qualitative characteristics determining the commercial decay (loss of freshness, low nutritional compounds etc.) (Gupta et al., 2022).

The moisture content after 21 days of storage was significantly ($p < 0.05$) lower in CTR (83.2%) than AL (86.77%), AL-LPE 2 % (86.58%) and AL-LPE 4% (86.15%). These trends showed that the barrier provided by alginate prevents the reduction of moisture as already observed in ready-to-eat fruits and vegetables

(Ghidelli et al., 2018; Nair et al., 2020), such as melon (Sipahi et al., 2013)and strawberries (Peretto et al., 2010).

Table 3 Chemical parameters in ready-to-eat Clementine fruits during storage

Parameter	Sample	Time					
		0	**3**	**7**	**14**	**21**	**Sig.**
TSS (Brix °)	CTRL	12.10±0.05^{AB}	12.15±0.17^{AB}	10.25±0.72^{bC}	12.18±0.10^{A}	11.47±0.38^{B}	**
	AL	11.88 ± 0.13	12.20±0.23	12.57±0.78^{a}	12.12±0.07	11.85±0.06	n.s.
	AL-LPE 2%	12.13± 0.61	12.90±0.69	12.08±0.55^{a}	12.02±0.00	11.80±0.92	n.s.
	AL-LPE 4%	11.75± 0.40	12.30±0.69	11.37±0.43^{a}	11.60±0.00	11.20±0.58	n.s.
Sig.		n.s.	n.s.	**	n.s.	n.s.	
TTA (%)	CTRL	0.59±0.03^{bcAB}	0.64±0.08^{abA}	0.53±0.04^{bAB}	0.49±0.0^{bB}	0.51 ±0.02^{cB}	**
	AL	0.51±0.03^{bB}	0.55±0.02^{cAB}	0.58±0.05^{abA}	0.48±0.00^{bB}	0.54 ±0.01^{abAB}	*
	AL-LPE 2%	0.63± 0.04^{ab}	0.65±0.03^{a}	0.62±0.04^{a}	0.65± 0.03^{a}	0.63± 0.01^{a}	n.s.
	AL-LPE 4%	0.68± 0.08^{aA}	0.61±0.02^{abB}	0.64±0.04^{aAB}	0.69± 0.03^{aA}	0.61±0.02^{aB}	*
Sign.		**	*	**	**	*	
pH	CTRL	3.84±0.01^{aB}	3.85±0.01^{aB}	3.91±0.01^{bAB}	3.93±0.01^{aAB}	4.07±0.22^{aA}	*
	AL	3.75± 0.01^{abB}	3.77±0.02^{abB}	3.99±0.04^{aA}	3.78±0.02^{bB}	3.80±0.02^{cB}	**
	AL-LPE 2%	3.59± 0.07^{cC}	3.68±0.02^{abBC}	3.83±0.01^{cA}	3.81±0.03^{bAB}	3.69±0.13^{bcC}	**
	AL-LPE 4%	3.67± 0.08^{bc}	3.64±0.15^{b}	3.74±0.03^{d}	3.77±0.04^{b}	3.71±0.13^{bc}	n.s.
Sign.		**	*	**	**	*	
Moisture (g/100 g)	CTRL	85.29±0.55^{AB}	88.46±2.00^{A}	87.60±1.90^{AB}	85.59±1.85^{AB}	83.2±1.16^{Bb}	*
	AL	85.38±2.00	85.56±0.30	86.74±1.97	85.8±1.35	86.77±1.23^{a}	n.s.
	AL-LPE 2%	85.69±1.61	86.63±1.83	85.57±2.21	85.17±1.49	86.58±0.89^{a}	n.s.
	AL-LPE 4%	85.76±1.41	86.89±1.53	86.04±1.32	86.42±0.50	86.15±0.79^{a}	n.s.
Sign.		n.s.	n.s.	n.s.	n.s.	*	

Data are mean (n=3) ± s.d. Small letters within a column and capital letters within a row show significant differences as assessed by Tukey's post hoc test. Abbreviations: **, significance at $p < 0.01$; *, significance at $p < 0.05$; n.s., not significant.

The content of organic acids is considered an important quality index both for the nutritional aspect and the typical freshness taste of citrus fruits. In Clementine, the most representative acids are citric, ascorbic, and malic. Table 4 shows the trends of organic acids during 21 days of refrigerated storage.

The application of edible coatings on clementine segments resulted in a significant variation ($p>0.01$) of malic and ascorbic acid up to 14 and 21 days of storage respectively while for citric acid, no statistically significant differences between samples were found up to 21 days of storage. The detected organic acid composition was in accordance with the TTA values previously shown in this study (Table 3), expressed as % of citric acid (the most abundant acid present in citrus) but it doesn't include all organic acids that can interact with NaOH indeed ascorbic and malic ones.

The combined action of enriched edible coatings and storage conditions favour the maintenance of citric acid levels and the increase of malic acid levels, due to the regulation of genetic expression of citrate hydrolyse, citrate synthase, NADP-malic enzyme, and NAD-malate dehydrogenase enzyme responsible for changes of malic and citric acid in fruits during the storage Valero et al., 2013; Ehteshami et al., 2022; Khan et al., 2013; Zhou et al., 2019; Tang et al., 2016). In agreement with it, the results obtained in this study showed that from 3 days of storage, an increase of malic acid level was recorded in AL-LPE 2% and AL-LPE 4%, while no statistically significant differences were observed throughout the storage of CTR and AL.

CTR showed a marked and significant ($p >0.01$) ascorbic acid loss from 152.99 mg 100 g^{-1} to 90.51 mg 100 g^{-1} after 21 days, instead, it remained stable until the end storage of AL, as already observed by authors in previous studies [35]. The enrichment with LPE improved the level of ascorbic acid for AL-LPE 2% and AL-LPE 4% by about 35% respect to AL and CTR and these quantities were maintained until 21 days. Significant differences ($p>0.01$) were found at the last

storage time among samples, with the highest content in AL-LPE 2% (202.96 mg 100 g^{-1}), followed by AL-LPE 4% (184.37 mg 100 g^{-1}), AL (124.28 mg 100 g^{-1}) and CTR (90.51 mg 100 g^{-1}).

The degradation of ascorbic acid is a natural process that occurs during the preservation of fruits, due to oxidation and respiration rate and can be speeded up by storage conditions (Kharchoufi at al., 2018; Cebadera Miranda et al., 2020; Rey et al., 2020). The presence of good levels of ascorbic acid in the fruits gives the food excellent health characteristics. It is strictly recommended the intake of 80-100 mg of ascorbic acid per day to counteract the onset of various human diseases (Naidu et al., 2003).

Table 4 Organic acids composition in ready-to-eat Clementine fruits during storage

Organic acids (mg 100g $^{-1}$)	**Sample**	**Time (days)**					
		0	3	7	14	21	Sig.
Citric acid	CTRL	473.77±19.74	455.9±43.93	501.37±2.88	444±3.43	425.6±66.4	n.s.
	AL	500.52±2.07	423.52±21.23	499.74±53.59	463.21±7.20	464.46±2.75	n.s.
	AL-LPE 2%	516.75±18.34	477.42±43.89	540.55±66.43	482.49±56.63	483.04±67.26	n.s.
	AL-LPE 4%	489.48±2.82	426.6±21.78	422.11±8.95	465.44±0.00	439.97±23.93	n.s.
Sign.		n.s.	n.s.	n.s.	n.s.	n.s.	
Malic acid	CTRL	86.2±4.28^{a}	82.57±6.28^{a}	71.24±5.40^{ab}	63.88±14.29^{ab}	68.13±11.13	n.s.
	AL	62.45±5.43^{b}	59.37±4.90^{b}	55.38±9.19^{b}	48.31±0.03^{b}	62.55±6.71	n.s.
	AL-LPE 2%	57.99±11.6^{Bb}	84.84±16.66^{Aa}	76.52±0.31^{ABa}	71.26±0.15^{ABab}	76.07±13.11^{AB}	*
	AL-LPE 4%	59.43±5.64^{bB}	87.74±13.37^{Aa}	52.21±4.34^{Bb}	75.8±0.26^{Aba}	52.63±23.29^{B}	*
Sign.		**	*	*	**	n.s.	
Ascorbic acid	CTRL	152.99+3.19^{aA}	104.62+13.14^{bB}	94.56+1.85^{bB}	97.19+1.65^{bB}	90.51+0.99^{cB}	**
	AL	124.49+18.75^{b}	112.18+35.52^{b}	169.82+14.13^{a}	170.78±3.11^{a}	124.28±1.21^{bc}	n.s.
	AL-LPE 2%	205.69+36.80^{ab}	175.41+4.95^{a}	176.35+5.72^{a}	177.99±2.52^{a}	202.96±12.10^{a}	n.s.
	AL-LPE 4%	172.31+7.61^{ab}	180.92+11.50^{a}	175.31+6.37^{a}	180.61±1.98^{a}	184.37±8.26^{ab}	n.s.
Sign.		**	*	**	**	**	

Data are mean (n=3) ± s.d. Small and capital letters,**, *, n.s. see Table 3.

Citrus fruit and by-products resulting from their processing were recognised as a source of phenolic compounds, with flavonoids as the most representative class (Ghasemi et al., 2009). Immediately after the application of the edible coating enriched with LPE, samples AL-LPE2% and AL-LPE4% showed a significantly higher TFC than CTR while no significant differences were found among samples for TPC (Figure 2).

TPC and TFC tended to increase during the storage in AL, AL-LPE2%, and AL-LPE4%, raising the highest levels after 7 days. These results are according to a previous study by authors that showed a faster phenolic loss during time on coated clementine segments (Boninsegna et al., 2023). The increased TPC and TFC is therefore a natural physiological process that occurs during fruit storage whose main cause is the biosynthesis of new compounds catalyzed by enzymatic reactions at the expense of unavailable high molecular weight compounds, converted into available low molecular weight compounds (Simonne et al., 2011; Milella et al., 2011). Nevertheless, if appropriate preservation strategies aren't used, the increase could be followed by a drastic decrease due to the susceptibility of these compounds to oxidation (Lu et al., 2023; Choi et al., 2011). The data in Figure 1 clearly show that the application of an alginate-based edible coating (AL) preserves the TPC and TFC in segments for up to 21 days of cold storage. Clementines are naturally a source of flavonoids with hesperidin and naringin among the most abundant (Petriccione et al.,2025; Gattuso et al., 2023; Imeneo et al., 2022; Lu et al., 2023). The data obtained in this study showed that the addition of LPE to the formulation of the alginate-based edible coating resulted in a significant dose-dependent increase in hesperidin and eriocitrin, according to the chemical characterization of

LPE mentioned above (Table 2). Regarding narirutin, no significant change was observed after the application of the edible coating (Table 5), since narirutin was present in low quantity in LPE, while it is instead strictly related to the chemical composition of Clementine fruits (Villa-Rodriguez et al.,2015; Singh et al., 2024) .

In recent literature, there are no studies about the application of edible coatings on ready-to-eat Clementine fruits, whereas studies on whole clementine fruit proved that different post-harvest preservation treatments prevent the loss and determine the synthesis of new compounds(Lafuente et al., 2011; De Ancos et a., 2017).

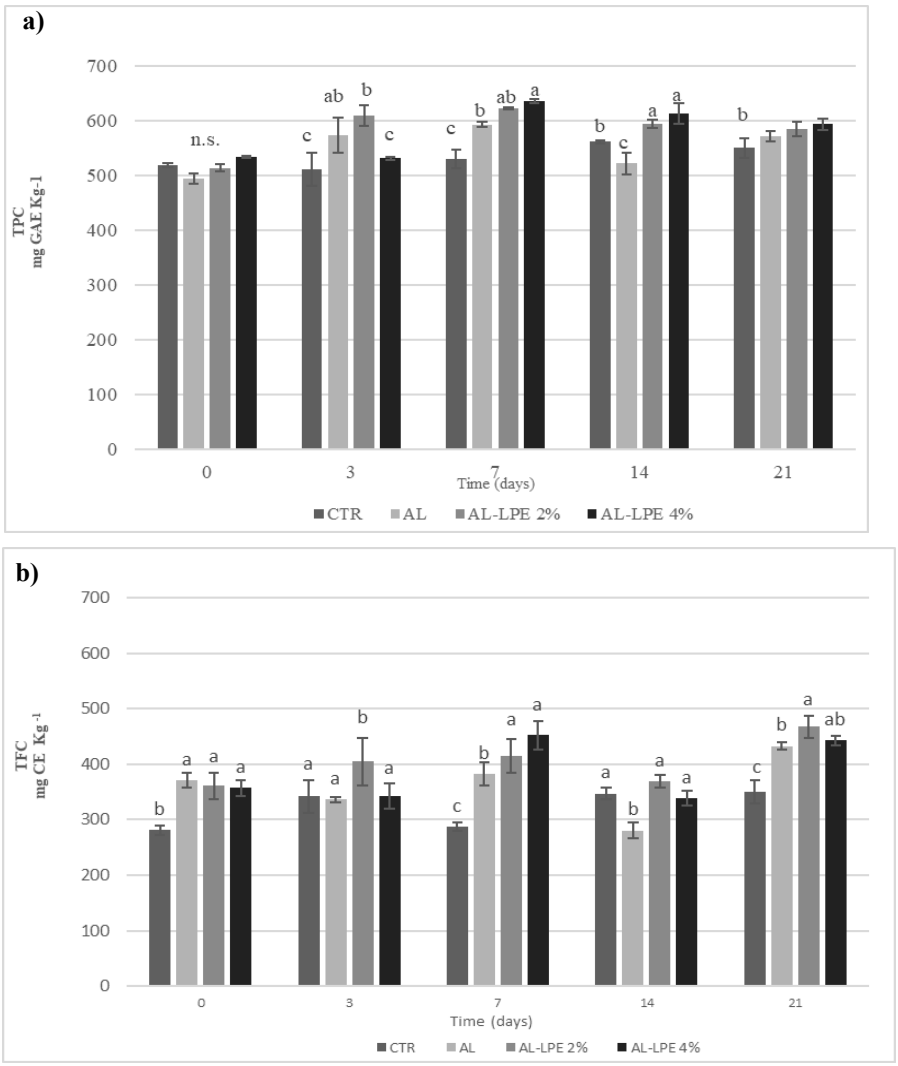

Figure 2 Total Phenolic Content (TPC) (a) Total Flavonoid Content (TFC) (b) of ready-to-eat Clementine fruits during storage

Table 5 Flavonoids composition (mg Kg^{-1}) in ready-to-eat Clementine fruits during storage

	Sample	Time (days)					
		0	3	7	14	21	Sign.
Narirutin	CTRL	170.01 ± 3.37	190.44 ± 17.82	170.62 ± 9.56	165.35 ± 3.01 ^{b}	168.80 ± 3.53	n.s.
	AL	175.32 ± 6.74 ^{CB}	207.38 ± 6.02 ^{A}	175.04 ± 0.42 ^{B}	152.61 ± 0.66 ^{cC}	173.02 ± 4.91 ^{BC}	**
	AL-LPE 2%	187.50 ± 2.36 ^{A}	213.05 ± 6.46 ^{B}	181.76 ± 4.60 ^{AB}	192.56 ± 2.01 ^{aAB}	188.78 ± 8.24 ^{AB}	*
	AL-LPE 4%	191.65 ± 10.11	201.86 ± 0.09	183.02 ± 13.55	167.81 ± 0.76 ^{b}	178.98 ± 2.12	n.s.
Sig.		n.s.	n.s.	n.s.	**	n.s.	
Hesperidin	CTRL	96.39 ± 13.34 ^{cC}	124.56 ± 12.21 ^{A}	96.24 ± 12.83 ^{cC}	102.23 ± 12.83 ^{cCB}	116.90 ± 13.65 ^{AB}	*
	AL	123.61 ± 6.08 ^{bBC}	128.44 ± 4.95 ^{A}	125.03 ± 18.47 ^{bA}	104.75 ± 18.74 ^{bcC}	115.57 ± 6.08 ^{B}	**
	AL-LPE 2%	125.54 ± 13.68 ^{bA}	138.62 ± 12.65 ^{B}	126.49 ± 6.20 ^{bC}	141.77 ± 6.52 ^{aAB}	117.35 ± 12.52 ^{C}	**
	AL-LPE 4%	142.78 ± 17.21 ^{aA}	129.11 ± 7.62 ^{AB}	142.92 ± 8.91 ^{aA}	115.22 ± 8.91 ^{bBC}	110.90 ± 21.21 ^{C}	**
Sign.		**	n.s.	**	**	n.s.	
Eriocitrin	CTRL	0.39 ± 0.01 ^{cA}	0.40 ± 0.00 ^{bA}	0.22 ± 0.01 ^{cB}	0.07 ± 0.00 ^{cC}	0.08 ± 0.00 ^{bC}	**
	AL	0.38 ± 0.00 ^{dA}	0.37 ± 0.01 ^{bA}	0.36 ± 0.00 ^{abA}	0.06 ± 0.00 ^{cB}	0.08 ± 0.00 ^{bB}	**
	AL-LPE 2%	0.99 ± 0.01 ^{bA}	0.76 ± 0.02 ^{abB}	0.46 ± 0.00 ^{abC}	0.35 ± 0.00 ^{bD}	0.49 ± 0.00 ^{aC}	**
	AL-LPE 4%	1.06 ± 0.01 ^{aAB}	1.19 ± 0.02 ^{aA}	0.65 ± 0.01 ^{aAB}	0.55 ± 0.00 ^{aAB}	0.51 ± 0.00 ^{aB}	*
Sign.		**	*	*	**	**	

Data are mean (n=3) ± s.d. Small and capital letters,**, *, n.s. see Table 3.

The antioxidant potential of foods depends on the synergistic action of many compounds and, particularly, in citrus fruits is mainly due to the action of phenolic compounds and ascorbic acid (Rey at al., 2020). In this study, ABTS and DPPH were applied to test the activity of molecules with different polarities (Wołosiak et al., 2021; Glucin et al., 2020).

An increment of antioxidant activity was detected immediately after treatments with LPE on Clementine fruits until 3 days of storage (Figure 3). After 7 days of storage, a decrement was recorded at ABTS assay and a slight rise at DPPH assay probably due to the synthesis of new antioxidant compounds with different antioxidant actions. However, given the nature of the compounds present in citrus, the scavenging activity registered against ABTS radical was always higher than DPPH radical.

The coated Clementine segments (AL, AL-LPE 2%, and AL-LPE 4%) showed higher antioxidant activity than CTR after 21 days of storage coherently with the discussed trend of TPC, TFC, and organic acids

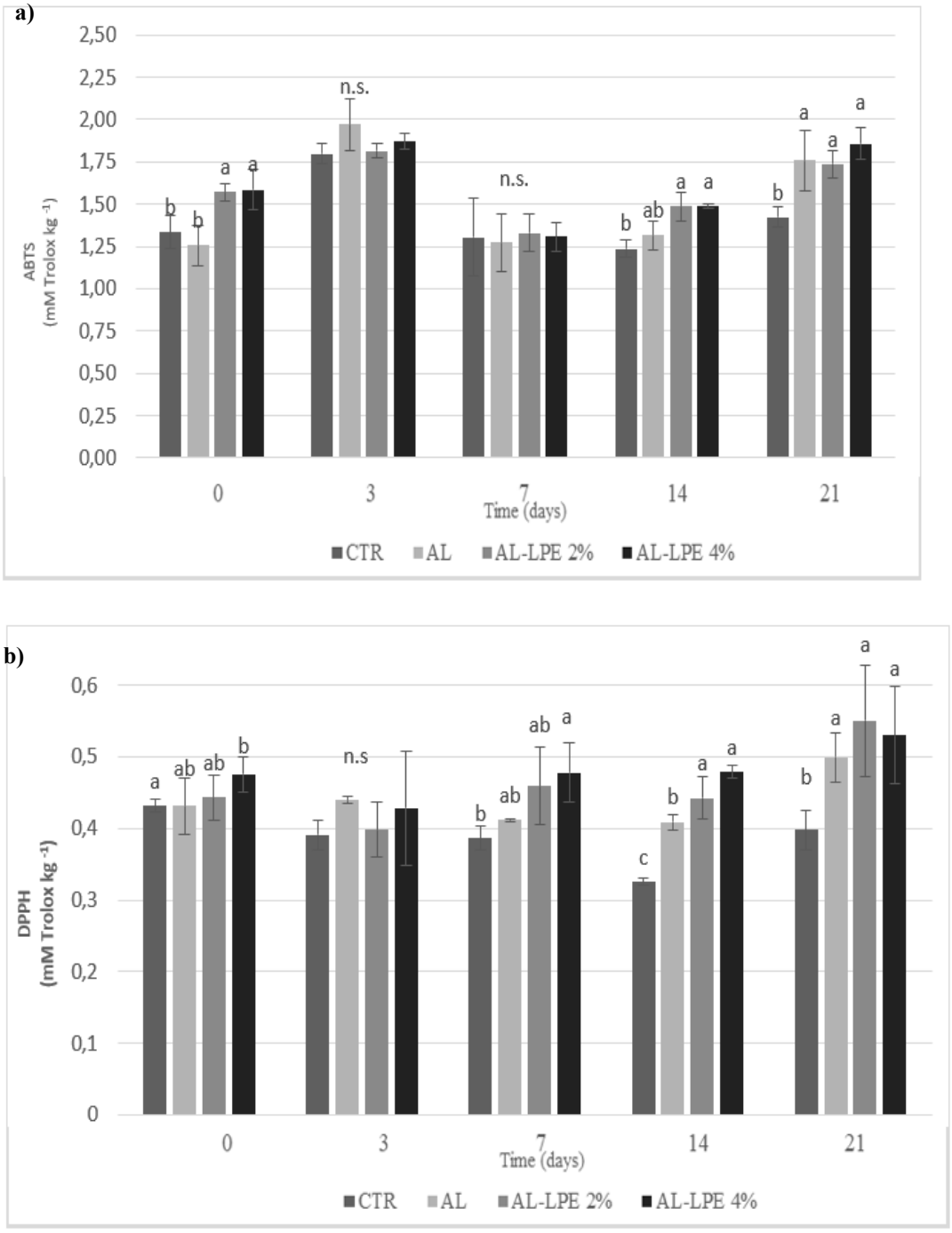

Figure 3. Antioxidant activity of ready-to-eat Clementine fruits during storage Letters shows the significant differences among samples for each monitoring time by Tukey's *post-hoc* test. n.s. see Table 3.

Regarding the colour parameters, no differences were noted among samples and during storage, as evidenced in Table 6.

Table 6 Colour parameters in ready-to-eat Clementine fruits during storage

Parameter	Sample	Time (days)					
		0	3	7	14	21	Sig.
L*	CTRL	50.19 ± 1.69	50.72 ± 1.42	53.25 ± 1.22	52.86 ± 2.65	51.71 ± 1.67	n.s.
	AL	54.80 ± 1.33	52.62 ± 1.69	53.84 ± 1.16	52.57 ± 1.54	50.92 ± 2.10	n.s.
	AL-LPE 2%	49.67 ± 5.70	53.15 ± 1.80	52.31 ± 1.83	51.99 ± 2.10	51.58 ± 6.11	n.s.
	AL-LPE 4%	51.90 ± 5.86	50.54 ± 2.39	50.98 ± 2.48	50.70 ± 3.19	50.40 ± 2.09	n.s.
Sign.		n.s.	n.s.	n.s.	n.s.	n.s.	
a*	CTRL	8.30 ± 1.21	8.41 ± 1.27	8.74 ± 1.01	8.85 ± 2.14	8.67 ± 1.32	n.s.
	AL	8.18 ± 1.30	9.27 ± 2.01	9.42 ± 2.38	8.60 ± 1.96	9.46 ± 1.56	n.s.
	AL-LPE 2%	8.80 ± 3.06	8.12 ± 1.50	8.91 ± 2.29	8.39 ± 1.47	8.79 ± 2.29	n.s.
	AL-LPE 4%	8.31 ± 2.95	8.88 ± 2.10	8.54 ± 1.90	7.84 ± 1.89	8.19 ± 1.21	n.s.
Sign.		n.s.	n.s.	n.s.	n.s.	n.s.	
b*	CTRL	19.48 ± 1.07	20.90 ± 1.54	19.78 ± 1.43	21.71 ± 1.97	20.21 ± 1.82	n.s.
	AL	20.27 ± 1.50	20.48 ± 1.41	24.23 ± 2.40	20.60 ± 2.63	21.39 ± 1.58	n.s.
	AL-LPE 2%	19.39 ± 2.16	19.66 ± 2.07	21.43 ± 1.53	20.71 ± 2.08	21.47 ± 2.96	n.s.
	AL-LPE 4%	20.83 ± 1.74	21.9 ± 1.45	21.07 ± 2.30	18.67 ± 2.75	19.21 ± 2.81	n.s.
Sign.		n.s.	n.s.	n.s.	n.s.	n.s.	

Data are mean (n=3 ; n=12 for colour parameters) ± s.d. Small and capital letters, **, *, n.s. see Table 3.

The contribution made by LPE addition to edible coating was more evident for weight loss, headspace gas composition (Table 7) , and microbiological count.(Table 8) A natural decrease in weight was observed in all samples during the storage and significant differences ($p < 0.05$) were noted particularly after 21 days in AL-LPE 2% and AL-LPE 4% which showed a slower dehydration. Regarding the O_2: CO_2 ratio in the headspace of trays, significant changes were found from the 14th day of storage with evident differentiation between uncoated segments (CTR), coated ones with alginate (AL), coated ones with LPE enriched alginate. The concentration of oxygen and carbon dioxide is due to the combined action of tissue metabolic activity after peeling and respiration of microbial flora [85]. In this study

the differences recorded between coated and no coated samples (Table 7) for weight loss and O_2: CO_2 ratio in the headspace of trays were due to the simultaneous action of alginate and LPE; the application of the edible alginate-based coating on the fruit surface determines a modification of the atmosphere surrounding each coated segment slowing down its metabolic activity. LPE moreover slowed or inhibited the proliferation of microorganisms and, consequently, the quality changes associated to (Rojas-Graü et al., 2007; Raybaudi-Massilia et al., 2007).

Table 7 Weight loss and trays' headspace parameters of ready-to-eat Clementine fruits during storage

Parameter	Sample	Time (days)					
		0	**3**	**7**	**14**	**21**	**Sig.**
Weight loss (g 100 g^{-1})	CTRL	0.00 ± 0.00 ^{D}	0.03 ± 0.01 ^{C}	0.02 ± 0.01 ^{Cb}	0.07 ± 0.02 ^{B}	0.10 ± 0.01 ^{Aa}	**
	AL	0.00 ± 0.00 ^{C}	0.05 ± 0.02 ^{B}	0.05 ± 0.01 ^{Ba}	0.07 ± 0.01 ^{AB}	0.10 ± 0.01 ^{Aa}	**
	AL-LPE 2%	0.00 ± 0.00 ^{D}	0.04 ± 0.02 ^{BC}	0.03 ± 0.01 ^{ABCab}	0.06 ± 0.02 ^{AB}	0.08 ± 0.00 ^{Aab}	*
	AL-LPE 4%	0.00 ± 0.00 ^{B}	0.05 ± 0.03 ^{A}	0.05 ± 0.01 ^{Aa}	0.07 ± 0.02 ^{A}	0.05 ± 0.02 ^{Ab}	*
Sign.		n.s.	n.s.	*	n.s.	*	
O_2 (%)	CTRL	21.00 ± 0.00 ^{A}	14.52 ± 1.11 ^{Bb}	14.30 ± 1.90 ^{B}	7.10 ± 0.99 ^{Cb}	5.6 ± 0.71 ^{Cc}	**
	AL	21.00 ± 0.00 ^{A}	16.40 ± 0.43 ^{Ba}	13.30 ± 0.63 ^{C}	8.25 ± 0.57 ^{Dab}	8.30 ± 0.68 ^{Db}	**
	AL-LPE 2%	21.00 ± 0.00 ^{A}	17.40 ± 0.38 ^{Ba}	14.70 ± 1.86 ^{C}	9.40 ± 0.14 ^{Cab}	10.60 ± 0.21 ^{Ca}	**
	AL-LPE 4%	21.00 ± 0.00 ^{A}	17.70 ± 0.75 ^{Ba}	14.92 ± 1.06 ^{C}	12.50 ± 2.12 ^{Da}	8.80 ± 0.64 ^{Eb}	**
Sign.		n.s.	**	n.s.	**	**	
CO_2 (%)	CTRL	0.02 ± 0.00 ^{D}	9.40 ± 1.37 ^{C}	10.00 ± 2.30 ^{BC}	13.00 ± 1.13 ^{ABb}	19.90 ± 0.14 ^{Aa}	**
	AL	0.02 ± 0.00 ^{D}	9.20 ± 0.43 ^{C}	9.80 ± 0.81 ^{C}	14.90 ± 0.10 ^{Ba}	18.25 ± 0.51 ^{Aab}	**
	AL-LPE 2%	0.02 ± 0.00 ^{C}	7.75 ± 1.82 ^{B}	8.90 ± 1.69 ^{B}	16.80 ± 0.92 ^{Aa}	17.30 ± 0.78 ^{Ab}	**
	AL-LPE 4%	0.02 ± 0.00 ^{D}	7.70 ± 0.79 ^{C}	10.30 ± 1.22 ^{C}	13.50 ± 2.25 ^{Bab}	17.50 ± 1.82 ^{Aab}	**
Sign.		n.s.	n.s.	n.s.	*	*	

Data are mean (n=3 ; n=12 for colour parameters) ± s.d. Small and capital letters, **, *, n.s. see Table 3.

Microbiological analysis of ready-to-eat Clementine fruits (Table 8)also confirmed its antimicrobial activity, as evidenced by the slow growth of mesophilic bacteria (TBC) and mould with a dose-dependent effect. Yeasts were not detected

in all samples and for each time of storage. The hydroalcoholic extract obtained from lemon by-products was already indicated by several authors as antimicrobial against many microorganisms that cause spoilage problems and food safety (*Bacillus spp., Salmonella spp., Staphylococcus spp, Listeria monocytogenes, Enterobacteraerogenes, Escherichia coli, Pseudomonas aeruginosa, Alternaria sp, Aspergillus and Rhizopus sp*.) in accordance with the results obtained in this study (Gupta et al., 2017; Imeneo et al., 2022).

Table 8 Trays' headspace and microbiological parameters of ready-to-eat Clementine fruits during storage (Log_{10} CFU g^{-1})

Parameter	Sample	Time (days)					
		0	3	7	14	21	Sig.
CBT	CTRL	0.00±0.00^{D}	0.00±0.00^{D}	1.45±0.26^{abC}	3.00±0.04^{aB}	5.11±0.00^{aA}	**
	AL	0.00±0.00^{C}	0.00±0.00^{C}	1.89±0.35^{aB}	1.50±0.28^{bB}	2.75±0.25^{bA}	**
	AL-LPE 2%	0.00±0.00^{C}	0.00±0.00^{C}	1.10±0.17^{bB}	1.09±0.12^{bcB}	2.45±0.50^{bA}	**
	AL-LPE 4%	0.00±0.00^{C}	0.00±0.00^{C}	0.96±0.24^{bB}	1.00±0.00^{cB}	2.32±0.50^{bA}	**
Sign.		n.s.	n.s.	*	**	**	
Mould	CTRL	0.00±0.00^{C}	0.00±0.00^{C}	0.00 ± 0.00^{C}	1.77±0.10^{aB}	2.15±0.63^{aA}	**
	AL	0.00±0.00^{C}	0.00±0.00^{C}	0.00 ± 0.00^{C}	1.03±0.10^{aB}	1.94±0.34^{aA}	**
	AL-LPE 2%	0.00±0.00^{B}	0.00±0.00^{B}	0.00 ± 0.00^{B}	0.00±0.00^{bB}	1.66±0.00^{aA}	**
	AL-LPE 4%	0.00±0.00	0.00±0.00	0.00 ± 0.00	0.00 ± 0.00^{b}	0.00±0.00 ^{b}	n.s.
Sign.		n.s.	n.s.	n.s.	**	*	

Data are mean (n=3 ; n=12 for colour parameters) ± s.d. Small and capital letters, **, *, n.s. see Table 3.

Textural analyses (Figure 4) showed that all tested coatings significantly ($p<0.01$) improved the firmness of the Clementine segments, as reported in previous studies on similar coatings (Najafi Marghmaleki et al., 2021; Reyes-Avalos et al., 20216). Predictably, CTR showed a lower firmness than AL, AL-LPE 2%, and AL-LPE 4%, due to the natural softening process after peel removing and dissection in citrus segments. During the storage period, different trends were observed: AL

showed a stronger increase of firmness (49.18%) from 1.83 N to 2.73 N, whereas no significant variations were noted in AL-LPE 2% and AL-LPE 4%. The trends of AL could be due both to interactions between alginate and cell wall pectins and to the chemical structure of edible coating which causes excessive drying and hardening of vesicles (Huang et al., 2023). The results of our study seem coherent with literature that reports an improvement in physical performance of coating by the addition of vegetable extracts ranging from 3-5 pH (Bazban et al., 2017).

Several studies show that edible alginate coatings can improve the visual and structural properties of ready-to-eat fruits over long shelf life, avoiding structural changes in the cell wall and visual appearance due to the degradation of valuable constituents (Zhang et al., 2024; Alharaty et al., 2020).

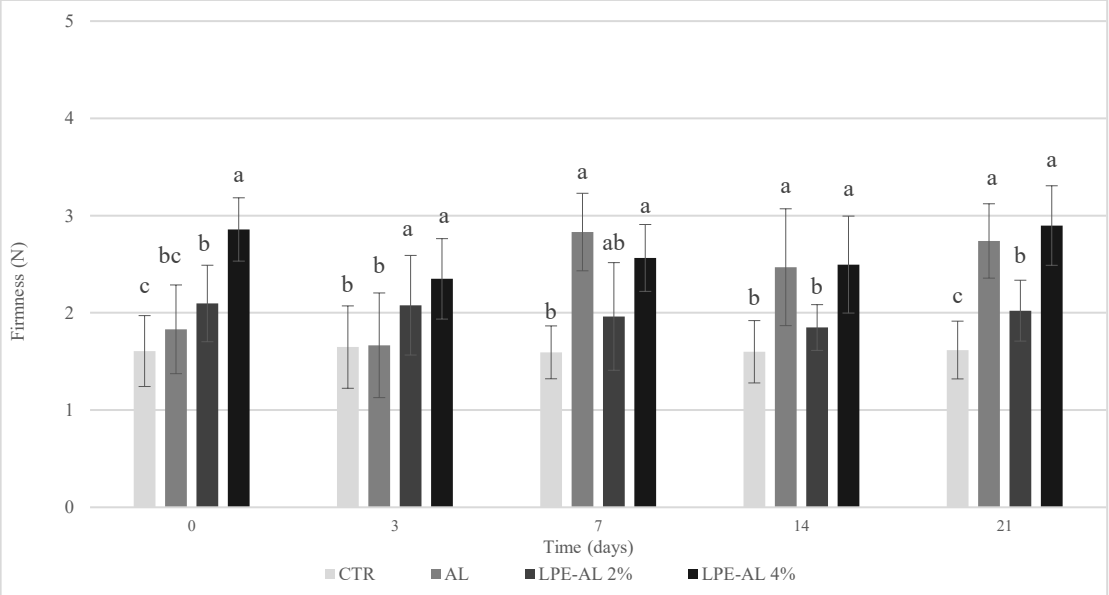

Figure 4 Firmness (N) of ready-to-eat Clementine fruits during the storage Letters shows the significant differences among samples for each monitoring time by Tukey's *post-hoc* test. n.s. see Table 3.

The sensory evaluation confirmed the results obtained for physical analysis (Table 7 and Figure 5). In particular, the treatments applied to AL, AL-LPE 2%, and AL-

LPE 4% statistically improved ($p<0.01$) the colour and firmness of the Clementine segments compared to CTR immediately after their application while the parameters related to the olfactory and gustatory sensations were unchanged, as well as total acceptability (Table 7 , Figure 5a and 5c). The results obtained at the beginning of storage indicated that LPE did not affect negatively the visual characteristics and the flavour of the fruits, which is one of the main requirements that edible coatings must have to be applied to food (Baldwin et al., 2011).

The data at the end of storage (Table 7) showed that the alginate-based coating and the addition of LPE improved the sensory acceptability for colour, fruitiness, and turgidity at 21 days of storage at 4 °C (Figure 5b), with overall acceptability scores at the end of storage of 6.83 and 7.0 for Al-LPE 2% and AL-LPE 4% respectively (Figure 5c).

Table 7 Sensory evaluation of Clementine segments during the storage

	Days	CTR	AL	AL-LPE2%	AL-LPE4%	Sign.
Colour	0	7.00±0.50^{b}	8.67±0.47^{a}	8.33±0.47^{a}	8.50±0.49^{a}	**
	21	6.83±0.86^{b}	8.00±0.82^{a}	7.5±0.96^{ab}	8.00±0.63^{a}	*
Visual Appearance	0	7.83±0.76	8.5±0.50	8.5±0.76	8.33±0.49	n.s.
	21	8.17±0.69	8.17±0.37	8.33±0.94	7.67±1.02	n.s.
Fruity	0	7.17±1.34	6.33±0.47	6.83±0.69	7.00±0.63	n.s.
	21	6.33±0.47^{ab}	6.00±0.57^{b}	7.00±0.58^{a}	6.50±0.49^{ab}	*
Citrusy	0	6.50±0.76	6.67±0.75	6.83±0.69	6.83±0.80	n.s.
	21	5.83±0.68	6.50±0.05	6.83±0.68	6.67±0.80	n.s.
Firmness	0	6.83±1.07^{b}	8.00±0.58^{a}	8.33±0.47^{a}	8.17±0.63^{a}	**
	21	6.17±0.75^{b}	7.00±0.95^{ab}	8.17±0.37^{a}	8.00±0.75^{a}	**
Overall acceptability	0	6.60±0.80	6.40±0.49	6.80±0.75	6.80±0.40	n.s.
	21	5.33±0.82^{b}	6.17±0.41^{ab}	6.83±0.41^{a}	7.00±0.89^{a}	**

Data are mean (n=10) ± s.d. Small letters, **, *, n.s. see Table 3.

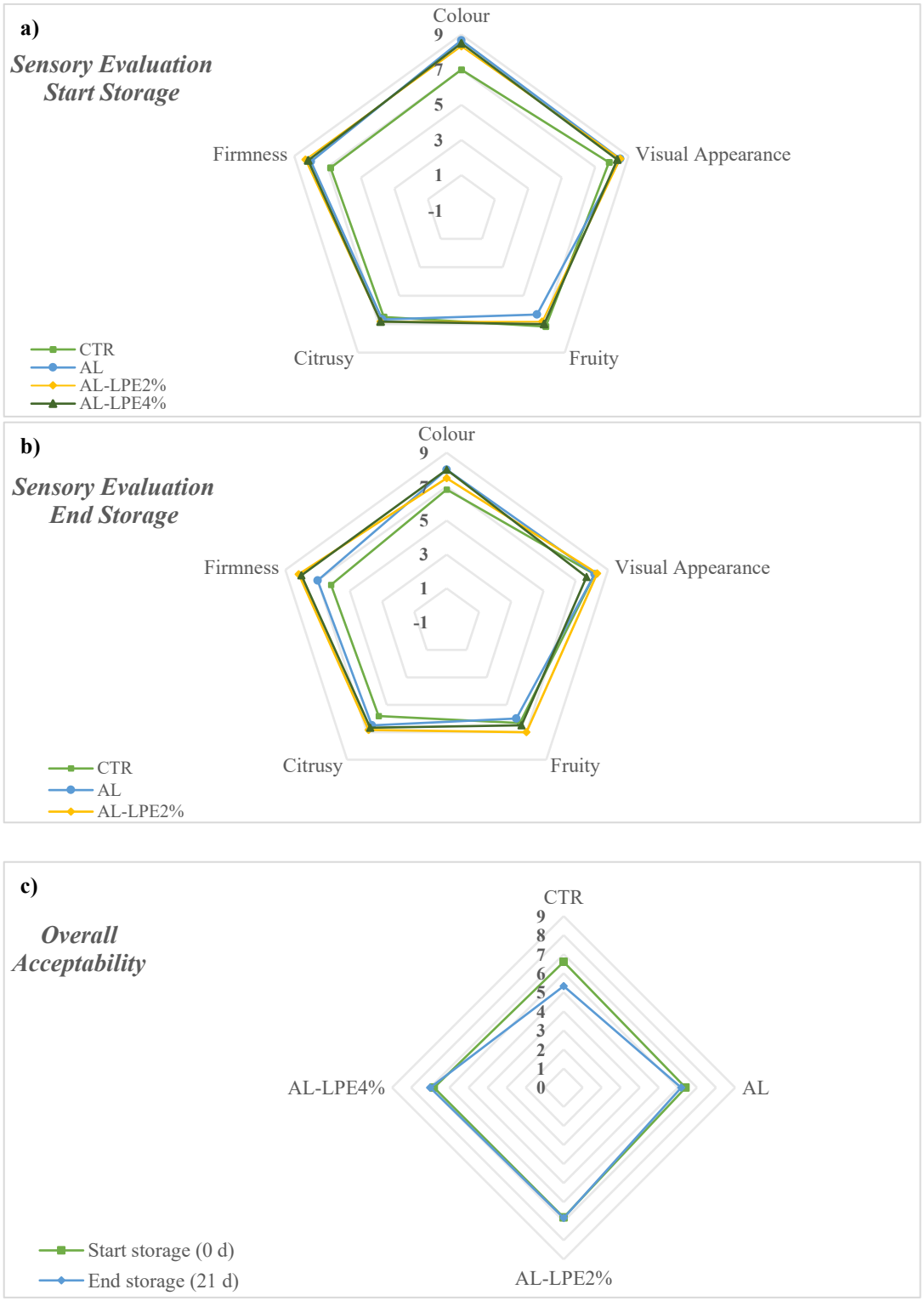

Figure 5. Spider plot of Sensory evaluation of Clementine Segments at start (a), end (b) storage and overall acceptability (c)

5.2.4. Conclusions

The synergistic action of alginate-based coating and lemon by-product extract has considerably improved the storage and safety of ready-to-eat Clementine fruits up to 21 days of storage at 4°C. These parameters are intimately linked to the visual acceptability perceived by the consumer at the moment of purchase regarding both the status of the trays (swelling, fog, etc.) and the appearance of the fruit (intensity colour, turgidity, wrinkling, presence of moulds, etc.) as confirmed by sensorial analysis. Among the tested treatments, the addition of both concentrations of LPE to the coating formulation permitted to obtaining a structure that favoured in Clementine fruits the retention of antioxidant compounds, microbial safety and good sensory acceptance.

In the modern consumer scenario, the challenge is to meet consumer demand for high-value fruit products: the presence of LPE in the edible coating allowed an increase of high levels of ascorbic acid and prevented its degradation for 21 days of cold storage, making these foods an excellent and beneficial food for the final consumer. Eating 50 grams of ready-to-eat Clementine segments AL-LPE 2% and AL-LPE 4% can support the daily requirement of ascorbic acid. Moreover, the combination of alginate and LPE is an efficient and sustainable natural treatment to preserve ready-to-eat fruits by satisfying consumer demand for natural preservatives and the environmental need for reuse sustainable by-products of food processes.

The use of LPE can therefore allow for the preservation of the total quality of the ready-to-eat fruits for 21 days and, at the same time, the reuse of lemon by-product could encourage the rapid transition of the citrus industry from a linear

economy to a circular, thus promoting the sustainability of production and the reduction of food waste with a high environmental impact. Future studies could focus on the *in vivo* activity of compounds recovered from lemon by-products.

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Chapter 7 Conclusions and Future Perspectives

The scientific evidence obtained during these three years of the PhD project has highlighted the strategic value of coffee and citrus agro-industrial by-products as sustainable sources of bioactive compounds. The innovative use of these by-products has demonstrated the potential to improve food quality, safety and shelf life, meeting both consumer needs in terms of naturalness and functionality of foods, both the environmental requirements related to reducing by-products and the rapid transition of the food sector towards a circular economy.

Among the most relevant aspects revealed by the PhD research counts himself the success of the extraction of bioactive compounds from Coffee Silverskin (CSE), obtaining extracts rich in bioactive compounds (polyphenols, flavonoids, chlorogenic acid, and caffeic acid) with high antioxidant activity. The efficacy of these extracts has been validated through several food applications, including dipping treatments for fresh-cut fennel slices and the enrichment of gummy candies. The results obtained from these studies showed that the inclusion of CSE improved the chemical, physical, and sensory properties of products, contributing to greater stability over time and enhanced antioxidant activities. Furthermore, the use of CSE as a functional ingredient in food applications has been shown to positively influence the microbiological characteristics of processed foods, reducing spoilage and prolonging shelf-life, as well as in the increase of valuable bioactive compounds and health promoters such as organic acids and phenolic acids.

Similarly, the recovery of bioactive compounds from lemon pomace (LPE) and their incorporation into edible coatings alginate based have shown promising results for the preservation of ready-to-eat clementine segments up to 14 days of storage.

This new form of edible coatings has proved to be an effective strategy for extending the shelf-life of the product while maintaining unchanged nutritional, microbiological and sensory characteristics, surpassing in some aspects even traditional modified atmosphere packaging techniques (MAP).

Therefore, the potential of CSE and LPE could be extended to other applications in the food industry as baking, dairy products, or beverages to offer an innovative solution for boosting the stability and nutritional value of these foods. Further studies could explore the use of CSE and LPE in synergy with other natural extracts, obtained from other food by-products, to develop even more effective food formulations with long-term beneficial effects on the quality of various categories of food. Moreover, further study could be focused on the use of food by-products for the production of biopolymers for food packaging and edible films and coatings, and also the specific isolation of bioactive compounds from food by-products, to obtain purified ingredients with targeted applications in food, pharmaceutical and cosmetics sector. Identification and isolation of highly effective bioactive fractions from agri-food by-products could open new frontiers in their incorporation into biodegradable packaging material, to confer antimicrobial and antioxidant properties, as well as in the formulation of high-value-added food as natural and nutraceutical ingredients.

However, although the results of this PhD research lay the foundations for an industrial application of agri-food by-products, providing concrete solutions for reducing waste and exploiting materials that would otherwise be considered as waste, there are still some critical areas that deserve further investigation.

An aspect crucial for the effective exploitation of agro-industrial by-products the aspect that needs to be further investigated is the in vivo and in vitro activity of the bioactive compounds extracted from agri-food industrial by-products. Assessment of the absorption, metabolism and biological effects of these compounds in the human body is essential to determine their actual effectiveness and safety for the final consumer, facilitating their application in food products to confer nutraceutical and functional activity. However, it is also mandatory to raise awareness among producers and consumers of the benefits of using by-products, thereby promoting their acceptance in the food market.

In conclusion, this study has shown that agro-industrial by-products can be a valuable resource for the food industry, contributing to sustainability and innovation of the sector. However, their full exploitation requires further scientific research and an integrated approach involving research, industry and regulators to turn waste into resources and promote a more efficient and sustainable food system.

SCIENTIFIC ACTIVITIES CONDUCTED DURING PHD COURSE

Publications on international peer reviewed journals during PhD course

1. Boninsegna, M. A., De Bruno, A., & Piscopo, A. (2023). Quality Evaluation of Ready-to-Eat Coated Clementine (Citrus x Clementina) Fruits. Coatings, 13(9), 1562. https://doi.org/10.3390/coatings1309156223-25
2. Boninsegna, M. A., De Bruno, A. & Piscopo, A. (2024). Improving the Storage Quality of Ready-to-Eat Clementine Fruits Using Lemon By-Products. Agriculture, 14(9), 1488. https://doi.org/10.3390/agriculture14091488
3. Boninsegna, M. A., Cilea, I., Piscopo, A., De Bruno, A., & Poiana, M. (2024). Sustainable use of coffee roasting by-products: development of high value-added gummy candies. Journal of Food Measurement and Characterization, 18(11), 9519-9531. https://doi.org/10.1007/s11694-024-02898-9

Manuscripts Under Review

1. Boninsegna, M. A., De Bruno, A., Piscopo, A. & Poiana, M. (2024) Use of Coffee roasting by-products (Coffee Silverskin) as a natural preservative to prevent the storage decay of fresh-cut fennel slices. Food Packaging and Shelf-life. (Under Review)

2. Boninsegna, M.A., Piscopo A., Bandić, L. M & Jurić S. (2024) Development of edible film enriched with bioactive compounds from Mandarin peel. Food Chemistry. (Under Review)
3. Boninsegna, M. A., Piscopo, A., De Bruno, A., Crea, G., Chinè, V., and Poiana, M. (2025) Sustainable recovery of bioactive compounds from Coffee silverskin by Food grade solvents. Journal of Environmental Science and Health, Part B (Under review)
4. Boninsegna, M. A. Maslov Bandićb, L., Donsì, F., Piscopo, A., Jurić, S. (2025). Optimization of Ultrasound-Assisted Extraction of Bioactive Compounds from Satsuma Mandarin Pulp Agro-Industrial Residue using Water and Water/Ethanol Solvent Mixtures. Chemical Engineering Transactions (Under Review)
5. Boninsegna, M.A., Piscopo A., De Bruno, A., Bandić, L. M & Jurić S. (2025) Application of edible coatings enriched with bioactive compounds from Mandarin by-products to extend the shelf life of ready-to-eat onions. Journal of the Science of Food and Agriculture (Under Review)
6. Giacondino C., Boninsegna M.A., De Bruno, A. & Piscopo, A. (2024) Effect of Bio-based and Petroleum-based Packaging on quality Change of fresh-cut fennel. Journal of the Science of Food and Agriculture. (Under Review)
7. Giacondino C., Boninsegna M.A., De Bruno, A. & Piscopo, A. (2024) Application of intelligent packaging in the quality assessment of fruits and fresh-cut vegetables. Food Packaging and Shelf Life. (Under Review)

Contributions to national and international conferences

1. 27th Workshop on the developments in the Italian PhD Research on Food, “University of Naples- Federico II”, Portici, Naples, Italy. (2023). By-products from the agri-food industrial sector: resource or waste? An eco-friendly utilization to preserve the quality of food.
2. 6th ISEKI E-Conference "Food Production Based on Food Safety, Sustainable Development, and Circular Economy". "Lucian Blaga" University of Sibiu, Sibiu, Romania. (2023) Sustainable use of coffee roasting by-products: development of high value-added gummy candies
3. Conference SISTAL 2024, “Aldo Moro University”, Bari, Italy (2024). Sustainable use of Lemon by-products to improve the shelf-life of ready-to-eat citrus fruit
4. 28th Workshop on the developments in the Italian PhD Research on Food, “University of Catania”, Catania, Italy. (2024) By-products from the agri-food industrial sector: resource or waste? An eco-friendly utilization to preserve the quality of food.

Other PhD activities

University of Zagreb (Croatia), Agricultural Chemistry Department (January-July 2024)

Study of by-products from the agri-food industrial sector for food application: Optimization of the recovery of bioactive compounds from Mandarin waste and use for edible film formulation and hydrogel

Other academic activities

1. "Subject expert" to the teachings of Fundaments of Agricultural Industries and Food Quality Management. Course in Food Science and Technology, Agriculture Department, University Mediterranean of Reggio Calabria (Italy);

2. "Subject expert" to the teachings of Statistical Processing of experimental data. Course in Food Science and Technology, Agriculture Department, University Mediterranean of Reggio Calabria (Italy);

3. "Subject expert" to the teachings of Physical and Sensory analyses of food products. Course in Food Science and Technology, Agriculture Department, University Mediterranean of Reggio Calabria (Italy);

4. "Subject expert" to the teachings of Conditioning and Distribution of food products Technologies. Course in Food Science and Technology, Agriculture Department, University Mediterranean of Reggio Calabria (Italy);

5. "Subject expert" to the teachings of Quality Assessment and Management in Gastronomy and Catering. Course in Food Science and Technology, Agriculture Department, University Mediterranean of Reggio Calabria (Italy).